THE BOTANICAL GAZETTE

EDITOR
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ERRATA

VOL. LXXII

P. 341, line 5, omit sweet pea

P. 372, line 4 from bottom, for most read moist

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P. 62, lines 3 to 5, delete sentence referring the Brazilian rust on *Lygodium* to the genus *Desmella*

P. 85, line 4 from bottom, for LIPMAN read LIPMAN and GERICKE

P. 87, line 8, for ROBINSON (**59**, **60**) read ROBINSON (**59**) and ROBINSON, STEINKONIG, and FRY (**60**)

P. 201, line 2 from bottom, for following read preceding

P. 223, Fig. 3, for 1.19 read 11.9

P. 278, line 10 from bottom, for equal read comparable

P. 285, line 2 under Literature Cited, for Am. So. read Ann. Sci.

P. 382, Table III, last column, for 0.1240 read 0.0240

P. 383, Fig. 3 is inverted, making the legend incorrect as to right and left

VOLUME LXXIII NUMBER 1

THE BOTANICAL GAZETTE

January 1922

NONSYMBIOTIC GERMINATION OF ORCHID SEEDS

LEWIS KNUDSON

(WITH THREE FIGURES)

The germination of orchid seeds for a long time has been recognized as difficult and generally uncertain of attainment. Practical orchid growers for years have attempted to find a method which will insure germination. They meet with success at times, but fail utterly on a second attempt with the same method. Moreover, two sowings made at the same time and under apparently identical conditions may result in germination in the one case and failure in the other. There are growers in England, France, and also the United States who, if one may believe reports, are consistently successful in germinating the seeds of the commercially important orchids. The grower, however, is naturally unwilling to part with the details of his method. From the scientific aspect it is doubtful whether he can explain the cause of his success. Generally speaking it may be stated that practical orchid growers have not yet solved the problem of producing orchid plants from seeds.

The difficulty of germinating seeds of orchids is due in part to inherent causes, but undoubtedly is due also to environmental factors. The extremely small size of the embryo renders it liable to death if it becomes desiccated. Generally the seeds are sown on a substratum rich in organic matter, such as sawdust, leaf mold, wood or bark, peat, sphagnum, or mixtures of the two last-named substances. These substances are favorable for the growth

of fungi and algae, and the embryos may be killed because of being covered by these organisms, or more likely by injurious substances produced by the decomposition of these organisms. The work of BURGEFF (4) and BERNARD (2) demonstrates that death may be due to pathogenic fungi, and the writer's experiments in transplanting seedlings from tubes to open pots demonstrate clearly this danger. In addition to these factors, attention must be given to preventing loss due to insect pests. As suggested, however, there are apparently inherent characteristics of the seeds which make for refractory germination. It is this which attracted the attention of BERNARD, who in a number of publications presented evidence tending to show that the germination of the seeds and the subsequent growth of the seedlings are dependent upon infection by certain strains of the fungus which generally is found living in the orchid root, and which BERNARD considered to be *Rhizoctonia*. BURGEFF came to substantially the same conclusions, maintaining that germination was possible only when the embryo became infected with the proper strain of the fungus, to which he gave the name *Orcheomyces*, without attempting to classify it.

BERNARD and BURGEFF both pointed out that infection of the embryo began at the suspensor end of the seed, and that in the case of *Cattleya* and related forms the primary infection occurred through the delicate suspensor. Growth occurred if only the lower portion of the embryo became infected, and if the infection continued beyond approximately the lower third of the embryo, then death of the embryo resulted. It was also observed in germinating embryos that the fungus disintegrated in the infected zone, forming clumps of disintegrated hyphal material in the cells similar to the clumps found in cells of the root. It was the opinion of BERNARD that the fungus was digested by the orchid embryo. The essential point to be noted, however, is that a delicate balance between the host and the fungus apparently must be maintained in order to insure germination and also to prevent death of the embryo.

Granting for the present that a symbiotic relationship exists between the fungus and the embryo, it is nevertheless true that failure of germination is more common than success, even when the fungus is provided. BERNARD'S experiments reveal case after

case in which the introduction of the fungus was followed by death of the seeds or failure to germinate. He states as follows:

> The germination by inoculation is not obtained without certain difficulties. For five years I have sown seeds of diverse species of orchids in culture tubes, each of which contained 100 seeds, and these I have inoculated with *Rhizoctonia* obtained from the roots. Altogether, I have obtained a few hundreds of seedlings, but I underestimate when I place the number of seeds used in my experiments at 50,000. For the majority of the seeds, the association with the fungus that I have placed in their presence has been merely passive and without effect, or impossible or rapidly injurious to the embryos.

The explanation generally offered in these cases is that "activity" of the fungus was altered or the proper strain was not employed, so that the essentially delicate balance between the fungus and the embryo was not maintained.

In certain experiments BERNARD succeeded in germinating seeds of *Cattleya* and *Laelia* without the intervention of the fungus. This was accomplished by using a more concentrated solution of salep. Salep (KING, **6**) is the dry powder obtained by pulverizing tubers of certain orchids, and contains, principally, mucilage 48 per cent, starch 27 per cent, and proteins 5 per cent. It probably contains also some sugar as well as soluble mineral matter. The seedlings obtained in this way were in every respect normal and the germination was very regular. BERNARD suggests that some such method might be developed for practical purposes, since the results with the fungus are so unsatisfactory.

The increasing importance of orchid culture in America, the difficulties in and the restrictions on the importation of orchid plants, and the desirability of creating new hybrid forms, make particularly desirable a method for germinating the seeds. Certain data from the experiments of BERNARD and BURGEFF, indicating that soluble organic compounds might cause germination, and my own previous experiments (**7**) on the organic nutrition of plants, demonstrating that various sugars have a very favorable influence on growth, are indications that germination of orchid seeds might be obtained by the use of certain sugars. This proved to be true.

The results here reported describe a method for germinating the seeds under sterile conditions, the influence of certain sugars

on growth of the embryos, the influence of different concentrations of sugar on growth, the effects induced by certain plant extracts, the favorable influence of certain bacteria, and experiments on transplanting. In the discussion are treated critically the ideas expressed by BERNARD and BURGEFF with respect to the function of the fungus.

For a clearer understanding of the data that follow, it is desirable to trace briefly the mode of development in the germination of seeds of *Cattleya* and *Laelia*. For a detailed discussion, BERNARD'S paper should be consulted. The embryo is somewhat oval-shaped, and is undifferentiated except that the cells at the basal region are large, while those at the apical region are smaller. This is the meristematic region. At the base is subtended a delicate suspensor. The embryo is inclosed within a transparent integument with an opening at the lower end through which the suspensor may protrude. The maximum length of the embryo of *Cattleya* or *Laelia* is about 250μ and the width about 75μ.

Germination consists, first, in an enlargement of the embryo in a transverse direction until a small spherule stage is reached. Accompanying this development there is the formation of chlorophyll, generally more pronounced in the meristem region. The embryo when it ruptures the integument has a width of about 175μ and a length of about 270μ. At the time of rupturing the integument, absorbing hairs begin to grow out from the epidermis. Subsequent development consists in a further enlargement of the embryo, other absorbing hairs begin to develop near the basal region, and there is attained a large spherule or top-shaped structure characterized by a marked depression at the upper surface. Following this there appears in the middle of the depression the first leaf point, which subsequently develops into the first leaf. During this period there is a continued increase in the diameter of the embryo, so that a disklike structure is formed which has been termed by BERNARD the protocorm. At the meristematic region a second and a third leaf may unfold, elongation may occur, and a distinct stem is apparent. The first root may arise either from the protocorm or from the stem below the second or the third leaf. The period required for these developments is generally

from four to six months. Under greenhouse conditions this advanced stage has apparently been attained in some cases in a shorter time.

Methods

Unless otherwise indicated, all cultures were made using agar slopes in culture tubes 180 mm. × 18 mm. The nutrient solution used was either Pfeffer's or a modification referred to hereafter as solution B. The solutions were made up as follows:

Solution B	Pfeffer's
$Ca(NO_3)_2$, 1 gm.	$Ca(NO_3)_2$, 4 gm.
K_2HPO_4, 0.25 gm.	K_2HPO_4, 1 gm.
$MgSO_47H_2O$, 0.25 gm.	$MgSO_47H_2O$, 1 gm.
$Fe_2(PO_4)_3$, 0.05 gm.	KNO_3, 1 gm.
$(NH_4)_2SO_4$, 0.50 gm.	KCl, 0.5 gm.
Distilled H_2O, 1 l.	$FeCl_3$, 40 mgm.
	Distilled H_2O, 5 l.

Solution B was used because Burgeff stated that the orchid seeds utilized ammonium sulphate to better advantage than the nitrate salt. My own experience is not in accordance with this.

Generally 1.50 per cent agar was used, and all media and vessels were autoclaved at fifteen pounds pressure for thirty minutes. To prevent the lodging of spores and microorganisms on the cotton stopper of the culture tube, it was capped with a small vial which, fitting tightly over the cotton plug, inclosed the upper third of the tube. The use of the vial cap was essential because otherwise, under the moist greenhouse conditions, contamination resulted from spores growing down through the cotton plug or between the plug and the tube. By using the vial cap cultures remained pure even after a year in the greenhouse.

The cultures were all grown under aseptic conditions. For sterilizing the seeds, the calcium hypochlorite method of Wilson (13) was used. For this purpose 10 gm. of calcium hypochlorite was added to 140 cc. of distilled water. This was vigorously shaken for a few minutes and then filtered. The clear filtrate was used for sterilizing the seeds. The quantity of seeds desired was placed in a small test tube and the clear filtrate added. The tube was then shaken until each seed became moistened with the solution.

This was repeated several times, since the seeds generally float together in a mass at the surface of the liquid. The period of exposure was about fifteen minutes, although in some preliminary experiments with seeds of *Cattleya* and *Laelia* no injury was noted after a three hours' exposure. The seeds were transferred from the sterilizing solution, without any previous rinsing in water, by the use of a platinum needle. With the small loop used, it was possible to pick up about 100 seeds. These were scattered over the surface of the agar slope. The cultures were maintained in moist chambers in the greenhouse shaded by cheesecloth from direct sunlight, with the temperature between 15° and 35° C. In determining growth, the embryos were measured by means of an ocular micrometer. As was shown by both BERNARD and BURGEFF, the width of the embryo or the protocorm may be accepted as a good criterion of the degree of growth. Other data are included, such as percentage of germination, time of formation of first leaf, color, starch content, etc.

Preliminary experiments

EXPERIMENT 1.—On December 7, 1918, seeds of *Cattleya Schroederae*×*C. gigas* were sterilized by treating them for two hours with the calcium hypochlorite solution. The seeds were sown on agar slopes. The medium used in one case was an extract of peat, made by autoclaving 300 gm. of bog peat, such as is used for potting orchids, with 1200 cc. of tap water. This was filtered and the clear brownish filtrate used. The other medium was made by autoclaving 400 gm. of dormant canna tubers with 600 cc. of water for thirty minutes. By January 7, 1919, the seeds on both were in the small spherule stage and were green. On April 10, four months after planting, the seeds on the canna medium had germinated, the seedlings having one and two leaves. On the peat agar medium the embryos were a little larger than on January 7, 1919, but not significantly different.

EXPERIMENT 2.—The media used were extracts of carrot and garden beet. The carrot extract was made by autoclaving 70 gm. of young carrots (root) with 75 cc. of tap water, and the garden beet extract was made by autoclaving 50 gm. of young beets (root)

with 75 cc. of water. The extracts were filtered, and to the clear filtrate 1.25 per cent agar was added. Seeds of *Cattleya labiata* × *C. aurea* were sterilized and planted on February 14, 1919. On May 13 some of the seeds in each had germinated and the remainder were almost germinated, that is, they were just at the point of producing the first leaf.

EXPERIMENT 3.—The media used were Pfeffer's alone and Pfeffer's plus 1 per cent sucrose. Seeds of *Cattleya mossiae* were planted on January 14, 1919. On July 1 the seeds in the sucrose culture had germinated, one leaf showing. On the Pfeffer's alone the embryos were in a small green spherule stage, the diameter being about 250μ, while the diameter of the embryos on sucrose was about 1000μ.

EXPERIMENT 4.—Seeds of *Cattleya intermedia* × *C. Lawrenceana* were sown on July 18, 1919, on solution B plus 2 per cent glucose on the one hand and 2 per cent sucrose on the other. Owing to an absence from the University, the cultures were not examined until June 9, 1920. At that time, in both glucose and sucrose cultures, the seedlings were well developed, although the culture media had lost most of the water by evaporation. The seedlings had two or three leaves and one or two roots, some of the roots being 4 mm. in length.

Influence of certain sugars and plant extracts on germination

The preliminary experiments show that germination of seeds of *Cattleya* and *Laelia* is possible without the aid of the fungus, provided soluble organic substances are present, particularly sugars. In all these cases the leaf point appeared only after three months, and yet under practical greenhouse conditions, when the seeds are sown merely on a compost of peat and sphagnum or other organic material, the leaf points may appear in a shorter time. For example, according to Mr. T. L. MEAD, of Oviedo, Florida, seeds of *Cattleya* have shown leaf points in as short a period as thirty-five days. Some of the media used by him were oak bark, magnolia bark, and a compost of decayed leaves and sphagnum.

It is possible, of course, that under these practical conditions the fungus is a factor in the growth. Is it possible also that certain

of the products produced on decomposition of the organic substances, such as the auximones described by BOTTOMLEY (3) or the vitamine water-soluble B, are involved in the germination of orchid seeds? Of course other factors may be involved, such as the hydrogen ion concentration, mineral salts, or the rate of transpiration, particularly as influencing the organic composition of the plant.

That a full nutrient medium plus sugar is not capable of sustaining continued growth of higher plants was shown by KNUDSON and LINDSTROM (8) in their experiments with albino corn. The plants kept either in the light or in the dark and supplied with one of several different sugars all died after a month or two. These experiments, together with the work of BOTTOMLEY on auximones, the beneficial influence of vegetable extracts on the growth of fungi recently described by DUGGAR (5) and by WILLAMAN (11), and the beneficial influence of vegetable extracts on the growth of yeast as described by WILLIAMS (12) and by BACHMANN (1) suggest that more rapid germination and more vigorous plants could be obtained if a vegetable extract was added to the nutrient medium.

With no idea of determining what specific substances are involved in stimulating growth, but in the endeavor to develop a rapid and effective method for the germination of seeds of certain orchids, the experiments described in table I were made. The nutrient solution used was solution B.

The extracts used were prepared as follows. Potato extract: 200 gm. of new potato with the skin removed, with 300 cc. of distilled water; wheat extract: 200 gm. of air-dried soft wheat, with 300 cc. of distilled water; beet extract: 200 gm. of a red garden beet cut into small pieces, with 200 cc. of distilled water. Extraction was made by autoclaving for fifteen minutes at fifteen pounds pressure, and the extracts obtained by filtration. The yeast juice was obtained as follows: three four-liter flasks, each containing three liters of WILLIAMS' solutions, were inoculated with a cake of Fleischman's yeast, and after a week the yeast was filtered from the solutions, autolyzed at 37° for twenty-four hours, and then dried by suction and washing with ether. Seventy gm. of yeast was then steamed for ten minutes with 250 cc. of distilled

water. The liquid was filtered and made up to a liter volume by the addition of distilled water.

All cultures were made in quadruplicate. The individual cultures of each series were strikingly uniform in growth, so that

TABLE I

Laelia-Cattleya HYBRID NO. 1;* SEEDS PLANTED AUGUST 31, 1920; MEASUREMENTS MADE JANUARY 27, 1921

CULTURE NO.	CULTURE SOLUTION	WIDTH OF EMBRYO IN MICRONS			PERCENTAGE OF GERMINATION	ORDER OF SUPERIORITY, MARCH 27
		Minimum	Maximum	Average		
B 53...	Full nutrient 50 cc.+50 cc. beet extract	242	582	407	0	5
B 17...	Full nutrient 50 cc.+50 cc. potato extract	339	630	459	0	5
B 60...	Full nutrient 50 cc.+50 cc. wheat extract	174	436	291	0	7
B 39...	Full nutrient+2% glucose	485	1261	970	20	4
B 28...	Full nutrient solution alone	194	242	213	0	8
B 27...	Full nutrient+2% glucose 90 cc.+10 cc. beet extract	485	1358	814	70	3
B 34...	Full nutrient +2% glucose 90 cc.+10 cc. wheat extract	582	1358	979	90	1
B 55...	Full nutrient+2% glucose 98 cc.+2 cc. yeast extract	582	1552	1076	90	1
B 66...	Full nutrient+2% fructose	776	1260	940	60	1
B 48...	Full nutrient+2% fructose 90 cc.+10 cc. beet extract	485	1358	902	80	2
B 22...	Full nutrient+2% fructose 90 cc.+10 cc. potato extract	485	1202	1008	90	1
B 31...	Full nutrient+2% fructose 90 cc.+10 cc. wheat extract	582	1260	902	70	2
B 5...	Full nutrient+2% fructose 98 cc.+2 cc. yeast extract	582	1358	1047	80	1
B 43...	Full nutrient+2% fructose 96 cc.+4 cc. yeast extract	582	1746	970	60	2
B 14...	Beet extract alone	242	872	388	0	5
B 69...	Potato extract alone	339	679	504	0	5
B 6...	Wheat extract alone	242	436	358	0	6
B 74...	Yeast extract alone	87	339	194	0	7

* Composition: *L. Perrinii* Lindl. ¼; *C. labiata* Lindl. ¼; *C. amethystoglossa* Reichb. ¼; *C. intermedia* Grah. ¼.

for the first measurements only one culture for each series was taken, and forty individual measurements were made for each culture. The measurements given in table I were made on January 27,1921. The order of superiority of the cultures on March 27 is recorded in the last column of the table. Similar data, not

included, were obtained in a like experiment with *Laelia-Cattleya* hybrid no. 2.

The degree of development represented by the numbers 1 to 8 is as follows: (1) dark green seedlings, most of these with two leaves and a few showing roots; (2) seedlings the same as no. 1, but light green; (3) seedlings light green, most of them with one leaf and a few showing two leaves; (4) seedlings light green, with only one leaf and that leaf short; (5) about 50 per cent of embryos showing leaf point; (6) embryos just showing a depression in meristem region; (7) advanced spherule stage; (8) smaller spherule stage.

The data in table I show that fructose is more favorable for growth of the embryos than glucose. This is apparent not only in the percentage showing leaves, but in the general appearance of the cultures. The embryos in the glucose cultures were whitish or yellowish in color. On the other hand, the fructose cultures were dark green. A more striking difference was noted on March 27, when in the glucose cultures the embryos were still yellowish and had shown no appreciable gain since January 27. The fructose cultures, on the other hand, had progressed and were still more markedly superior to the glucose cultures than on January 27. Fig. 1 shows the fructose culture and the nutrient solution culture minus sugar.

The addition of a plant extract to the glucose cultures has a marked effect on growth and chlorophyll development. In each case the percentage of germination is higher than with glucose alone, and the ranking of glucose-containing cultures on March 27 indicates that those with yeast or wheat extract rank with the best cultures, that with beet extract ranks in the third group, and the cultures with glucose alone fall in the fourth group. The addition of plant extract to the fructose-containing media is practically without any beneficial effect.

The loss or lack of development when glucose is supplied in the nutrient solution has been noted by MAZÉ and PERRIER (9) for corn, and by SERVETTAZ (10) in nutrition experiments with moss. In the case of orchid embryos the chlorophyll makes its appearance only when the leaf is developing, and then generally only in the leaf. Even then the leaves are only of a light green

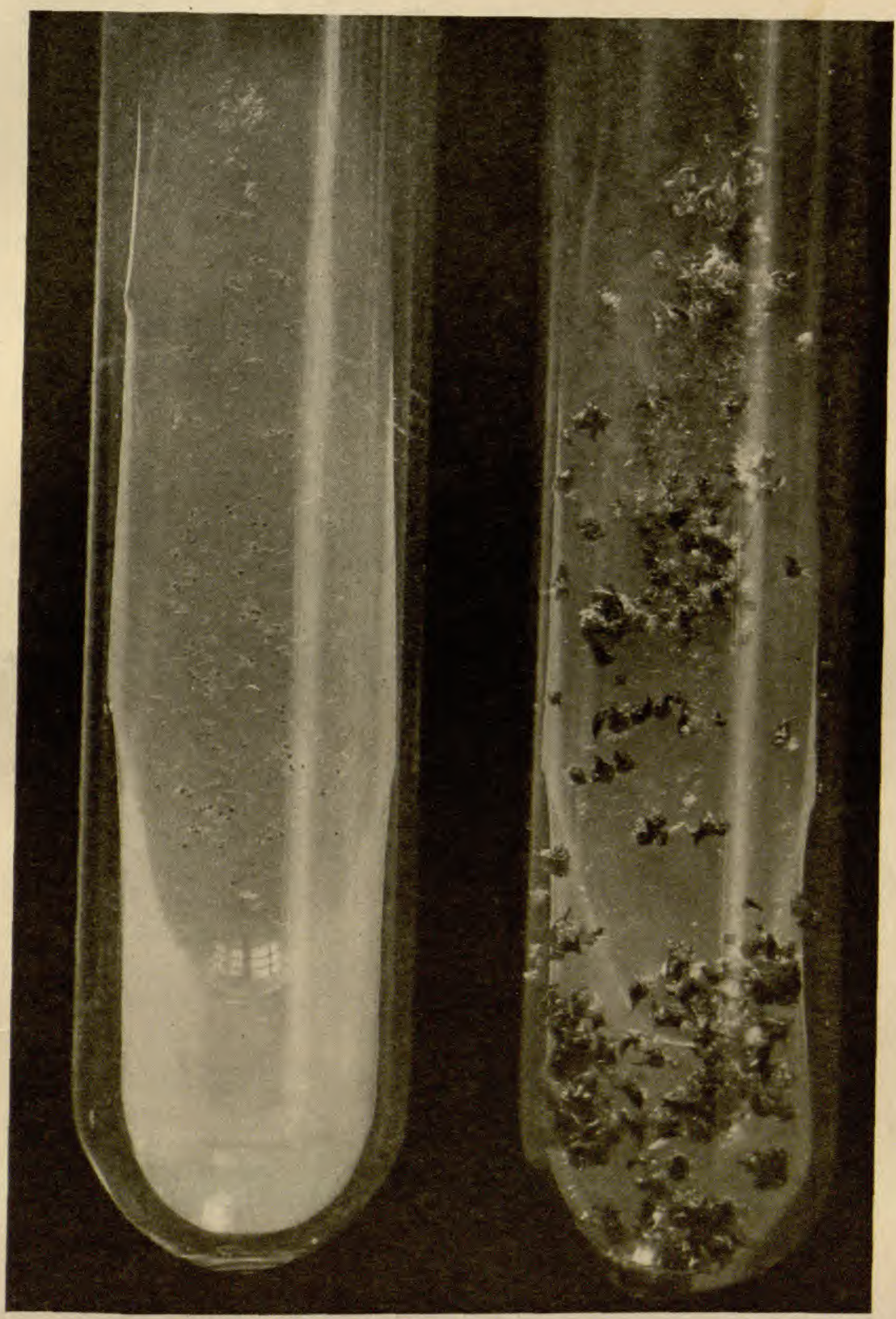

FIG. 1.—Solution B, embryos in small spherule stage; solution B+2 per cent fructose, seedling stage; ×2.

color. Is chlorosis due to a non-utilization of nitrogen or iron in the presence of glucose, or in the upbuilding of chlorophyll is glucose less favorable than fructose? Certain experiments now in progress may throw some light on this interesting point, and it is therefore desirable to await the results before speculating any further.

On January 27 the leaf point was not yet evident in any of the plant extract cultures. On March 10, however, in the wheat, beet, and potato extracts, embryos with leaf points were apparent, and a little later the same was noted for the yeast extract cultures. After several months more, seedlings with one and two leaves were to be noted in all these cultures.

Is germination on these extracts due to sugars or to other substances? Analyses made of the different extracts show that the potato, wheat, and yeast extracts had a sugar content of less than 0.025 per cent. The extracts diluted one-half with the nutrient solution, therefore, practically speaking had no sugar. The beet extract had a sugar content of 0.80 per cent, yet it did not permit any better germination than did the potato extract with merely a trace of sugar. As indicated previously, it should be borne in mind that the beet extract contains some substance injurious to the embryo. Furthermore, the stimulating effect of the plant extracts when added to the glucose solutions must be due to substances other than sugars. In the experiments on the influence of concentration of sugar, it will be noted that on the concentration of 0.05 per cent no germination has occurred even after four months.

Influence of concentration of sugar

In view of the fact that germination is possible when sugar is supplied in the culture media, it seemed desirable to determine the concentration most favorable for growth. Accordingly several series of experiments were made, a number of which are here reported.

In the first experiment seeds of *Laelia-Cattleya* hybrid no. 2 were used. These were planted November 12, 1920, and notes were made December 16, 1920, and January 11, February 15, and March 15, 1921. Each of the average figures given represents the average

of thirty separate measurements. The data are given in table II. The slow growth is well shown. There is in general a corresponding increase with increase in concentration, but the increase in concentration beyond 0.80 per cent is without any significant effect. On February 15 the embryos of all the cultures were examined for starch. It was found only in those cultures with 0.80 per cent glucose or higher. This fact is evidence that the absorption of glucose at a concentration of 0.80 per cent is in excess of the utilization, and consequently a higher concentration

TABLE II

INFLUENCE OF CONCENTRATION OF GLUCOSE, *Laelia-Cattleya* HYBRID NO. 2;* SEEDS SOWN NOVEMBER 12

CULTURE SOLUTION	AVERAGE WIDTH OF EMBRYOS IN MICRONS		
	December 16	January 11	March 15
Solution B	126	145	174
Solution B 0.05% glucose	184	232	247
Solution B 0.10% glucose	200	252	339
Solution B 0.20% glucose	242	281	475
Solution B 0.40% glucose	310	291	455
Solution B 0.80% glucose	291	339	533
Solution B 1.00% glucose	320	417	543
Solution B 2.00% glucose	320	436	523

* Composition: *C. Trianaei* Reichb. ½; *C. Loddigesii* Lindl. ¼; *L. purpurata* Lindl. ¼.

should be without any increased beneficial effect. It should be borne in mind that glucose used with solution B is not particularly suited for the germination of orchid seedlings, since there is induced constantly in the embryos a distinct chlorosis. It is probable that higher concentrations of sucrose or fructose would permit of a more rapid germination.

The results of several other experiments on the influence of different concentrations of glucose on the germination of seeds of *Cattleya* are in agreement with these results, and need no repetition. In an experiment with seeds of *Epidendron*, germination was obtained with a concentration of 0.2 per cent glucose. In the cultures with less than 0.1 per cent glucose, not only was there a less development of the embryos, but a large percentage of the seeds never showed any initial swelling and development of chlorophyll.

The detailed data are given in table III. The figures given under average width represent the averages of forty individual measurements. Only seeds that had shown an initial increase in diameter and were green were included.

In this experiment another interesting observation was made. Just previous to the formation of the leaf point, the embryos were gorged with starch. With the formation of the leaf, however, there was a disappearance of the starch, it having been converted into

TABLE III

INFLUENCE OF CONCENTRATION OF GLUCOSE, *Epidendron tampense*×*E. inosmun*; SEEDS PLANTED DECEMBER 8, 1920; NOTES TAKEN MARCH 17, 1921

CULTURE SOLUTION	CULTURE NO.	WIDTH OF EMBRYOS IN MICRONS			PERCENTAGE WITH LEAVES	REMARKS
		Minimum	Maximum	Average		
Full nutrient.....	P 2	116	203	145	.....	95% no change
Full nutrient+0.05% glucose	P 72	116	291	178	.....	90% no change
Full nutrient+0.1 % glucose	P 78	189	407	281	.....	80 %no change
Full nutrient+0.2 % glucose	P 81	339	630	465	10	30% no change
Full nutrient+0.4 % glucose	P 86	291	582	397	10	40% no change
Full nutrient+0.8 % glucose	P 91	194	582	446	20	50% no change
Full nutrient+1.00% glucose	P 99	339	582	446	10	30% no change
Full nutrient+2.00% glucose	P 104	291	630	446	10	25% no change

sugar, as evidenced by the fact that some of the embryos still showed a slight presence of dextrins.

Influence of microorganisms

Throughout the various experiments made a few cultures always became contaminated. Generally, if the contamination was a *Penicillium*, the embryos became covered by the mycelium and death resulted. Those embryos not covered often showed a marked increase in growth over the embryos in the corresponding uncontaminated cultures. The increase in growth may have been due to one or a combination of the following: an increase in the carbon dioxide content of the tube; a change in the chemical character of the nutrient medium, brought about either by secretion of organic substances from the fungus or by products produced on decomposition of the fungus; or changes in the sugar effected by extracellular enzyme action.

In an experiment with seeds of *Epidendron* growing on solution B plus 0.80 per cent glucose, one of the cultures became contaminated with a species of *Actinomyces*. The result was that when the embryos in the contaminated culture were dark green, with one and two leaves, the embryos in corresponding uncontaminated cultures were still white or yellowish and only one or two of them showing the leaf point. In the contaminated culture the embryo

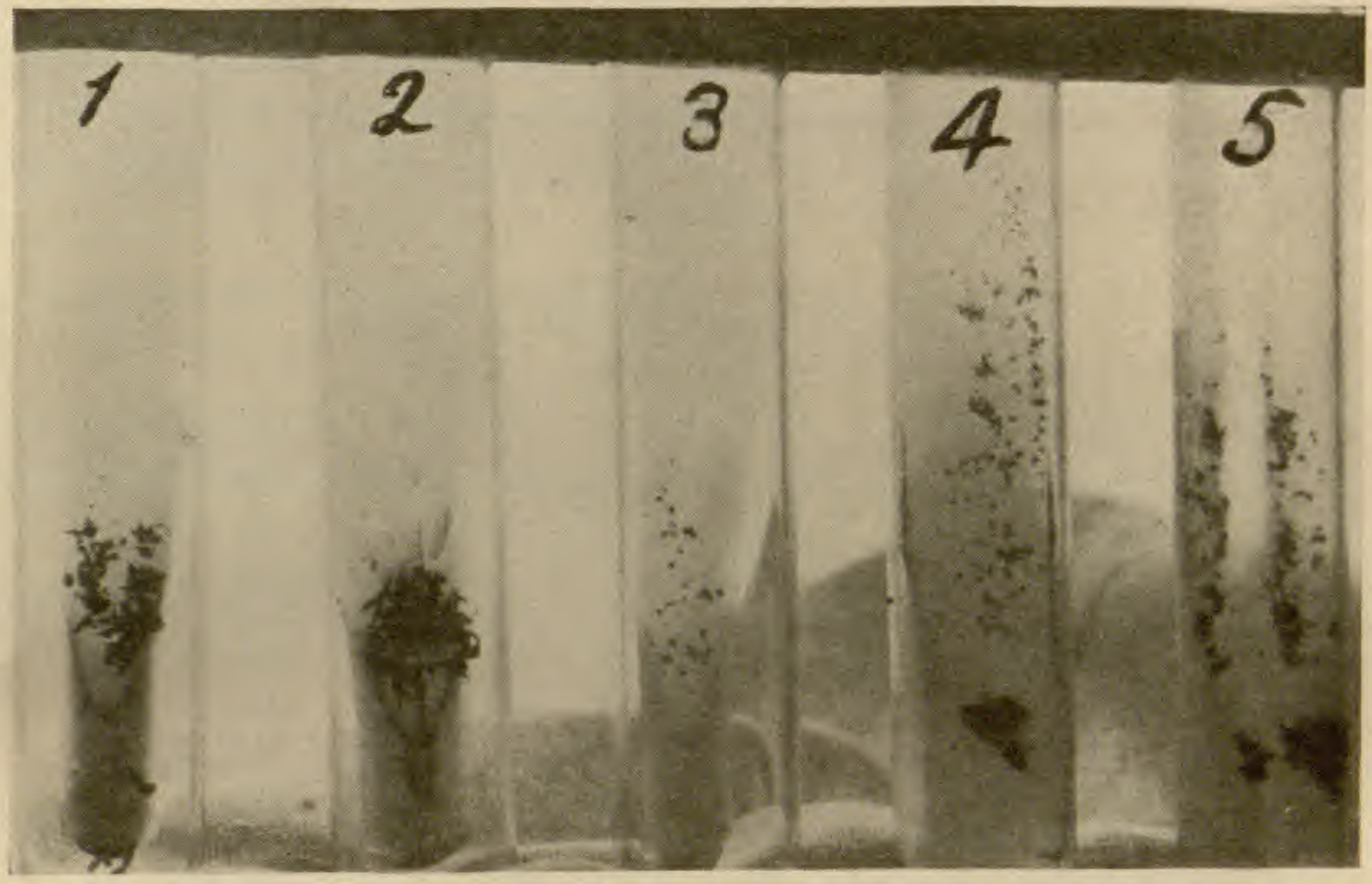

FIG. 2.—Culture no. 1, solution B+2 per cent glucose contaminated by *Actinomyces* sp. (corresponding check like no 3); culture no. 2, Pfeffer's +2 per cent sucrose inoculated with *Bacillus radicicola;* culture no. 3, same but not inoculated; culture no. 4, solution B+2 per cent glucose; culture no. 5, solution B+2 per cent glucose inoculated with *B. radicicola*; $\times\frac{2}{3}$.

had an average width of 975 μ, while in the uncontaminated cultures the average width of the embryos was 600 μ.

In view of these observations, and since BOTTOMLEY reported a beneficial effect on the growth of *Lemna* by the addition to the nutrient solution of an aqueous extract of *Azotobacter* or of *Bacillus radicicola*, an experiment was made to determine the influence of the latter organism on germination. Ten tubes were prepared with Pfeffer's solution plus 2 per cent sucrose; five were inoculated with *B. radicicola* from alfalfa, and five were left uninoculated. Seeds of *Epidendron* were sown on December 18, 1920. On March 5, 1921, 80 per cent of all the seeds in the inoculated tubes had

germinated and possessed one or two leaves. On the cultures not inoculated, the embryos lacked chlorophyll, and not one had produced even the leaf point. Most of the embryos exhibited the depression at the meristem region. Two months later some of the embryos, although still lacking chlorophyll, had formed the leaf point; while in the inoculated cultures the seedlings had two and three leaves and some had already produced roots (fig. 2).

The results appeared so unusual that the experiment was repeated with seeds of *Laelia-Catileya* hybrid no. 3, using solution B and also Ashby's solution. The composition of the nutrient

TABLE IV

INFLUENCE OF *Bacillus radicicola* ON *Laelia-Cattleya* HYBRID NO. 3;* SEEDS SOWN MARCH 14, 1921; NOTES TAKEN AUGUST 10, 1921

Nutrient medium	Average width in microns
Solution B	271
Solution B+0.1% glucose	407
Solution B+1.0% glucose	814
Solution B+inoculated	194
Solution B+0.1 glucose inoculated	378
Solution B+1.0% glucose inoculated	834
Ashby's solution	194
Ashby's solution+0.1% glucose	446
Ashby's solution+1.0% glucose	698
Ashby's solution+inoculated	164
Ashby's solution+0.1% glucose inoculated	397
Ashby's solution+1.0% glucose inoculated	970

* Composition: *C. superba* Schomb. ¼; *C. Dormaniana* Reichb. ¼; *C. Warscewiczii* Reichb. ¼; *L. purpurata* Lindl. ¼.

solution and the width of embryos are given in table IV. The favorable influence of *Bacillus radicicola* was noted only in the cultures having 1 per cent glucose. On solution B+1 per cent glucose the diameters of the embryos averaged the same for both the inoculated and the uninoculated seeds, but there was a striking difference in the color and the number with leaves. In the uninoculated, 20 per cent of the embryos were showing the leaf point, but the embryos were whitish in color. In the inoculated, 50 per cent of the embryos showed leaves, the leaf development was greater, and the embryos were green.

On Ashby's medium plus 1 per cent glucose, the beneficial effect of the organism was more apparent. There was a marked increase in the width of the embryos. In the inoculated cultures all the embryos had produced one or two leaves and the embryos were dark green. In the uninoculated cultures only 25 per cent of the embryos had produced a leaf point and the embryos were chlorotic (fig. 2). On both solution B and Ashby's+1 per cent glucose, the influence of *Bacillus radicicola* was so strikingly beneficial that it was observable immediately.

In the cultures with 0.10 per cent glucose or no glucose, the influence of *B. radicicola* seemed to be injurious, for in all the inoculated cultures the average width of the embryos was less than in the uninoculated cultures.

The cause of this favorable influence of *Bacillus radicicola* on the growth of orchid embryos remains yet to be determined. Some experiments were also made in which the cultures were inoculated with *Azotobacter* sp. In every case, however, there was a marked retardation in growth.

Transplanting experiments

On July 12, seeds of *Laelia-Cattleya* hybrid no. 2 were sown on solution B plus 2 per cent fructose plus 1.5 per cent agar. As culture vessels, Erlenmeyer flasks of 150 cc. capacity were used, and 30 cc. of the medium was employed. On October 14 the embryos were just on the verge of producing the leaf point. They were then transferred to six Erlenmeyer flasks (D 18 to D 23) containing 50 cc. of nutrient media, as follows: Pfeffer's solution+2 per cent glucose+0.1 cc. carrot decoction.

On March 1, 1920, the seedlings in cultures D 18 to D 23 had two and three leaves with a pronounced protocorm, and from the protocorm one and two roots had grown out, the roots varying from 1 to 3 cm. in length; whereas in the corresponding tube cultures, four seedlings in a hundred had produced roots, and these roots were only 2 mm. and 3 mm. in length. On March 11, 1921, seedlings were transferred from cultures D 22 and D 23 to liter Erlenmeyer flasks containing 300 cc. of solution B plus or minus sugar. Eight cultures were made, four with 2.0 per cent sucrose

and four without sugar. On August 20, 1921, notes were made on these cultures. In the sucrose cultures the seedlings had made a marked development. The largest seedlings had four and five leaves, some of the leaves being 2 cm. in length; while the roots of these seedlings, two or three in number, were 2–5 cm. in length (fig. 3). In the cultures lacking sugar the growth was less striking, the leaves being 2–4 mm. and the roots 2–10 mm. in length.

FIG. 3.—Seedlings one year old on solution B+2 per cent sucrose; $\times\frac{2}{3}$.

Seedlings were transplanted at the same time from D 23 to a compost of peat and sphagnum in ordinary flower pots. These seedlings, on August 20, 1921, showed leaves 5–7 mm. and roots 1–2 cm. in length. These seedlings were better than those planted on solution B in the liter flasks, but not so good as the seedlings on solution B plus 2 per cent sucrose in the liter flasks.

That better growth is possible in 150 cc. to 500 cc. Erlenmeyer flasks than in culture tubes was demonstrated repeatedly during these experiments. Erlenmeyer flasks of from 150 to 500 cc. capacity, containing the culture media left over after supplying the tubes with the requisite amount, were generally planted with the seeds that remained after the tubes were sown. In practically every instance germination took place sooner in the flasks than in the tubes. The probable explanation is that in the tube cultures the inward diffusion of carbon dioxide is impeded to a certain extent by the cotton plug, and, the volume of air in the tube being small, the carbon

dioxide is soon exhausted. In the larger flasks, however, the volume of air, and therefore the volume of CO_2, is much greater, from six to twenty-five times as great, and furthermore, the area through which the CO_2 can diffuse is greater by virtue of the larger mouths of the flasks. That the diffusion of CO_2 is impeded by a cotton stopper was shown in a previous paper (KNUDSON 7).

From the practical standpoint this would seem to be a method for the propagation of orchid seeds. The seeds may be germinated in the small culture tubes or in larger containers, and when roots are produced they may be transplanted either to pots in the open or transferred to sterile culture in larger flasks. My efforts to develop the seedlings on peat sphagnum mixture in flower pots in the open resulted in failure on several different occasions, due in one case to the temperature running up to 40° C., which permitted a pathogenic fungus to destroy utterly the seedlings in about twenty-five pots. In another case, during an absence from the city the seedlings were destroyed by insects. Previous to the misfortunes which the seedlings experienced they had been growing for periods of three and four months and were making satisfactory development. Other experiments are now in progress on this phase of the question. Some tubes were sent to Mr. T. L. MEAD, of Oviedo, Florida. Some of these were transplanted four and five months ago, and according to a recent communication from Mr. MEAD, the seedlings transferred are continuing growth, and but few seedlings were lost as a result of transplanting. The results of certain experiments now in progress indicate that more rapid growth will be obtained if the culture seedlings are transferred to sterile media containing sugar and grown for a year or two under these conditions. This method, moreover, has the advantage that the seedlings are not exposed to the depredations of insects or the ravages of parasitic fungi. Furthermore, contamination of the cultures by *Penicillium* or *Aspergillus* is without any injurious effect, provided that at the time of transplanting the seedlings have roots.

Discussion

What is the significance of these results in relation to the views advanced by BERNARD and BURGEFF, and quite generally believed

today, that for the germination of orchid seeds infection of the embryo by the appropriate fungus is essential? BERNARD believed that the action of the fungus was a physicochemical one, in that the fungus would cause an increase in a concentration of the cell sap, which increase in concentration would induce germination and the formation of a protocorm in somewhat the same way that the form of algae could change by increasing the concentration of the external solution. He points out that the fungus can invert sucrose and this may occur in the embryo.

The writer believes that the fungus may bring about germination in another way. As previously pointed out, in all his media BERNARD used a substance known as salep. This is a powder derived by grinding the dried tubers of certain species of orchids, and is rich in pentosans and starch, containing also about 5 per cent of organic nitrogenous substances. It probably contains some soluble organic and inorganic matter, judging from freezing-point determinations made by BERNARD. In view of the fact that organic matter is present, it is conceivable that the influence of the fungus might be to digest some of the starch, pentosans, and nitrogenous substances; which digestion products, together with secretions from the fungus or products produced on decomposition of the fungus, might be the cause of germination. In brief, it is conceivable that germination is induced not by any action of the fungus within the embryo, but by products produced externally on digestion or secreted by the fungus. Unfortunately I have not as yet succeeded in satisfactorily isolating the organism stated as necessary by BERNARD, nor has it been possible to purchase salep. Work is still in progress on this problem. There are, however, certain facts which support the idea that the action of the fungus is not necessarily internal. BERNARD does not give any analyses of the medium used, but he does give certain cryoscopic data. The medium generally used, made with salep, had a freezing-point depression of 0.01° C. Assuming that this depression ($\triangle$) is produced largely by hexose sugars, it would indicate at the outset of the experiment a concentration of hexose sugars equivalent to 0.1 per cent glucose. It is not possible, of course, to say that this depression is due entirely to hexose sugars; perhaps other sugars are present,

as well as other soluble organic and inorganic substances. The significant fact is that at the outset some soluble organic substances are present.

In addition to the soluble substances present, which apparently are not sufficient in quantity nor suitable as regards quality to permit of germination, there are to be considered the insoluble organic substances, pentosans, starch, and organic nitrogenous substances. Digestion of starch by the fungus would augment the concentration of sugar, and digestion of the organic nitrogenous substances might produce certain products which would make possible the germination of the seeds.

In my experiments it is true that the sugar used generally was of a relatively high concentration, but in the case of *Epidendron*, germination was obtained on 0.2 per cent glucose, which sugar is not particularly favorable for growth. That other substances besides sugar exert a pronounced influence is shown by the experiments on the beneficial effects of adding certain plant extracts to the glucose-containing solutions. The fact that other substances besides sugars may be important in the germination is shown by the experiments in which germination was obtained on decoctions of yeast, wheat grains, or of potato. All of these extracts contained less than 0.02 per cent total sugar. The experiments on the influence of *Bacillus radicicola* also lend weight to the idea that certain extraneous products may markedly influence germination.

BURGEFF, in certain of his experiments, used 2 per cent salep, but in other experiments he used starch, sucrose, or glucose. The explanations offered with respect to the function of the fungus in discussing BERNARD'S work may be used to account for the results obtained by BURGEFF. There may appear to be rather more difficulty in explaining the function of the fungus in the cultures containing either glucose or sucrose. It will be necessary to discuss these in more detail.

In one experiment, seeds of *Cattleya* were sown in a tube containing a nutrient solution plus 0.33 per cent sucrose. After three months the embryos were 0.4–0.5 mm. in width. Then, according to BURGEFF, they remained stationary. Cultures four months old, inoculated and maintained in the dark at 23° C., produced the first

leaf at the age of eight months. BURGEFF does not state that the uninoculated cultures were maintained under the same condition, but presumably they were. Granting that the uninoculated culture did not produce leaves, it is possible to explain the germination on the basis of the inversion of sucrose, which would yield approximately concentrations of both glucose and fructose molecularly equivalent to the original sucrose concentration. In addition there is to be considered the possible influence of products secreted by the fungus or produced on decomposition of the fungus.

The favorable influence of saprophytic fungi and bacteria demonstrated by my experiments is paralleled by certain cultures of BURGEFF. He transplanted four months' old seedlings to a mineral nutrient medium containing salep. Some of the cultures became contaminated with saprophytic organisms. The uncontaminated culture showed little growth, if any, after three months; while the culture contaminated by *Penicillium* made a marked growth, the leaves being 4 cm. in length. Another saprophytic fungus in another culture likewise caused a marked increase in growth, the leaves being about 9 mm. in length; and in a third culture contaminated by bacteria the seedlings were of similar character to those of the pure culture, but apparently darker green in color. All of these seedlings had produced vigorous roots. In the pure culture some of the seedlings had died. BURGEFF considers that the more favorable growth in the tube with fungus contamination was due to the development of an acid reaction in the medium. There is a possibility that increased carbon dioxide content and other products produced by the organism are partly responsible. It should be stated that the seedlings originally transferred to these tubes had previously been infected by the essential fungus.

Another experiment of BURGEFF lends weight, however, to the idea that the fungus is effective in inducing germination as a result of certain reactions brought about within the embryo. In this experiment the culture medium consisted of a weak nutrient solution plus $\frac{1}{20}$ per cent starch. Seeds of a *Laelia-Cattleya* cross were planted, and the root fungus from each of seventeen different orchids was tested for its ability to induce germination. In the cultures uninoculated the embryos attained a width of 0.45 mm. in four months. In certain cultures only the suspensor became

infected. The diameter of the embryos in this case reached from 0.6 to 0.8 mm. in the same time. With infection still more advanced, but less than normal, a few seeds had leaves after seven months. Normally infected embryos produced leaves, and embryos that adhered to the wall of the tube likewise germinated. An infection more advanced than normal caused the same development as an infection slightly less than normal. When from one-half to two-thirds of the embryos became invaded, growth was less than in the embryos not infected, and in another case the seeds were killed outright.

As stated, certain facts from these experiments make it difficult to explain the action of the fungus as purely external. If so, why should the fungi behave so differently in inducing or retarding germination? Unfortunately BURGEFF gives no details so that one may judge whether or not these results could be duplicated on second trial. Another difficulty is an adequate explanation for the germination of seeds adhering to the inner surface of the culture tube. It is possible, of course, that decomposition products of the fungus growing on the surface of the tube may have been the cause. It is desirable to await experiments with the fungus before attempting to discuss these points further.

There are other phases of the problem presented by BERNARD, especially the loss by the fungus of its capacity to induce germination after prolonged culture in the laboratory. It is entirely possible that there has been no loss in the fungus, but that at the time of inoculating the culture the physiological state of the embryos was such as to resist or permit of infection. Those in which the infection was confined to the lower cell could still germinate despite the fungus. Those invaded to a greater extent would be killed. These and other experiments of BERNARD and BURGEFF suggest that one of the causes for the failure of germination is the parasitic character of the fungus. In other words, it is possible that the fungus, instead of being an aid in normal germination, is a factor in the death of the embryos and consequently in the failure of germination.

In conclusion, it may be stated that the evidence for the necessity of the fungus for germination has not yet been conclusively proved. The evidence is conclusive that under conditions of pure

culture employed by both BERNARD and BURGEFF germination of the seeds is dependent on the fungus. There is still considerable work to be done, however, before the validity of the fungus hypothesis can be proved or disproved.

Summary

1. A method is given for sterilizing seeds of certain orchids and for growing them under sterile conditions.

2. Germination of seeds of *Laelia*, *Cattleya*, and related forms is possible without the aid of any fungus when certain sugars are supplied.

3. Fructose appears more favorable than glucose.

4. In the presence of glucose, chlorosis of the embryo generally results.

5. Germination is possible on certain plant extracts containing merely traces of sugar.

6. Embryos in sugar-containing cultures accumulate a considerable reserve of starch.

7. The concentration of glucose is important in the growth of the embryo.

8. *Bacillus radicicola* from alfalfa and certain other microorganisms on certain media have a favorable influence on the development of chlorophyll and germination.

9. Seedlings have been transplanted from tubes to large flasks and growth has continued.

10. The results thus far obtained indicate that the method is of value in the propagation of orchids from seeds.

11. The idea is advanced that the necessity of fungus infection for germination has not yet been proved.

12. One cause of failure of germination may be the pathogenic character of some of the endophytic fungi.

Mr. T. L. MEAD, of Oviedo, Florida, who has worked for many years on the practical problems of germinating orchid seeds, has supplied all the seeds used in these experiments. He has likewise supplied certain information on the practical difficulties and on

various results obtained by him. For all of these favors and for constant interest I wish to express my thanks.

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YELLOW-WHITE PINE FORMATION AT LITTLE MANISTEE, MICHIGAN

LEROY H. HARVEY

(WITH SIX FIGURES)

In a previous paper the writer[1] presented the inadequacy of current phytogeographical classification of the northern half of the Southern Peninsula of Michigan, and at that time advanced a scheme consonant with observational and experimental data. This region was shown to lie within that phytogeographical area whose climax (eschatophytic) forest type is of the maple-beech or mixed hardwood formation. It was shown that within this great transitional area there were at least two edaphic climax formation-complexes, the yellow-white pine and the black-white oak.

This paper is limited to a consideration of certain aspects of the "Big Pines" formation as it is exhibited at Little Manistee in Lake County, Michigan. The formation is now limited to two isolated stands one and one-half miles apart in the valley of the Little Manistee River which traverses each stand, the formation occupying its terraces and at a few points extending up on to the contiguous glacial highlands. These two stands are but the remnants of a once magnificent forest which occupied this general region. They are known locally as the Upper Pines and Lower Pines, and contain respectively about 160 and 60 acres. A general idea of these stands may be obtained from figs. 1–4.

During the preparation of this paper it was learned that the Upper Pines had suffered the inevitable, and that the saw and ax were rapidly resolving it into a historical fact. In light of this it has seemed wise to present rather fully all data available as a matter of record. Through the personal interest and activity of Judge HARRY D. JEWELL, of Grand Rapids, these magnificent stands of pines in 1914 were temporarily saved from complete deforestation, the logging operations actually being in progress at

[1] HARVEY, LEROY H., Some phytogeographical observations in Lake County, Michigan. Mich. Acad. Sci. Rept. 213–217. 1919.

the time of his intervention. Steps were taken toward the formation of a company to conserve this area, and the owner, E. GORDEN FILER, agreed to contribute a substantial part of the value of the standing timber. The State Legislature of 1915 sought to encourage the preservation of such tracts of timber, and passed a bill with the amendment to this effect, as proposed by Judge

FIG. 1.—Upper Pines from west at edge of glacial upland: shrubby-herbaceous association of reforestation occupies foreground; this will terminate in black-white oak complex.

JEWELL. Unfortunately the Governor, through the mistaken advice of one of the State Tax Commissioners who did not understand the merits of the measure, misconstrued its purpose, and vetoed the bill. It was hoped that this tract would eventually be made a part of the Public Domain. Action was delayed, however, and Michigan's archaic and prodigal method of forest taxation left no alternative. The Upper Pines are gone; a crime to be laid at the door of our state officials. Nevertheless, the efforts of these public spirited men cannot be commended too highly, and should

serve as a stimulus for other efforts all over the state looking toward the preservation in Public Domain of original stands of forest types and plant associations. This is not wholly a matter of nature sentiment, but one of scientific necessity.

FIG. 2.—Upper Pines, view within formation which here occupies a high terrace: dominant form of herbaceous layer is *Pteris aquilina;* atmometer station 7.

To Judge JEWELL I wish to express my deep appreciation of his hospitality during the prosecution of my field work. I also wish to express my thanks to my colleague, Dr. WILLIAM MCCRACKEN, who assisted me throughout in taking data.

Climatic factors

The climatic conditions of the region in all probability are fairly revealed in the data taken at Luther (table I), less than ten miles to the southeast, at a United States Volunteer Station. It will be noted that the maximum range in temperature for the four years is 136°, from 100° in August 1918 to −36° in February 1918. The average yearly rainfall for the four years is 33.65 inches, ranging from 28.29 inches in 1917 to 39.08 in 1920. The last killing frost in spring was on June 23, 1920, and the first killing frost in autumn was on September 11, 1917. The shortest growing period was 87 days in 1917, and the longest 106 days in 1919–1920, with an average of 98 days. While means and averages convey certain pertinent facts, it is the extremes which indicate the critical points of climatic influence on vegetation.

TABLE I

CLIMATIC FACTORS 1917–1920*

Factors	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Mean maximum temperature	24.2	26.4	42.3	56.1	66.8	77.5	80.7	78.6	70.1	54.6	43.7	30.8
Mean minimum temperature	8.45	7.7	21.0	30.7	40.7	52.0	55.1	53.5	46.2	39.6	28.9	16.8
Maximum temperature.....	45	45	68	72	92	92	98	100	89	80	65	65
Minimum temperature.....	−27	−36	−18	11	22	27	33	34	27	18	6	−19
Mean precipitation	1.37	1.07	2.49	2.93	3.35	3.38	3.68	3.49	3.06	4.00	2.44	2.38
Minimum precipitation.....	1.07	0.65	1.50	2.45	2.03	2.00	1.36	2.23	2.32	3.09	1.17	1.28

* The temperature records are in Fahrenheit and the precipitation in inches.

These climatic conditions are adequate to support the maple-beech or mixed hardwood formation which are found in typical development within the region. It is apparent, therefore, that the yellow-white pine complex is the expression of a combination of edaphic factors which exclude the climax type. The main critical factor, aside from the question of post-glacial invasion and pre-occupation, is undoubtedly the wilting coefficient of the soil.

Ecological factors

The ecological factors investigated included soil moisture, evaporation, and light intensity. Records were made over a period of twenty-eight days, August 3–31, 1918. As August–September practically represents the minimum of favorable ecological conditions for the growing period, the factorial values of either month

FIG. 3.—Lower Pines, northern end of stand: white oak in left foreground; reforestation from white oak grubs and invasion of black oak.

FIG. 4.—Lower Pines: fires excluded for a number of years, and there is a well developed shrubby layer in which species of *Cornus*, *Viburnum*, *Amelanchier*, *Crataegus*, and *Prunus*, as well as saplings of red and white oak are common; seedlings and saplings of white and yellow common in open places.

will adequately serve to indicate the critical ecological conditions. It was expected that some light would be thrown by such data upon the questions of the relation of evaporation to succession and upon the classical conception of the successional development of the formations of a region. In my paper already referred to, the data seemed to show that evaporation had no causal relation to succession. It was argued that the measurements of the ecological factors of any association are more largely a result of that stage of the succession rather than the cause of it. Specifically, the evaporation is less in the Big Pines formation than in the open herbaceous association, because of the greater protection in the former from factors enhancing water loss; the Big Pines formation is the cause of lowered evaporation and not the result of it. The evaporation conditions of any pioneer association obviously represent the conditions which must be tolerated by the invaders from any higher ecological type of association. These invaders help to reduce the evaporation, and finally, as the next step in the succession is established, it comes to possess its own evaporation ratio determined by its own canopy, shrubby, and herbaceous layers.

SOIL WATER.—Soil samples were taken in two series at depths of 7.5 cm. and 25 cm. by means of a knife-blade trowel, and placed at once in friction top cans of 350 cc. capacity. The samples were collected on August 4, 11, 18, and 25. Analyses of the samples were completed within the following month. The moisture-holding capacity (MHC) was computed upon the basis of dry weight as well as upon the basis of equal volumes, according to HILGARD'S[2] method. Each determination represents the average of the four samples for each station and series. The total field capacity (TFC) was computed directly upon the basis of dry weight. The wilting coefficient (WC) was calculated by the formula of BRIGGS and SHANTZ.[3] $WC = \frac{\text{moisture holding capacity} - 21}{2.90(\pm 0.021)}$, in which the MHC is determined by volume per cent. It is recognized that the WC data thus computed are only an approximation, as such data preferably should be based either upon experimental

[2] HILGARD, E. W., Soils. New York. 1906.

[3] BRIGGS, L. J., and SHANTZ, H. L., The wilting coefficient for different plants and its indirect determination. U.S. Dept. Agric., Bur. Plant Ind. Bull. 230; BOT. GAZ. **51**:210–219. 1911; **53**:20–37, 229–235. 1912.

determination or computed from the moisture equivalent mechanically determined. These data will be found in table II. For purpose of comparison similar data from the jack pine, black-oak, and the Kalamazoo maple-beech associations are added.

A study of these data brings out certain facts which are quite in agreement with field observations. The range in TFC in the Big Pines at 7.5 cm. from 7.1 to 14.9 per cent, with a corresponding range in WHC (column 3) from 48.5 to 52.5 per cent, is a direct expression of the effect of fires on the humus layer. At 25 cm. this effect is less apparent in the WHC, but the absence of protection from a good humus layer is shown in the TFC at the same depth.

TABLE II

SOIL WATER DETERMINATIONS IN PERCENTAGES

Station and association	TFC by DW		WHC by DW		WHC by volume		WC by formula	
	7.5 cm.	25 cm.	7.5 cm.	25 cm.	7.5 cm.	25 cm.	7.5 cm.	25 cm.
6. Upper Pines.....	14.9	6.6	44.4	28.7	53.5	41.8	11.2	7.1
7. Upper Pines.....	9.9	5.3	43.4	38.2	52.3	43.4	10.8	7.5
9. Upper Pines.....	7.1	4.3	36.3	30.5	48.5	43.1	9.5	7.5
5. Jack pine.....	14.7	6.1	50.9	34.9	60.4	42.7	13.6	7.5
3. Black-white oak.....	6.4	4.7	33.1	30.1	45.2	42.7	8.4	7.5
Maple-beech (Kalamazoo)	.....	.....	62.1	33.4	62.7	45.7	14.5	8.5

The soil is often so baked that it is grayish and "dead" to a depth of 4 or 5 cm. These oft repeated fires must have had marked influence upon growth increment of the facies, as well as largely determining the presence or absence and nature of the shrubby, herbaceous, and ground layers. The WC (column 4) exhibits in another way the same regressive influence due to humus destruction, producing a soil condition which must act selectively upon the various invading propagules. The uniform WHC and WC at 25 cm. in the Big Pines, as well as in the jack pines and oak associations, are very significant and form the evidence for the conclusion advanced in the former paper that the soil conditions reveal no differences of causal magnitude, and are thus clearly inadequate to explain present forest distribution, or to give any indication of a causal successional factor.

Fig. 5 is an attempt to exhibit to scale the soil-water relations in the Big Pines formation as they existed in August 1918. That the data may be comparable they are all computed on the basis of dry weight. The critical factor is the growth-water, whose theoretical possible maximum would be the difference between the WC and the WHC, or 29 per cent. This of course would scarcely ever be realized under field conditions. Actually the growth-water is the difference between the TFC and the WC, which in August 1918 was only 1.5 per cent, a quantity dangerously near the zero point of available water.

Evaporation.—The evaporation data were obtained in the usual way, Livingston standardized 8 cm. atmometers being used. During the month 284 mm. of rain fell on seven days, 250 mm. of

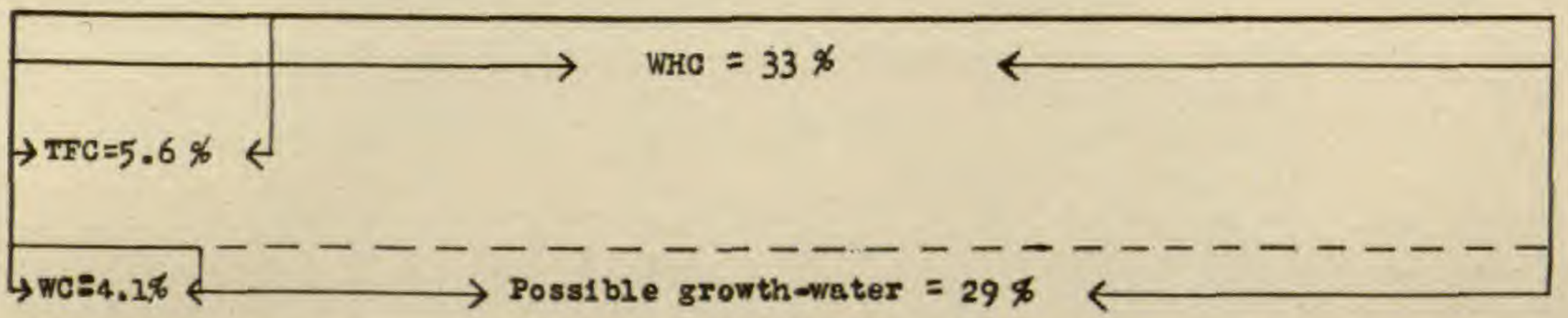

Fig. 5.—Soil-water relations (shown to scale): data on basis of dry weight.

this falling in twenty minutes. Rain correcting atmometers were not available, but it is thought that the use of these would not have given essentially different results. For five of the stations the records were taken daily; for the others every seven days. The 250 cc. reservoir bottles were all set slightly in the ground and stabilized with 12 inch length of $\frac{3}{16}$ inch mile steel to which they were bound by two bands of electric tape. Readings were made on an index mark on the neck of the bottle, and loss of water was supplied from a 50 cc. burette. Distilled water was used throughout. Stations were selected to represent various conditions of cover and canopy. The results are tabulated in table III.

The records in table III give evaporation rates only for the low herbaceous layer, and the average daily range from 8.3 to 18.6 cc. (124 per cent) is solely an expression of the degree of exposure, varying directly with the amount of insolation afforded by the herbaceous and shrubby layer. In connection with the

record of station 1*B* (18.6 cc.) and of station 1 (19.5 cc.), a difference of only 0.9 cc. (4.3 per cent) would seem to indicate that the canopy exercises only a slight effect in reducing evaporation from the herbaceous layer. "On the basis of these results it would appear entirely unwarranted to assign evaporation as a causal factor in succession."

LIGHT.—The light determinations were made with a Waynne's Infallible Meter, as this was the only device available. All readings seek to represent the maximum shade conditions due to the canopy; all part shadows due to the trunks were avoided. The

TABLE III

AVERAGE DAILY EVAPORATION IN CC., AUGUST 4–31, 1918

Station	Association	Number of atmometers	Evaporation (cc.)	Average for Big Pines (cc.)
1*B*.....	Lower Pines, B.	1	18.6	.....
1*C*.....	Lower Pines, C.	1	8.3	.....
2.....	Lower Pines Terrace	2	10.4	.....
6.....	Upper Pines, W. C.	2	12.5	.....
7.....	Upper Pines, S.	2	8.7	.....
8.....	Upper Pines, E. T.	2	14.7	.....
9.....	Upper Pines, E. C.	2	16.8	12.79
5.....	Jack pine	2	12.7	.....
3.....	Black-white oak	2	19.0	.....
1.....	Open-standard	1	19.5	.....
11.....	Garden, Kalamazoo	1	31.6	.....

meter was placed upon the ground and the stopwatch held behind the back; both were released simultaneously. When the solio paper merged imperceptibly into the darker standard, the watch was stopped and the reading recorded. The results represent the averages of eight or ten readings (table IV). All light data are referred to full sunlight, which for convenience of comparison are reduced to the standard one, and all habitat records are correspondingly reduced to the same standard and expressed in the form of a ratio. Thus in station 1 the canopy reduces the sunlight to one-fifteenth its full intensity.

The range in the light ratios is largely a question of density of stand and so density of canopy. It is interesting to note that the yellow pine canopy with the same density of individuals produces a light screen of only about one-half the value of that of the white

pine canopy. It would be of interest to know whether these shade differences are correlated with floristic or vegetational differences. This problem unfortunately escaped attention in the field.

Growth forms

Since the appearance of RAUNKIAER'S work in 1916, ecologists have viewed the influence of temperature factors from a radically new viewpoint. He points out that climatic conditions are reflected essentially in the biological nature of the vegetation, that is, in the nature and extent of the protection possessed by the perennating growth points during the winter or critical season. Upon the basis of this generalization he classifies vegetation into a series of

TABLE IV

LIGHT DETERMINATIONS, BIG PINES FORMATION

Station	Association	Ratio
1*B*	Lower Pines	1:15
2*A*	Lower Pines	1:12
7	Upper Pines	1:63
7	White pine canopy	1:57
7	Yellow pine canopy	1:30

life (or growth) forms. The following characterization of life forms and the abbreviations used are added for convenience of reference.[4]

Phanerophytes.—Woody plants of all types, both evergreen and deciduous, and exhibiting the least amount of protection from the cold, as showing the greatest amount of exposure. The group may be divided into *Megaphanerophytes* (MG), trees over 30 m.; *Mesophanerophytes* (MS), trees 8–30 m.; *Microphanerophytes* (MC), shrubs or trees 2–8 m.; *Nanophanerophytes* (N), shrubs under 2 m.

Chamaephytes (CH).—Perennial by virtue of the fact that the buds are just above the ground, or on the surface, and are thus often protected by the snow blanket.

Hemicryptophytes (H).—With dormant buds in the upper crust of the soil, the top of the plant dying down in winter.

[4] TAYLOR, N., The growth forms of the flora of New York and vicinity. Amer. Jour. Bot. 2:23–31. 1915.

Geophytes (G).—Perennial by bulbs, rhizomes, tubers, or by root buds.

Helophytes and *Hydrophytes* (HH).—The former has buds at the bottom of the water. Hydrophytes have perennating rhizomes or winter buds and are truly aquatic.

Therophytes (T).—Annuals.

On the basis of 400 species carefully selected from 1000 representative species, RAUNKIAER prepared what he calls a "normal spectrum," which shows the percentage distribution of these 400 species into the several life forms. This is now accepted as a basis of comparison.

The areas contiguous to the Big Pines have long been cleared, and are now covered with either grassy or shrubby plains or second growth oak associations. Considerable invasion has naturally occurred from these surrounding regions. Also repeated ground fires have run their course through both stands, although a part of the Lower Pines has been spared for several years. The north exposure of the immediate river banks furnishes many interesting isolated colonies of glacial relicts, but these, as well as the evident invasions, have been eliminated in the summary of species. An attempt has been made to include only those species which are evidently the natural components of the formation. Obviously this introduces the unchecked personal factor, and doubtless some errors of judgment have been made, but it is hoped not enough to essentially modify the results. As the study was made in August, doubtless some spring forms may also have been missed. On the basis of four separate lists, however, 100 species have been included (table V). In table VI the ecological spectrum of these 100 species is recorded. RAUNKIAER'S normal spectrum and TAYLOR'S (see footnote 4) spectrum of the northern elements of the flora within 100 miles of New York City are added for comparison. It should be noted further that only twenty-seven species have not been recorded outside of the Big Pines formation (table V). It is highly probable that careful search would further reduce this number.

A survey of the ecological spectrum of the Big Pines indicates that the mesophanerophytes determine the facies of the formation.

TABLE V

Species of the Big Pines formation

Species	MS	MC	N	CH	H	G	T	Peculiar to form
Pteris aquilina*						×		
Pinus resinosa	×							
Pinus Strobus	×							
Agropyron tenerum						×		
Bromus ciliatus						×		
Bromus Kalmii						×		
Calamagrostis canadensis						×		×
Danthonia spicata						×		
Deschampsia flexuosa						×		
Koeleria cristata						×		
Melica striata						×		×
Muhlenbergia racemosa						×		
Oryzopsis pungens						×		
Panicum latifolium						×		
Poa triflora						×		×
Maianthemum canadense						×		
Polygonatum biflorum						×		
Smilacina racemosa						×		
Smilax hispida			×					
Cypripedium acaule						×		
Cypripedium hirsutum						×		×
Epipactis pubescens						×		×
Salix humilis			×					
Myrica asplenifolia			×					
Quercus alba	×							
Quercus rubra	×							
Actaea alba					×			×
Aquilegia canadensis					×			
Hepatica triloba				×				×
Arabis brachycarpa							×	
Ribes Cynosbati			×					×
Hamamelis virginiana		×						
Amelanchier canadensis		×						
Amelanchier oblongifolia		×						
Amelanchier spicata		×						×
Crataegus no. 1		×						
Crataegus no. 2		×						
Crataegus no. 3		×						
Fragaria virginiana				×				
Potentilla canadensis					×			
Prunus americana		×						
Prunus serotina	×							
Prunus virginiana		×						
Rosa humilis			×					
Rubus allegheniensis			×					
Rubus Idaeus			×					
Rubus triflorus			×					
Lespedeza procumbens							×	×
Xanthoxylum americanum			×					×
Polygala paucifolia						×		×
Acer rubrum	×							

*The nomenclature and sequence of the seventh edition of Gray's *Manual* have been followed. Specimens are preserved in the author's private herbarium.

TABLE V—*Continued*

Species	MS	MC	N	CH	H	G	T	Peculiar to form
Acer saccharum	×							×
Ceanothus americanus			×					
Viola arenaria						×		
Viola pubescens						×		×
Viola scabriuscula						×		
Aralia nudicaulis						×		×
Sanicula gregaria					×			×
Cornus circinata		×						×
Cornus paniculata		×						
Arctostaphylos Uva-ursi				×				
Chimaphila umbellata				×				
Epigaea repens				×				
Gaultheria procumbens					×			
Gaylussacia baccata			×					
Pyrola americana						×		
Pyrola secunda						×		
Vaccinium pennsylvanicum			×					
Trientalis americana					×			
Asclepias purpurascens						×		×
Monarda mollis					×			
Prunella vulgaris					×			
Satureja vulgaris				×				
Melampyrum lineare					×			
Pedicularis canadensis				×				
Galium pilosum					×			
Mitchella repens				×				×
Diervilla Lonicera			×					
Lonicera canadensis			×					×
Lonicera glaucescens			×					×
Sambucus racemosa			×					×
Triosteum aurantiacum					×			×
Viburnum acerifolium			×					×
Viburnum pubescens			×					×
Campanula rotundifolia				×				
Antennaria neodioica				×				
Antennaria Parlinii				×				
Antennaria plantaginifolia				×				
Aster laevis					×			
Aster Lindleyanus					×			
Aster macrophyllus					×			×
Helianthus mollis					×			
Hieracium canadense					×			
Hieracium longipilum					×			
Hieracium venosum					×			
Prenanthes trifoliolata					×			
Solidago caesia					×			
Solidago hispida					×			
Solidago rugosa					×			×
Solidago serotina					×			
Total: 100 species	7	11	18	11	24	27	2	27

It has been noted that the influence of oft repeated fires regulates the "presence or absence and nature of the shrubby and herbaceous layers." The large percentage of hemicryptophytes and geophytes in a forest formation are thought to be in large part an expression of this influence.

Valences

The biological spectrum furnishes one of the essentials in the ecological characterization of a formation. The method of valences also offers an objective means of presenting an equally important picture, that of the numerical abundance of the various floristic elements of the formation. It is the frequency percentage of the various elements which determines the aspect of the formation or its physiognomic character.

TABLE VI

PERCENTAGES OF GROWTH FORMS IN BIOLOGICAL SPECTRA

Type of growth form	MG	MS	MC	N	CH	H	G	HH	T
Big Pines.....	0	7	11	18	11	24	27	0	2
Normal spectrum.	7		17	20	9	27	3	1	13
New York City...	0	1.31	3.94	8.55	8.55	26.31	24.34	23	3.94

Various methods have been employed since the introduction of the quadrat method in such statistical investigation. The method used in this study in an adaptation of that used by RAUNKIAER.[5] As only the facies was considered, it was possible to devise a rapid method of record. A rope 5 m. long held in the hand of an assistant formed the radius of a circle whose species were recorded as the investigator moved the radius through 360°. The center of the next area was obtained by swinging the radius through 180°, and with this as a fixed point the assistant then took his stand 5 m. farther on in the same axis. Thus areas are tangent to each other. In this way a transect of 200 m. was run. The valence records are thus based upon twenty such areas (table VII).

[5] For accounts of RAUNKIAER'S work see: SMITH, W. G., RAUNKIAER'S life forms, and statistical methods. Jour. Ecol. 1:16–26. 1913; and FULLER, G. D., and BAKKE, A. L., RAUNKIAER'S "life forms," "leaf classes," and statistical methods. Plant World 21:25–37. 1918.

The valence table shows that the yellow pine is slightly the dominant species in the Upper Pines. In the Lower Pines the reverse is true. It will be noted that this is due to the grouping of individuals of a species in certain parts of the stand, as is shown by transects IV and V. Similar grouping of the white pine is shown in transects I and II, while transect III shows an almost equal abundance of the two dominant species. These facts of abundance are not correlated with any soil differences as far as could be determined. It probably represents the influence of

TABLE VII

VALENCE OF FACIES IN FREQUENCY PERCENTAGE, UPPER PINES

SPECIES	TRANSECT NO.					TOTAL	RATIO
	I	II	III	IV	V		
Yellow pine	25	27	50	72	54	228	100
White pine	45	49	53	26	37	210	92
White oak	6	15	4	5	4	34	15
Red oak	0	1	0	0	0	1	0.4
Red maple	0	0	1	0	1	2	0.8
Total	76	92	108	103	96	465	.....

persistent seed trees of the original stand which predominantly determined the nature of the succession within their respective areas.

The red and white oaks are rather constant members of the formation, the white oak always predominating. While they do not influence the physiognomy of the facies, yet in case of cutting or burning, the white oak is the persistent species and largely determines the nature of the reforestation, giving rise to the oak or mixed oak formation in which the red oak fails to reappear, being replaced by the black oak and in some cases by the additional invasion of the jack pine, neither of which, so far as observed, is ever found in the Big Pines formation. The red maple represents sporadic invasion from the not far distant margin or floodplain of the Little Manistee River. It was not recorded from the upland portions of the formation.

Age distribution of facies

The ecological spectrum (table VI) and valences (table VII) convey a very definite concept of the Big Pines formation. Nevertheless the picture is incomplete. It is to supply this deficiency in the visualization of the formation that the following method of analysis has been devised. It is essential that the age of the facies and the relative abundance of the individuals of each age be known, for therein is recorded not only the present status, but much of the

TABLE VIII

AGE DISTRIBUTION OF FACIES, UPPER PINES, STATIONS 6 AND 7

Circumference classes in inches	10–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70
Pinus resinosa	4	6	7	5	8	6	11	6	6	....	....	.....
Pinus Strobus	2	7	8	8	3	6	6	6	7	2	....	1
Quercus alba	....	....	3	3	....	1	....	2	1	....	....	1
Quercus rubra	....	....	....	....	....	....	....	2	....	....	1	.....
Acer rubrum	....	....	....	....	1	....	....	....	....	....	....	.....

Circumference classes in inches	71–75	76–80	81–85	86–90	91–95	96–100	101–105	106–110	111–115	116–120	Total	Ratio
Pinus resinosa	1	1	2	....	....	....	....	....	....	....	63	100
Pinus Strobus	....	....	....	....	1	1	....	....	....	1	59	92.6
Quercus alba	....	....	....	....	....	....	....	....	....	....	11	17.2
Quercus rubra	....	....	....	....	....	....	....	....	....	....	3	4.7
Acer rubrum	....	....	....	....	....	....	....	....	....	....	1	1.5

past history, as well as the future ecological tendencies of the formation.

The data were obtained by running transects through several portions of the formation, measuring the circumference (BH) of the trees as met. Numerous cores were taken with an increment borer in order to establish an increment factor which could be used to convert the circumferences into age equivalents. This, however, gave no ratio entirely satisfactory for all sizes, probably due to too few samplings. It is believed, however, that the circumference data reveal the relative age distribution, although actual age determinations are greatly to be preferred. Several transects gave essentially identical results. The record of one of these transects, run

in a portion of the formation where the shrubby layer was entirely lacking, is shown in table VIII.

Fig. 6 reveals the facts in an even more striking way. Before considering this graph of the age distribution, it should be stated that the five inch circumference classes are arbitrarily chosen. Each class represents approximately fifteen years. If actual age determinations were at hand, it would be desirable to use a one year class, thus more exactly recording the facts. It is thus

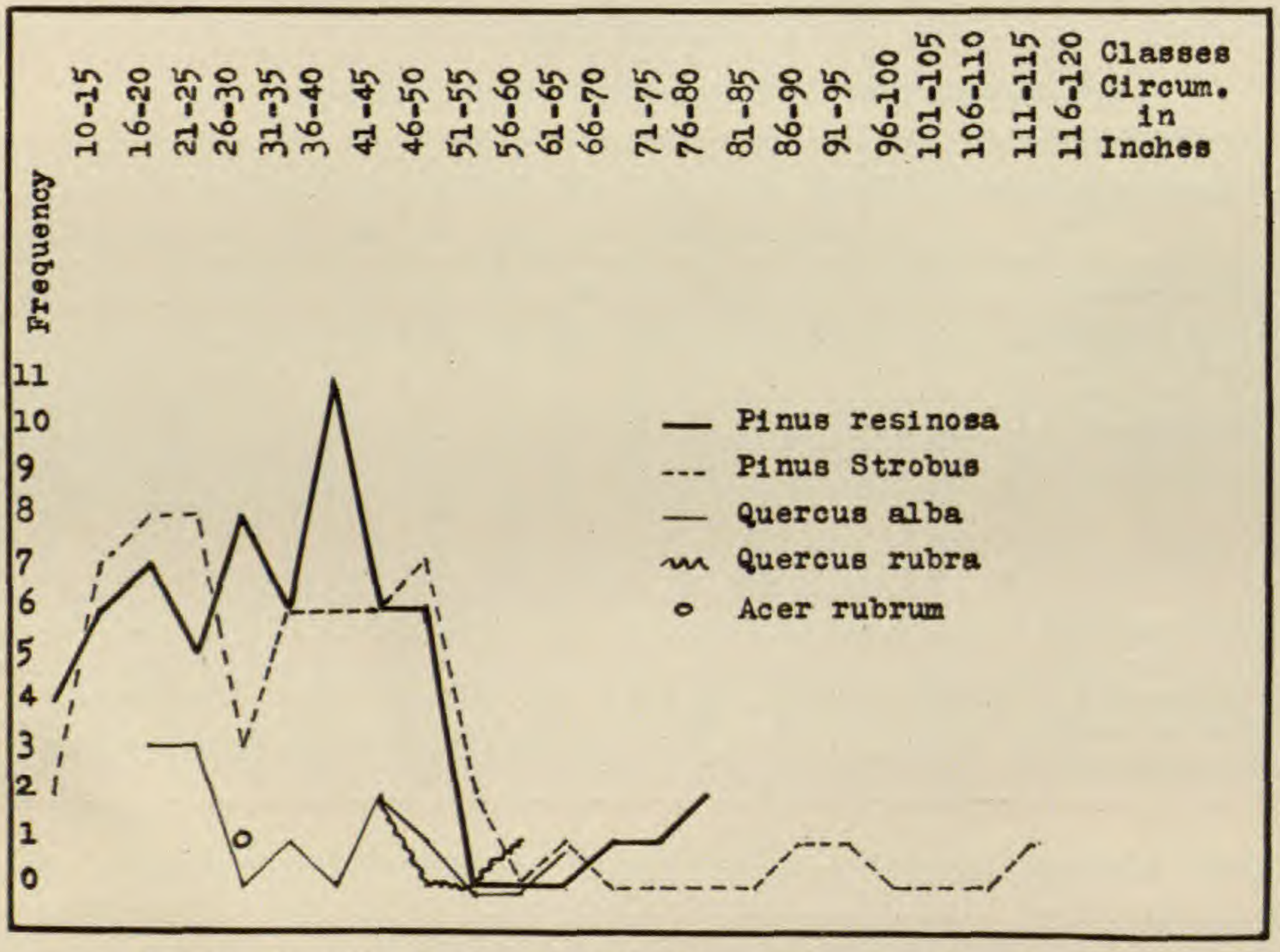

FIG. 6.—Age distribution of facies, Upper Pines, stations 6 and 7.

realized that the conclusions drawn from the graph are perhaps more valuable as a suggestion of a valid method of analysis than as a scientific representation of the actual conditions. It will be noted that the ratios of the three principal species of the facies (table VIII) are in almost perfect agreement with the ratios of frequency percentages as shown in table VII. It would appear that the transect analyzed is fairly representative of the formation.

Fig. 6 shows that the great mass of the individuals of the yellow pine lie between circumferences of 10 to 55 inches, which represents an approximate age range of 30–175 years. There are a few isolated

individuals whose circumferences (75–85 inches) indicate an age range of 240–275 years. The graph shows three fairly well marked modal points which are thought to represent periods of more abundant seeding or ecesis. With actual age data these periods could be accurately indicated as to years.

The white pine shows essentially the same range in age, 30–190 years, with two modal points less clearly established. Taking both stands into consideration, there are a few isolated ancient trees of white pine not included in the transect, whose circumferences measure 100, 115, 134, and 146 inches, and whose ages range from 350 to 450 years.

The total absence of seedling and sapling pines in this transect is probably due to the influence of shade and fires, for in certain open stations protected from fires for a period of years germination of both species appears to be common and reforestation is in process.

Consideration has already been given to the white oak, red oak, and red maple in a previous section.

On the basis of the preceding data one may venture to reconstruct somewhat the history of the formation. The original stand of the Big Pines would appear to extend back some 400 or 450 or more years, and doubtless the formation had then been self-perpetuating for centuries. The break in age between these persisting patriarchs and the present stand, whose mass falls under 200 years of age, is very striking, although it is bridged somewhat by a broken series of isolated trees. What is the explanation of this break in history? The date of the original logging of this region would not appear to account for this hiatus. It would rather seem that there had been an almost entire destruction of this formation some 200 years ago, possibly through fire of lightning origin, which then became reestablished through a few isolated individuals of various ages escaping the devastation. The well distributed range of ages down to 30 years would apparently indicate that the formation has been and is under natural conditions permanently self-perpetuating. It represents an ecological climax (eschatophytic) formation.

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FORMULAS FOR CALCULATING NUMBER OF FRUITS REQUIRED FOR ADEQUATE SAMPLE FOR ANALYSIS[1]

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When taking samples of variable fruits, as oranges for example, it is important to obtain an approximation of the number of fruits that should be included in the sample, in order that the results of the analyses shall be sufficiently accurate for the purpose of the investigation. It is the object of this paper to give formulas which may be used in such cases; to illustrate their use by numerical examples; to indicate the reliability that may be placed upon them; and to show the results that were obtained in applying them to the analysis of citrus fruits.

The first step consisted in obtaining a measure of the variability of the fruit in question. In the case of citrus, this was accomplished by analyzing individual fruits, since one fruit was found to yield enough material for the analytical work performed. If smaller fruits, such as plums, were used, it would be necessary to increase the sample to half a dozen, or a dozen, or some other number that would make a convenient sample with which to work, but the results of the analysis of each of the chosen units should be tabulated separately. From these data the probable error of a single sample was found, and this value formed the starting point for the calculations made in formulas described in later paragraphs.

Variability in composition of individual oranges in single sample

Fifty-one oranges were taken at random from a single tree. These fruits were all of good marketable quality, and were apparently free from diseases, insect injuries, and bruises. They were uniform in color, but of course variable in size. The fruits were analyzed individually and the results for each fruit tabulated

[1] Published by permission of the Secretary of Agriculture.

separately, as given in table I. At the bottom of the table will be found the values for the probable error of the mean and the probable error of a single observation. These were calculated from the following formulas: P.E. mean $= \pm 0.6745\sqrt{\frac{\Sigma d^2}{n(n-1)}}$; P.E. sing. $= \pm 0.6745\sqrt{\frac{\Sigma d^2}{(n-1)}}$; where "n" is the number of variates (in this case fifty-one), and Σd^2 is the sum of the squares of the deviations of each measurement from the mean. For example, in the column under brix, table I, "d" is the deviation of 12.80 from 14.00, etc.

TABLE I

COMPOSITION OF FIFTY-ONE ORANGES, WASHINGTON NAVEL VARIETY

Orange no.	Degrees brix	Percentage of sugar	Percentage of acid	Sol. sol. / acid ratio	Orange no.	Degrees brix	Percentage of sugar	Percentage of acid	Sol. sol. / acid ratio
1	12.80	9.63	0.98	13.05	30	13.70	10.91	1.07	12.80
2	13.10	10.30	0.98	13.35	31	14.00	10.93	0.96	14.60
3	12.50	9.46	1.08	11.60	32	13.70	10.68	1.14	12.00
4	13.70	10.44	1.14	12.00	33	15.30	11.92	1.15	13.30
5	14.40	11.17	1.06	13.60	34	13.85	10.83	1.06	13.05
6	15.00	11.14	1.06	14.15	35	13.20	10.31	1.02	12.95
7	13.90	10.85	0.84	16.55	36	14.70	11.51	0.86	17.10
8	13.40	10.43	0.98	13.70	37	14.75	10.96	1.04	14.20
9	13.70	10.94	0.93	14.75	38	15.30	11.57	1.29	11.85
10	13.70	10.65	0.84	16.30	39	15.30	11.46	1.23	12.45
11	13.55	10.71	0.90	15.05	40	13.75	10.88	0.91	15.10
12	13.35	10.14	1.15	11.60	41	13.40	10.18	1.29	10.40
13	13.20	10.35	0.94	14.05	42	13.35	10.33	1.19	11.20
14	13.95	10.85	0.98	14.25	43	13.45	10.09	1.24	10.85
15	14.30	10.83	0.96	14.90	44	14.45	11.00	0.94	15.35
16	15.05	11.59	0.95	15.85	45	12.80	10.05	1.15	11.15
17	14.90	11.80	1.02	14.60	46	14.00	10.76	1.27	11.00
18	13.20	10.30	1.09	12.10	47	14.70	11.26	0.86	17.10
19	15.25	12.05	1.00	15.25	48	14.90	11.44	0.98	15.20
20	13.40	10.53	1.02	13.15	49	12.60	11.35	1.07	11.80
21	14.85	11.18	1.11	13.40	50	14.80	10.88	1.31	11.30
22	13.40	11.35	1.01	13.25	51	14.10	11.13	1.16	12.15
23	14.45	11.49	0.82	17.60					
24	13.80	10.99	0.91	15.15	Mean	14.00	10.89	1.05	13.60
25	13.00	10.20	1.14	11.40					
26	14.45	11.28	1.22	11.85	P.E. mean.	±0.07	±0.06	±0.01	±0.17
27	14.30	11.15	1.05	13.60					
28	14.60	11.61	1.12	13.05	P.E. sing.	±0.5	±0.4	±0.09	±1.3
29	14.55	11.40	0.87	16.70					

The probable error of a single sample and the probable error of the mean are connected in the following manner: P.E. mean $= \frac{\text{P.E. sing.}}{\sqrt{n}}$, so that after a value for P.E. sing. has been found, the

value of P.E. mean for any desired number of fruits may be calculated by substituting this number for "n" in the formula. Thus if P.E. sing. has been found to be 0.5, P.E. mean for a sample of twenty-five fruits is $\frac{0.5}{\sqrt{25}}=0.1$.

The values in table II, giving the odds, may be utilized under the two following conditions. In the first place, it may be used in connection with the analytical results obtained from a single lot of fruit to estimate the degree of assurance that an accuracy between certain limits has been attained. For example, the average sugar content (in table I) was 10.89. If a second sample of fifty-one fruits had been taken at the same time and under the same conditions, we would probably not have obtained exactly this value.

TABLE II*

TABLE OF ODDS

Coefficient	Odds	Coefficient	Odds
1.0	1.00 to 1	3.4	44.87 to 1
1.5	2.21 to 1	3.6	64.79 to 1
2.0	4.64 to 1	3.8	95.15 to 1
2.5	9.89 to 1	4.0	142.26 to 1
2.8	15.95 to 1	4.2	215.92 to 1
3.0	22.26 to 1	4.4	332.33 to 1
3.2	31.36 to 1	4.6	519.83 to 1

*The values in this table were selected from a table by PEARL and MINER (6). Original article should be consulted for a complete list of values.

But the P.E. mean, ±0.06, indicates that the chances are even (1 to 1) that the value found would have been between 10.95 and 10.83. In addition to this information, table II shows that the chances are 9.89 to 1 that the value would have been between 10.89 plus (2.5×0.06) and 10.89 minus (2.5×0.06), that is, between 11.04 and 10.74.

Considering the probable error of a single sample in connection with table II, the P.E. sing. was found to be 0.4. This means that if one more fruit had been taken, the chances are even that its value would have been between 10.89+0.4, and 10.89−0.4. In other words, half the fruits in table I should have sugar values between 11.29 and 10.49, and half should be outside these limits. Table I shows that twenty-four oranges are within these limits and

twenty-seven outside. Table II indicates further that the chances are 4.64 to 1 that no single sample would deviate from 10.89 by as much or more than 2.0 times 0.4: that is, of the fifty-one fruits in table I, about nine should be outside the limits 11.69 to 10.09, and forty-two should be within them. A count shows that in this case five are outside and forty-six within.

In the second place, table II may be applied in an entirely different case, namely, when comparing the analytical results from two different lots of fruit in order to estimate the degree of assurance that the difference shown between them is significant. For example, in table VII it is shown that the refractive index of the juice of the Eureka strain of lemons was 44.6 ± 0.2, while that of the Shade Tree strain was 45.7 ± 0.3. The difference is 1.1. What are the chances that this difference is significant and not due merely to a sampling error? This calculation is made from the following formula: $$\frac{\text{difference}}{\text{P.E. of difference}} = \frac{1.1}{\sqrt{(0.2)^2+(0.3)^2}} = \frac{1.1}{0.36} = 3.0.$$ The figure 3.0 is here termed the coefficient of odds, and its value is sought in column 1 in table II, from which it appears that the odds are about 22 to 1 (judging from these data, at least) that the juice of lemons from the Shade Tree strain is higher with respect to refractive index. Table II applies only in those cases in which the difference between two results may be expected to occur in either direction. For a table showing odds when it is known that the difference between two results will be in one direction only, see WOOD (**11**, p. 26).

Formulas for calculating number of fruits for sample

Two general sets of conditions may be recognized under which samples are collected for analysis: (1) When samples are taken from each of two or more different lots of fruit, with the object of later comparing them, to determine whether the differences between them are significant, and what the odds are that this is so. (2) When a sample is taken from a single lot of fruit for the purpose of obtaining a figure that will represent the composition of that lot, and to attain a certain assurance that this figure is correct within certain desired limits.

HAYNES and JUDD (3) have studied the requirements under the first condition. They proposed the following formula for use in calculating the number of individuals to include in a sample in order that a certain difference between two averages may be considered significant: $N = 2\left(\frac{3 \times p}{5}\right)^2$. N is the "number of samples which must be taken in order that there may be a probability of 0.957[2] that a 5 per cent difference is significant"; 3 is the coefficient in the "table of odds" (table II), and thus is equivalent to odds of 22 to 1; "p" is the probable error of a single sample and must be determined experimentally (in this case by analyzing individual fruits).

Other values than 3 and 5 may be assumed to meet the conditions of the experiment; therefore, in order to make comparisons with what is to follow, it is desired to express the preceding formula in general terms as follows: $N = 2\left(\frac{\text{coefficient of odds} \times \text{P.E. sing.}}{\text{difference}}\right)^2$ (*formula 1*). To illustrate the use of this formula, data may be taken from HAYNES and JUDD'S paper. Working with apples, they found the mean titration value to be 10.20 with a P.E. sing. of 0.78, and the latter is thus 7.7 per cent of the mean. To get an assurance of 30 to 1 that a 5 per cent difference is significant: $N = 2\left(\frac{3.2 \times 7.7}{5}\right)^2 = 49$ apples.

The problem under the second condition may now be considered. We wish a general formula that will connect the number in the sample with the probable error of a single fruit and with the coefficients in the "table of odds" (table II). In table I it was shown that the mean sugar content was 1089.±0.06. What are the chances that the "true" value is within the limits ±0.17? The chances are found in the following way (MERRIMAN 5): $\frac{0.17}{0.06} = 2.8$, and looking up the coefficient 2.8 in table II, we find the chances are about 16 to 1 that the error in 10.89 is not more than ±0.17.

[2] The expression 0.957 may be thought of as indicating a probability of 957 out of 1000, which represents a ratio of 957 to 43, or about 22 to 1.

This relation may now be expressed in general terms by putting "deviation" for ±0.17, where it is to be the deviation above or below the mean, which we wish to use as a limit for accuracy; then putting "P.E. mean" for 0.06, and "coefficient of odds" for 2.8, we have: $\frac{\text{deviation}}{\text{P.E. mean}} = \text{coefficient of odds}$, but P.E. mean $= \frac{\text{P.E. sing.}}{\sqrt{N}}$ (WOOD 11), and substituting this value, the equation becomes $\frac{\text{deviation}}{\frac{\text{P.E. sing.}}{\sqrt{N}}} = \text{coefficient of odds}$, from which

$$N = \left(\frac{\text{coefficient of odds} \times \text{P.E. sing.}}{\text{deviation}}\right)^2 \quad (\textit{formula 2}).$$

In illustration of the use of this formula, table VI shows that fifty grapefruits from tree no. 1 had an average brix of 13.15 and the P.E. sing. was 0.35. What number of fruits are required to give odds of 10 to 1 that the brix of that number will be correct to ±0.15? Table II shows that for odds of 10 to 1, the coefficient of odds is 2.5, therefore $N = \left(\frac{2.5 \times 0.35}{0.15}\right)^2 = $ thirty-four grapefruits. No account is taken of errors in the method of analysis, since in the present case analytical errors are small as compared with the variability of the individual fruits with respect to the constituent. If it is desired to take analytical errors into account also, see WAYNICK (10) and ROBINSON and LLOYD (7).

Comparison of formulas

Although formulas 1 and 2 appear to be very similar, the first in fact giving values just double those of the second, certain essential differences should be pointed out. Formula 1 applies when *two different lots* are being compared, in which case the significance of the difference between them is affected by the sampling error of each lot. Formula 2 applies to the analytical results of a *single lot* only, its own error being the only one involved. Such a condition arises when an analysis is made for the purpose of reporting the composition of a product with respect to a certain constituent, or when an analysis is made to determine whether a constituent has reached a certain required value.

Accuracy of formulas

In the preceding paragraphs it was found that the use of formulas 1 and 2 gave forty-nine fruits as the required number in one illustrative case, and thirty-four as the required number under the other set of conditions. We should not be justified, however, in concluding from this test that forty-seven would be too few in the first case, and thirty-six would be more than enough in the second. With either formula it is seen that the number N depends for its value upon the value of the probable error of a single sample, and therefore it becomes necessary to inquire how variable this value is, and what effect changes in its value have upon N.

TABLE III

DIFFERENT VALUES OBTAINABLE FROM SAME LOT OF FRUIT

CALCULATIONS AFTER THE FOLLOWING NUMBER OF FRUITS ANALYZED	TAKEN IN ORDER OF ANALYSIS		TAKEN IN ORDER REARRANGED BY LOT			
			First rearrangement		Second rearrangement	
	P.E. sing. found	No. of fruits required	P.E. sing. found	No. of fruits required	P.E. sing. found	No. of fruits required
10	1.1	22	1.4	36	1.0	18
15	1.1	22	1.4	36	1.2	26
20	1.0	18	1.4	36	1.2	26
25	1.1	22	1.4	36	1.2	26
30	1.1	22	1.4	36	1.4	36
35	1.1	22	1.4	36	1.4	36
40	1.1	22	1.4	36	1.3	31
45	1.2	26	1.3	31	1.3	31
51	1.3	31	1.3	31	1.3	31

It is instructive to note what values would have been obtained if the value of P.E. sing. had been taken, not after fifty-one fruits had been analyzed, but after the analysis of say ten fruits, or after fifteen, or twenty-five. The different values for P.E. sing. and N that were obtainable in this manner calculated from formula 1 are shown in table III. It is thus found the P.E. sing. varied from 1.0 to 1.3, which values, substituted in the formula, caused the value of N to vary from 18 to 31. Formula 2 would likewise have given variable values, but the actual figures would have been one-half as large.

The fruits in table I were analyzed in the order of size, number one being the largest. It may be urged that therefore we do not

have a true random sample, or that there is a correlation between size and composition. The correlation coefficient between size and the soluble-solids-acid ratio, however, was calculated by the method recommended by TOLLEY (9), and was found to be 0.158, with a probable error of 0.092, which does not indicate any significant correlation.

In order to partially eliminate the size of the fruit as a factor, the order in table I was rearranged by lot. With the new order,

TABLE IV

RESULTS OF CALCULATIONS OF PROBABLE ERROR BASED ON ANALYSIS OF GROUPS OF TEN FRUITS EACH

GROUPS OF 10 FRUITS EACH	SOLIDS-ACID RATIO	
	P.E. sing.	No. required for desired assurance
Group 1	1.0	18
2	0.9	15
3	1.3	31
4	1.0	18
5	1.6	47
6	1.4	36
7	1.3	31
8	1.3	31
9	1.3	31
10	0.5	5
11	1.8	59
12	1.3	31
13	1.1	22
14	0.9	15
15	1.2	26

the values of P.E. sing. and N were calculated after ten fruits were analyzed, after fifteen, etc. The results are shown in table III. P.E. sing. was found to vary from 1.3 to 1.4, causing N to vary from 31 to 36. Another rearrangement by lot is shown in the last two columns of table III. Values of P.E. sing. vary from 1.0 to 1.4, causing N to change from 18 to 36. In both these cases, values by formula 2 would also have been variable, but of course would have been just half as large numerically.

Use of small numbers to calculate probable error of single fruit

It may be inquired what the P.E. sing. would have been for different lots of ten fruits each. Groups of ten each were selected

by lot and the values of P.E. sing. and N calculated. Strictly speaking, when the number involved is small, say ten, the formula for P.E. only gives approximate results (BRUNT 1). The value of P.E. sing. for the ratio is thus shown to vary from 0.5 in group 10 to 1.8 in group 11, causing a change in N from 5 to 59 (table IV). One trial with a small number of fruits would not be adequate for the determination of the value of P.E. sing. and of N, at least with such variable material as oranges.

Probable error of a probable error

The preceding discussion indicates that variable values were found for N, depending on the value found for P.E. sing. To obtain an idea of the variability of P.E. sing. and of N in the manner described (that is, by obtaining the results given by several different groups containing different numbers) is tedious and unsatisfactory. A more convenient method of judging the accuracy of P.E. sing. and N is desired. It is plain that the probable error calculated from the analysis of fifty fruits is more representative of the lot than that calculated from ten fruits. The relation of the error in the probable error to the number of fruits analyzed is given by the expression (BRUNT 1, p. 57): Probable error of P.E. sing. = P.E. sing. $\times \frac{0.4769}{\sqrt{n-1}}$ (*formula 3*). Thus if 1.3 is the P.E. sing. for the soluble-solids-acid ratio (table I), then the probable error of $1.3 = 1.3 \times \frac{0.4769}{\sqrt{51-1}} = 0.09$, or about 0.1. In other words, the "true" value of P.E. sing. is probably between 1.2 and 1.4. We may obtain an estimate of the limits of N by substituting 1.2 and 1.4 successively in the formulas; in this case N is found to be 26 or 36 for formula 1, and 13 or 18 for formula 2.

Ordinarily it will be sufficient to consider the probable limits of the value of N by approximations made by the use of formula 3 in the manner indicated. If it is found desirable to do so, however, a formula may be used for the correction. If we rearrange formula 1 to read: $N = 2\left(\frac{\text{coefficient}}{\text{difference}}\right)^2 (\text{P.E. sing.})^2$, and apply the method described by GOODWIN (2), we find that deviation produced in the

value of N by an error in the value of P.E. sing. is as follows:

$$\text{Deviation in N} = \frac{d\left[2\left(\frac{\text{coefficient}}{\text{difference}}\right)^2(\text{P.E. sing.})^2\right]}{d(\text{P.E. sing.})} \times \text{error in P.E. sing.,} = 4\left(\frac{\text{coefficient}}{\text{difference}}\right)^2 \times \text{P.E. sing.} \times \text{error in P.E. sing. } (\textit{formula 4}).$$

To apply this formula to a particular case, we find from table III that the P.E. sing. for fifteen fruits was 1.1; the error in 1.1 is found by substituting in formula 3 to be $1.1 \times \frac{0.4769}{\sqrt{15-1}} = 0.14$. If we wish odds of 22 to 1 for a difference of 1.0 in ratio, we obtain, by substitution in formula 4: Deviation in $N = 4 \times \left(\frac{3.0}{1.0}\right)^2 \times 1.1 \times 0.14 =$ six fruits, therefore the corresponding value, 22, found in table III, is in error by six fruits, and the probable number extends from 16 to 28.

The corresponding formula for applying a correction to formula 2 is: Deviation in $N = 2 \times \left(\frac{\text{coefficient}}{\text{difference}}\right)^2 \times$ P.E. sing. $\times$ deviation in P.E. sing. (*formula 5*).

Data on other lots of oranges

The discussion thus far has related to the data from only one lot of oranges from a single tree. Fruits from four other trees were obtained and analyzed in the same manner. The number of fruits used was small, but some idea of the accuracy of the probable errors can be obtained by applying formula 3. The data are shown in table V, and serve to indicate values of P.E. sing. that may be expected in dealing with different lots of oranges.

Data on grapefruit

Fifty fruits were taken at random from a grapefruit tree in one grove, and a corresponding number from another tree located in another grove. The fruits were analyzed individually and the mean and P.E. sing. determined. To save space, the complete analyses are not given, but the results are summarized in table VI. From this table it is seen that P.E. sing. of the fruit from the two lots is

approximately the same with respect to brix and sugar, but P.E. sing. for acid and for ratio is considerably different in the two lots.

Data on lemons

That different lots of fruit show different values for P.E. sing. is also apparent from the analysis of individual lemons. In table VII will be found the results of the analysis of thirty lemons from two different lemon trees, each tree representing a different strain

TABLE V

SHOWING DIFFERENT VALUES OF P.E. SING. WITH DIFFERENT LOTS OF ORANGES

TREE NO.	NO. OF FRUITS IN SAMPLE	DEGREES BRIX		PERCENTAGE ACID		$\frac{\text{Sol. sol.}}{\text{acid}}$ ratio	
		Mean	P.E. sing.	Mean	P.E. sing.	Mean	P.E. sing.
2.....	12	13.70	0.5	0.87	0.07	15.9	1.1
3.....	13	15.00	0.5	0.86	0.04	17.4	0.8
4.....	12	11.80	0.4	0.79	0.08	15.2	1.3
5.....	9	12.45	0.3	1.46	0.10	8.6	0.7

TABLE VI

COMPARISON OF COMPOSITION OF FRUIT FROM TWO GRAPEFRUIT TREES

TREE NO.	TOTAL NO. OF FRUITS	BRIX		PERCENTAGE SUGAR		PERCENTAGE ACID		SOLIDS-ACID RATIO	
		Mean	P.E. sing.	Mean	P.E. sing.	Mean	P.E. sing.	Mean	P.E. sing.
1.....	50	13.15	0.35	8.16	0.27	2.29	0.01	5.8	0.2
2.....	50	12.30	0.35	7.89	0.29	1.65	0.09	7.5	0.4

of the Eureka variety. While too much reliance cannot be placed on the values obtained by analyzing fifteen fruits, it is seen from the table that the two lots of fruit probably have different values of P.E. sing. with respect to three of the characters of which analytical results were obtained.

Further precautions regarding use of formulas

Two further precautions may now be added regarding the use of the formulas. When the value of P.E. sing. has been found for one tree or lot of fruit, it must not be assumed that another tree

or lot will have the same value (compare acidity of two grapefruit trees, table VI). When two trees or lots of fruit are found to have the same value for P.E. sing with respect to one constituent, it must not be assumed that they agree also with respect to other constituents (compare trees no. 1 and no. 2, table VI, with respect to brix and ratio).

TABLE VII

VARIABILITY IN COMPOSITION OF INDIVIDUAL LEMONS, EUREKA VARIETY

EUREKA STRAIN*					SHADE TREE STRAIN*				
Lemon no.	Sp. Gr. of fruit	Percentage rind	Refrac. index of juice	Acidity of juice cc. NaOH	Lemon no.	Sp. Gr. of fruit	Percentage rind	Refrac. index of juice	Acidity of juice cc. NaOH
1.....	0.92	40	42.9	27.2	1.....	0.96	41	42.1	24.3
2.....	0.96	40	44.2	28.5	2.....	0.94	59	45.4	24.4
3.....	0.94	50	44.2	28.8	3.....	0.96	50	45.8	24.9
4.....	0.96	49	44.8	29.7	4.....	0.98	36	44.9	26.8
5.....	0.95	49	45.2	27.9	5.....	0.98	47	46.8	21.8
6.....	0.94	46	44.6	30.7	6.....	0.95	62	47.0	24.0
7.....	0.96	48	45.6	30.5	7.....	0.95	56	45.8	24.5
8.....	0.95	35	44.0	28.7	8.....	0.96	54	45.6	22.9
9.....	0.94	46	43.6	28.1	9.....	0.98	47	45.4	22.0
10.....	0.96	50	46.3	25.1	10.....	0.99	54	47.6	20.8
11.....	0.95	50	45.0	27.8	11.....	0.96	48	45.7	26.6
12.....	0.95	48	45.6	28.0	12.....	0.98	39	45.7	26.3
13.....	0.96	57	43.8	29.3	13.....	0.98	51	46.2	22.6
14.....	0.96	39	43.9	28.0	14.....	0.98	54	.....	20.9
15.....	0.97	49	45.4	28.7	15.....	0.97	59	.....	22.0
Mean....	0.95	46	44.6	28.5	Mean.....	0.97	50	45.7	23.7
P.E. mean	±0.002	±1.0	±0.2	±0.2	P.E. mean.	±0.003	±1.3	±0.3	±0.4
P.E. sing..	±0.007	±4.0	±0.6	±0.9	P.E. sing..	±0.010	±5.0	±0.9	±1.3

* Strains described by SHAMEL, SCOTT, and POMEROY (8).

Comparison of standard formula with Peter's formula for calculating probable error of single observation

Two general methods for calculating the value of P.E. sing. are as follows:

STANDARD FORMULA

$$\text{P.E. sing.} = \pm 0.6745 \sqrt{\frac{\Sigma d^2}{n-1}}$$

PETER'S FORMULA

$$\text{P.E. sing.} = \pm 0.8453 \frac{\Sigma d}{\sqrt{n(n-1)}}$$

Thus, to use the standard formula, the sum of the squares of the deviations must be found, while with Peter's formula only the

sum of the deviations (taken without regard to sign) is needed. Inasmuch as the latter method is more convenient, it seemed profitable to show the difference in the value of P.E. sing. given by the two methods. In table VIII are shown the comparative values found.[3] It is seen that the difference in the value of P.E. sing. by the two methods is at least not more than is shown between two groups of even the same lot of fruit. Hence no large error would have been introduced by the use of the more convenient Peter's formula.

TABLE VIII

COMPARISON OF STANDARD FORMULA WITH PETER'S FORMULA FOR CALCULATING PROBABLE ERROR OF SINGLE OBSERVATION

No. of fruits in sample	P.E. sing. observation		No. of fruits in sample	P.E. sing. observation	
	Solids-acid ratio			Percentage sugar	
	Standard formula	Peter's formula		Standard formula	Peter's formula
10	1.09	1.08	10	0.39	0.40
15	1.01	.99	15	0.34	0.34
25	1.09	1.10	20	0.44	0.42
30	1.10	1.13	25	0.42	0.43
40	1.09	1.02	35	0.40	0.40
45	1.20	1.22	45	0.40	0.40
51	1.26	1.29	51	0.39	0.38

Summary

1. Formulas are given, for use under two different conditions of sampling, to determine the number of fruits required in a sample in order to give a desired assurance that a certain accuracy has been attained.

2. Approximately 250 fruits of oranges, lemons, and grapefruit were analyzed individually, and the probable errors calculated. The data so obtained were applied to the formulas, and numerical examples worked out to illustrate their use.

3. It is shown that the values given by the formulas are only approximately correct. The sources of error are discussed, and formulas given by which the amount of this inaccuracy may be estimated under different conditions.

[3] Computations are made much easier by the use of tables given by MELLOR (4).

4. Analyses of fruits taken from different orange, lemon, and grapefruit trees are given, showing the variability of the fruits of different trees with respect to brix of juice, percentage of sugar, acidity, etc., and the values of the probable errors that such variability produced.

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UREDINALES COLLECTED BY R. THAXTER AND J. B. RORER IN TRINIDAD[1]

J. C. Arthur

(WITH FOUR FIGURES)

During a visit to Trinidad extending from December 19, 1912, to May 16, 1913, made by Dr. Roland Thaxter of Harvard University for the purpose of collecting insects and fungi, especially Pyrenomycetes, a number of Uredinales were secured, chiefly in the latter half of the period. The island of Tobago was also visited for a day in May. This island lies about thirty miles northeast of Trinidad, and geographically is to be considered with it in a study of the flora. The two islands lie close to the northern coast of South America, and in their faunal and floral relations belong to that continent.

The Uredinales, consisting of about forty-five numbers, including two from Tobago, were recently submitted to the writer for study by Thaxter. They have been found to represent thirty-seven species of rusts, and to fall into the unusually large percentage of fourteen genera. Two of the species appear to be undescribed, and one of these seems sufficiently distinctive to be the type of a new genus.

Little is known of the rusts of Trinidad, and the independent publication of this interesting list of species appears to be worth while. A few numbers have been added, which were collected in 1915 and 1916 and sent to the writer by Mr. James B. Rorer, Mycologist to the Board of Agriculture of Trinidad from 1909 to 1918, and earlier assistant in botany at Harvard University. He sent four numbers, which show the same high percentage of species and genera as those from Thaxter, there being four species, one of them a most interesting undescribed form, and three genera. All of the species and two of the genera are unrepresented in the Thaxter collection.

[1] Contribution from the Botanical Department of Purdue University Agricultural Experiment Station.

In the following list nos. 6, 8, 14, 18, 19, 20, 30–36, thirteen in all (that is, 30 per cent) are short cycle species, while the others drop into various categories of long cycle species, five of them, nos. 1, 17, 23, 26, and 28, being actually or potentially heteroecious. Altogether the list embraces forty-three species of rusts, under sixteen genera, an excellent beginning to the study of the Uredinales of Trinidad and its adjacent islands.

For those species which have received treatment in the seventh volume of the *North American Flora* reference is made to the page where the description and synonomy occur; for the other species reference is given to the place of the original description. Thanks are especially due to Dr. THAXTER and Mr. RORER for the drawing and photographs included in this paper, illustrating two of the new species.

1. COLEOSPORIUM IPOMOEAE (Schw.) Burr. N.A.F. 87.—On *Ipomoea glabra* (Aubl.) Choisy, St. Anns Valley, February 1913, II, *Thaxter* 4.

Somewhat common throughout the West Indies in its uredinial stage.

2. PHAKOPSORA CROTONIS (Cooke) Arth. N.A.F. 104.—On *Croton gossypifolium* Vahl, La Seiva Valley, May 5, 1913, II, *Thaxter* 43.

3. PHAKOPSORA VITIS (Thüm.) Sydow, N.A.F. 102 (*Uredo Vitis* Thüm.).—On cultivated grape (*Vitis* sp.), Port of Spain, October 1916, II, *Rorer*.

4. CEROTELIUM FICI (Cast.) Arth. Bull. Torr. Bot. Club **44**: 509. 1917.—On *Ficus radula* Willd., Maraval Valley, May 14, 1913, II, *Thaxter*.

5. **Cerotelium minutum,** sp. nov.

II. Uredinia hypophyllous, more or less grouped on discolored spots, hidden in the pubescence of the host, small, 0.1–0.3 mm. in diameter, soon naked, pulverulent, pale yellow becoming colorless; peridium and paraphyses wanting; urediniospores obovoid or globoid, small, 14–16×17–23 μ; wall pale yellow or colorless, thin, 1 μ or less, sparingly and prominently echinulate, the pores obscure.

III. Telia hypophyllous, hidden by the pubescence, minute, 0.03–0.08 mm. in diameter, erumpent, forming columns about

30–50μ high, fuscous; teliospores catenulate, adhering laterally to form a cylindric column, ellipsoid or cuboid, 12–14×13–16μ; wall pale cinnamon-brown, uniformly thin, 1 μ or less, smooth.

On an undetermined Bignoniaceous vine, La Seiva Valley, April 1913, II, iii, *Thaxter* 38.

The host has large trumpet-shaped flowers and large, ovate, pointed leaves, pale with dense pubescence beneath. The rust is unusually minute. The presence of telia was pointed out by THAXTER, who supplied a microscope slide with sections showing the telial structure.

Maravalia, gen. nov.

Cycle of development includes telia, and possibly pycnia.

Telia subepidermal, erumpent, somewhat indefinite. Teliospores free, one-celled, with apical germination, pedicellate; wall colorless, thin, smooth.

6. **Maravalia pallida** Arthur and Thaxter, sp. nov.

O. Pycnia unknown, probably not formed.

III. Telia hypophyllous, numerous in circular areas 5–10 mm. across on somewhat larger yellowish spots, at first pulvinate, roundish, 0.2–0.4 mm. across, becoming larger, elongate and branched, somewhat confluent, early naked, yellowish becoming white, velvety by germination, ruptured epidermis erect or somewhat overarching; teliospores elongate-oblong, clavate-oblong, or cylindroid, 13–22×58–67μ, rounded at both ends or narrowed below, germinating upon maturity; wall colorless, uniformly very thin, about 0.5μ, smooth; pedicel slender, 8–10μ in diameter, 20–35μ long, colorless.

On *Pithecolobium latifolium* (L.) Benth. (*Zygia latifolia* St. Hil.), Maraval Valley, April 1913, *Thaxter*.

The genus differs from *Chaconia*, established by JUEL for a white rust on *Pithecolobium divaricatum* (Bong.) Benth., from Paraguay, by the mode of origin of the spores. Both are short cycle species; in *Chaconia* the spores are sessile and clustered on a large basal cell, while in *Maravalia* they are pedicelled and arise directly from the hymenial layer of hyphae in the same manner as is usual in the large majority of rusts (fig. 1). The genus is apparently a short cycle condition of the genus *Spirechina*. *M. pallida* much resembles in gross appearance *S. epiphylla*, a Texan rust on *Rubus*. In its teliospores it also much resembles *S. epiphylla*, and there is even a closer resemblance to those of *S. Pittieriana*, another *Rubus* rust from Costa Rica. Figs. 1 and 2 well illustrate the structure and habit of the rust.

7. **Milesia Blechni** (Sydow), comb. nov. (*Melampsorella Blechni* Sydow, Ann. Myc. **1**:537. 1903; *Uredo Blechni* Diet. and Neg., Engler's Jahrb. **22**:358. 1896).—On *Lygodium polymorphum* (Cav.) H.B.K., St. Anns Valley, February 20, 1913, II, *Thaxter* 45.

A common rust in Europe, and also known from Chile.· The host is new for the species. Special interest attaches to the group of fern rusts to which this one belongs, as probably representing the most primitive characters of Melampsoraceae, and possibly of all the rusts as well.

8. CIONOTHRIX PRAELONGA (Wint.) Arth. N.A.F. 124.—On *Eupatorium odoratum* L., La Seiva Valley, April 1913, *Thaxter* 41.

A common short cycle rust occuring throughout tropical America on various species of *Eupatorium*.

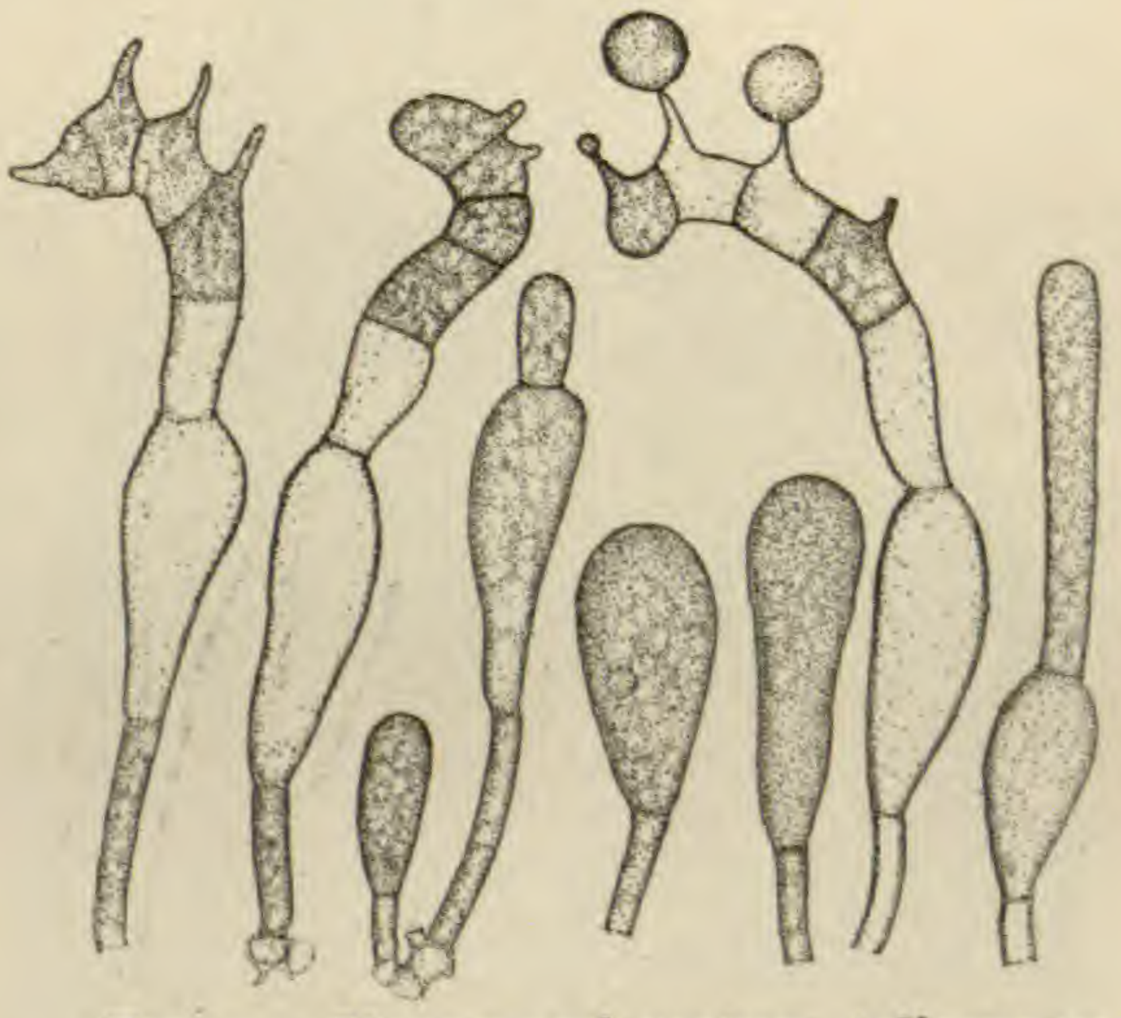

FIG. 1.—Young and mature teliospores of *Maravalia pallida*, the latter germinating.

9. RAVENELIA INDIGOFERAE Tranz. N.A.F. 144.—On *Indigo-fera suffruticosa* Mill. (*I. Anil* L.), Roxborough, Tobago, May 8–9, 1913, II, *Thaxter* 25.

10. DICHEIRINIA BINATA (Berk.) Arth. N.A.F. 147 (*Uredo Cabreriana* Kern and Kellerm.).—On *Erythrina umbrosa* H.B.K., Palmiste, San Fernando, October 24, 1916, II, *Rorer*.

· "The host is called Anauca Immortal, and is used to shade cacao. The rust attacks not only the leaf blades, but the midribs and petioles, sometimes causing distortion," as writes Mr. RORER. It is not uncommon in other West Indian islands and in Central America.

11. CTENODERMA CRISTATUM (Speg.) Sydow, Ann. Myc. **17**:103. 1919 (*Uromyces Cupaniae* Arth. and Johnst.).—On *Cupania americana* L., Maraval Valley, April 1913, III, *Thaxter* 40.

12. DESMELLA GYMNOGRAMMES (P. Henn.) Sydow, Ann. Myc. **16**:242. 1918.—On *Adiantum latifolium* Lam., Maraval Valley, March 1913, II, *Thaxter* 44; *Cyclopeltis semicordata* (Sw.) J. Sm., Maraval Valley, 1913, II, *Thaxter* 46.

This is a common fern rust in its uredinial stage throughout tropical America on many genera of ferns. It has been reported from Porto Rico on the first named host, but the second host is a new record. The teliospores have recently come to light on a collection from Brazil of *Lygodium polymorphum* (Cav.) H.B.K. in the National Herbarium. I am indebted to Mr. W. R. MAXON for the opportunity of studying this interesting material, and also for his kindness in determining the ferns mentioned in this paper. The genus *Desmella* possesses by far the most primitive characters of any rusts known in the family Aecidiaceae (Pucciniaceae), and consequently its members have unusual interest for the student of Uredinalean evolution.

FIG. 2.—Leaf of *Pithecolobium latifolium* showing groups of sori of *Maravalia pallida*, nearly natural size.

13. UROMYCES COLUMBIANUS Mayor, Mém. Soc. Neuch. **5**:467. 1913.—On *Melanthera aspera* (Jacq.) Steud., Manzanilla Beach, 1913, II, *Thaxter* 33; Maraval Valley, 1913, i, II, *Thaxter* 34.

14. UROMYCES JAMAICENSIS Vesterg. Ark. Bot. Stockh. 4^{15}:33. 1905.—On *Bauhinia Pauletia* Pers., St. Anns Valley, March-April 1913, *Thaxter* 20a; La Seiva Valley, April-May 1913, *Thaxter* 20b.

A short cycle species known heretofore only from the North American West Indies and Mexico.

15. UROMYCES WULFFIAE-STENOGLOSSAE Diet. Ann. Myc. **6**:96. 1908.—On *Wulffia baccata* (L.f.) Kuntze, St. Anns Valley, 1913, III, *Thaxter* 35; La Seiva Valley, 1913, III, *Thaxter* 36.

16. PUCCINIA ACNISTI Arth. N.A.F. 471.—On *Acnistus arborescens* Schlecht., Maraval Valley, 1913, I, *Thaxter* 23.

17. PUCCINIA ANTIOQUINENSIS Mayor, N.A.F. 347.—On *Cyperus diffusus* Vahl, Sangre Grande, April 1913, III, *Thaxter* 51.

18. PUCCINIA ARECHAVALETAE Speg. Ann. Soc. Ci. Arg. **12**:67. 1881.—On *Urvillea Seriana* (L.) H.B.K., St. Claire, Port of Spain, February–April 1913, *Thaxter* 28.

19. **Puccinia corticola** Arthur and Rorer, sp. nov.

O. Pycnia amphigenous, rather few, seen in groups of 2 or 3, inconspicuous, subepidermal, flask-shaped, 74–96μ broad by 110–115μ high; ostiolar filaments agglutinated.

III. Telia amphigenous and caulicolous, on leaves and young twigs, few, scattered, confluent in groups of 6–8 or more, on leaf blades rounded, on midribs and stems oblong or lenticular, 0.2–1.3 mm. long, on trunk and branches much larger, rather early naked, dark brown, pulverulent, ruptured epidermis evident; teliospores ellipsoid, 19–24×30–42μ, rounded above and below, slightly or not constricted at septum; wall cinnamon or chestnut brown, 2–2.5μ thick, slightly thicker at apex, coarsely and sparsely verrucose, the markings more pronounced above, pedicel colorless, fragile.

On *Cordia Gerascanthus* L. (Ehretiaceae), Arima Forest Reserve, December 1915, *Rorer*.

The host, Mr. RORER writes, is "a timber tree, which yields a wood known as Cypre. The disease is of economic importance here, as it not only attacks the leaves and young twigs, but the older branches as well, and causes large lesions on the main trunk of the tree." Figs. 3 and 4 show the injury to five year old trees. This interesting short cycle species is the only rust in *Puccinia* known to attack the trunks and larger branches of trees, although some species of *Gymnosporangium* show such behavior.

20. PUCCINIA EUPATORII DIET. Hedwigia **36**:32. 1897.—On *Eupatorium iresinoides* H.B.K., Diego Martin, 1913, *Thaxter* 32.

21. PUCCINIA GOUANIAE Holway, Ann. Myc. **3**:21. 1905.—On *Gouania polygama* (Jacq.) Urban, Maraval Valley, March 1913, II, *Thaxter* 30a; Roxborough, Tobago, May 8–9, 1913, II, iii, *Thaxter* 30b.

22. PUCCINIA HELICONIAE (Diet.) Arth. Bull. Torr. Bot. Club **45**:144. 1918.—On *Bihai Psittacorum* (L.) Kuntze (*Heliconia Psittacorum* L.), La Seiva Valley, April 1913, II, *Thaxter* 11.

23. **Puccinia** (?) **ignava** (Arth.), comb. nov. (*Uredo ignava* Arth.), N.A.F. 341.—On *Dendrocalamus giganteus* Munro (?), Maraval Valley, March 1913, II, *Thaxter* 49.

FIG. 3.—Base of trunk of a five-year-old tree of *Cordia Gerascanthus*, showing severe injury caused by *Puccinia corticola*.

The host is an introduced bamboo, and one not before recorded for the rust. The name is transferred to *Puccinia* for convenience of listing. There

is large probability that when the teliospores are found they will justify this change.

FIG. 4.—Trunk of five-year-old tree of *Cordia Gerascanthus*, showing lesions made by *Puccinia corticola*.

24. PUCCINIA INVAGINATA Arth. and Johnston, Mem. Torr. Club **17**: 146. 1918.—On *Gouania polygama* (Jacq.) Urban, St. Anns Valley, May 3, 1913, II, III, *Thaxter* 29.

Heretofore known from the North American West Indies and Guatemala, but not from South America.

25. PUCCINIA LEONOTIDIS (P. Henn.) Arth. N.A.F. 407.—On *Leonotis nepetaefolia* (L.) R. Br., Maraval Valley, March 1913, II, *Thaxter* 10.

Only the uredinia of this common tropical rust are known in America.

26. PUCCINIA PURPUREA Cooke, N.A.F. 284.—On Guinea corn, *Holcus Sorghum* L., Port of Spain, September 1916, II, *Rorer*.

27. PUCCINIA RUELLIAE (Berk. and Br.) Lagerh. N.A.F. 415.—On *Blechum Brownei* Juss., La Seiva Valley, 1913, II, *Thaxter* 2; *Dianthera pectoralis* (Jacq.) Gmel. (*Justicia pectoralis* Jacq.), Port of Spain, 1913, II, *Thaxter* 3.

28. PUCCINIA SCLERIAE (Paz.) Arth. N.A.F. 349.—On *Passiflora tuberosa* Jacq., St. Anns Valley, February-March 1913, I, *Thaxter* 18; *Scleria* sp., Sangre Grande, April 1913, II, *Thaxter* 52.

Cultures establishing the relation of the alternate hosts of this species were made by Mr. H. E. THOMAS in Porto Rico in 1917 (Phytopath. **8**:163–164. 1918).

29. PUCCINIA SMILACIS Schw. N.A.F. 377.—On *Smilax cumanensis* Willd. (?), La Seiva Valley, March-April 1913, II, *Thaxter* 17.

Not before reported for the South American region.

30. PUCCINIA SPEGAZZINII DeToni, in Sacc. Syll. Fung. **7**:704. 1888.—On *Mikania* sp., Maraval Valley, April 1913, *Thaxter* 7.

31. PUCCINIA SYNEDRELLAE P. Henn. Hedwigia **37**:277. 1898.—On *Emilia sonchifolia* (L.) DC., La Seiva Valley, April-May 1913, *Thaxter* 22; *Synedrella nodiflora* (L.) Gaertn., Sangre Grande, April 1913, *Thaxter* 42.

32. PUCCINIA TRIUMFETTAE Dietel and Holway; Holway, BOT. GAZ. **24**:30. 1897.—On *Triumfetta* sp., Maraval and La Seiva Valley, April-May 1913, *Thaxter*.

Heretofore scantily represented by collections from southern Mexico and Ecuador. The collection here cited also bore *Pucciniosira pallidula* rather intimately associated on the same leaves.

33. ENDOPHYLLUM GUTTATUM (Kunze) Sydow, Ann. Myc. **17**:179. 1920 (*E. circumscriptum* Whetzel and Olive).—On *Cissus sicyoides* L., Sangre Grande, February 1913, *Thaxter* 24.

34. ENDOPHYLLUM PUMILIO (Kunze) Sydow, Ann. Myc. **17**: 179. 1920. (*E. decoloratum* Whetzel and Olive.)—On *Clibadium surinamense* L., Maraval Valley, 1913, *Thaxter* 37.

35. ENDOPHYLLUM WEDELIAE (Earle) Whetzel and Olive, Amer. Jour. Bot. **4**: 49. 1917.—On *Wedelia trilobata* (L.) Hitchc., Manzanilla Beach, March 1913, *Thaxter* 5b.

36. PUCCINIOSIRA PALLIDULA (Speg.) Lagerh. N.A.F. 127.—On *Triumfetta* sp. Maraval Valley, 1913, *Thaxter* 27. Also associated with *Puccinia Triumfettae* in another collection.

37. UREDO ADENOCALYMMATIS P. Henn. Hedwigia **35**: 249. 1896.—On Bignoniaceae, La Seiva Valley, April 1913, *Thaxter* 39.

38. UREDO CHERIMOLIAE Lagerh. Bull. Soc. Myc. Fr. **11**: 215. 1895.—On *Rollinia multiflora* Splitg., La Seiva Valley, *Thaxter* 14.

A new host for this common Anonaceous rust.

39. UREDO MANDEVILLAE Mayor, Mém. Soc. Neuch. **5**: 591. 1913.—On *Mandevilla tomentosa* (Vahl) Kuntze, Aripo Savanna, April 1913, *Thaxter* 9a; La Seiva Valley, April 1913, *Thaxter* 9b.

40. UREDO PHYLLANTHI P. Henn. Hedwigia **35**: 248. 1896.—On *Phyllanthus Conami* Sw. (*P. acuminatus* Vahl), Maraval Valley, March 1913, *Thaxter* 31.

The species has previously been recorded from the vicinity of Rio de Janeiro, Brazil.

41. UREDO RUBESCENS Arth. Mycologia **7**: 327. 1915.—On *Dorstenia Contrajerva* L., Maraval Valley, January 1913, *Thaxter* 13.

Also known from Porto Rico and Guatemala.

42. UREDO SABICEICOLA Arth. Mycologia **7**: 323. 1915.—On *Sabicea aspera* Aubl., La Seiva Valley, March-April 1913, *Thaxter* 19a, b.

Heretofore only known from Porto Rico.

43. AECIDIUM BRASILIENSE Diet. Hedwigia **36**: 35. 1897.—On *Cordia* sp., Maraval Valley, 1913, *Thaxter* 15, 16.

Previously reported from southern Brazil.

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CLASSIFICATION OF THE ANAEROBIC BACTERIA[1]

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The classification of living forms should depend on an understanding of the laws of heredity as demonstrated in those forms. Preliminary classifications are made by applying the machinery of arrangement that has been worked out for other groups that are well understood, to groups of whose biological processes we know little. Preliminary classifications are necessary and are as desirable as are catalogues, and should be made to correspond to the known life processes of the organisms as nearly as possible, but they should only be offered tentatively.

The study of the biology of the anaerobic bacilli is in its early morning twilight. Today the scientific world holds two widely opposite opinions in regard to the classification of these organisms. The view held in Western Europe and in America is that the anaerobic species are many, distinguishable, and not highly variable; that held by many workers in Central Europe is that the species are practically indistinguishable, are highly variable, and may be changed one into another. It is impossible to bring these two points of view into alignment. They are not to be attributed to diverse interpretation of differences that shade into one another. They are themselves the outcome of an evolutionary process, depending on a mutation in thought, followed by the throwing up of a geographical barrier in 1914 that isolated the mutant thought and permitted it to overgrow its ancestral types of reasoning in a peculiarly favorable soil. The crux of the matter lies in the purity of the cultures studied by the classifier. It is notorious that the casual worker with anaerobic organisms knows neither how to purify them, nor how to tell when they are pure or impure. The anaerobes are not difficult to isolate when one knows how, but usually workers do not know how. Anaerobes have occasionally been isolated in pure culture since the early days of bacteriology.

[1] From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.

A number of descriptions of pure cultures exist, but it was the exceptionally studious worker who was responsible for such descriptions, and casual workers were apparently in the majority.

In 1901 GRASSBERGER and SCHATTENFROH (**18, 19**) propounded their theory of the transmutability of anaerobic species. In the period following they corroborated and extended their findings, and their work was pushed with so many publications (**16, 17, 20–23**) and with so much assertion that by 1914 it was seriously quoted in at least one well known German textbook (**39**), and the doctrine was thoroughly distributed throughout Central Europe. Under pressure of war, work is not carefully done. The casual workers found it necessary to make many anaerobic diagnoses from gas gangrene cases, which they made rapidly, and then turned to their new found collections to classify them. Many of these war workers corroborated the general findings of GRASSBERGER and SCHATTENFROH, namely, that the characters of anaerobes are highly variable, and that species among these organisms are not to be seriously studied.

CONRADI and BIELING (**4, 5**) were the most extreme in their contentions, claiming that one labile species was responsible for all gas gangrene cases. They described two cycles, one developing on carbohydrate media, the other on protein media, and claimed that immune sera identified all the strains in each cycle, but that when a strain was changed over to the other cycle culturally, it was also changed immunologically to that cycle. Such contentions struck so forcibly against all conceptions of immunity that they did not long go unchallenged. A number of workers (**12, 38**) corroborated the transmutability findings but not the immunological ones. In fact KLOSE (**31, 32**), working with highly variable impure cultures, used toxin formation to distinguish his strains. Some, such as ASCHOFF (**1**), who worked with slightly impure cultures, remained on the fence in regard to the transformations. Most assertive in their contentions that anaerobic organisms are highly variable in their reactions and are transmutable were the school of KOLLE (**34–36**), especially SCHLOSSBERGER (**43**), who suggested that the anaerobes may represent a single species. These workers sent cultures to VON WASSERMANN (**49**), who declared them indistinguishable. He

refused to submit anaerobic cultures to such drastic treatment as transatlantic shipment because of their fragility (48), and yet an anaerobic organism taken in muscle from a whale by NIELSEN (40) in 1888 responded satisfactorily to routine anaerobic technique in this laboratory in 1918, killing its guinea pig promptly and resembling exactly its original description! In the veterinary field VAN HEELSBERGEN (24) recently aligned himself with the German workers in human pathology.

The alteration of scientific attitude brought about by the adherence to such a theory as this is most interesting. Things become so simple under such an explanation, many technical difficulties are eliminated, immunization of animals for therapeutic purposes is made easy, and the scientific world, from the point of view of these workers, is so much the better off. For, if anaerobes may be changed one into another, why bother about isolating them? They will not stay pure. Anaerobic cultures from Central Europe that have found their way to this country are seldom pure, and frequently do not contain the type of organism for which they were named. If they contain a pathogen, two or three types may be isolated from the cultures, and these types behave consistently and do not do the queer things they were supposed to do by their first students. Central European anaerobic studies are struggling in the dark, the days of NÄGELI and BILLROTH have returned, the land of KOCH (33), of GHON and SACHS (15), and of VON HIBLER (30) has shifted from the careful work of older days, GHON himself (14) is converted to the new theories, and but a few constructive workers with abundant material have come out openly to combat them. Chief among these is ZEISSLER (51), a pupil of and coworker with FRAENKEL (6–11), who has always maintained that gas gangrene is due to various distinguishable anaerobic organisms. PFEIFFER and BESSAU (41) and GAEHTGENS (13) clearly distinguish various types. Early in the period of the war ZACHERL (50) and KÖVES (37) gave good descriptions of pure cultures of the vibrion septique type of organism.

As is to be expected, museum cultures from Central Europe are more badly mixed than any others. A collection of ten strains of anaerobes from KRAL'S in Vienna apparently did not contain a

single species for which the cultures were named. Unfortunately the anaerobe strains of some of our own institutions are but little better.

It would be difficult for the systematist employed in the study of higher plants whose major characters are well understood, whose mutations are today being scientifically studied, whose formal structure of classification was laid down many years ago and has been systematically developed, to imagine the complexity of the problem confronting one desirous of bringing order into the chaos represented not only by this war literature, but also by thirty years of anaerobic literature written before it. The time is more than ripe for some organization to enable new students to set to work with some clearness and assurance, an organization with a synopsis or index to the enormous literature that they must consult. This should give them an idea of the multiplicity of the species they will encounter, and should consider the biological factors relating to morphology, chemical behavior, and mutation as they are understood today.

Several workers have stated that anaerobic bacilli do not mutate. This is their natural reaction in denying the existence of the type of mutation that was described by the workers with impure cultures. To state that a living strain does not mutate would be to claim that it lacks one of the best recognized attributes of living matter. Obviously, it is necessary to determine where the mutations of bacteria lie, and what range of possible change they cover, before one can tell what characters are stable enough to be used for systematic purposes. The enzymes of the anaerobic bacilli are among the most highly active chemical agents known. Some of the anaerobes will be found among the most active splitters of carbohydrates, others have almost unbelievable proteolytic powers. It is to be expected that mutations will frequently be encountered in highly specialized organisms of this sort, and that these mutations will be chemical in their nature. When a mutation occurs that enables the organism to render assimilable a substance that its parent was unable to utilize, the mutant is readily detected because of the larger colony that it produces. Likewise a bacillus that loses a metabolic power forms smaller colonies than its parents. Data

pertaining to such mutations may be recorded photographically (**28**), and the possibilities thus afforded for the study of bacterial mutations in certain groups, notably in that of the tetanus bacillus (**29**), are unlimited.

The Society of American Bacteriologists (**2**) proposes the use of the botanical rules adopted at the Vienna Congress (**44**) in 1908 for the purposes of bacterial classification. In many ways the scheme formulated during a century and a half by the botanists is excellent for the purpose, although in some ways we are not ready for it. It is composed of stems and twigs and branches. When we pick up a bacterial group, we do not know whether to call it a stem or a twig or a branch, for the leaves have mostly grown on trunks. The tendency has been to work downward, to call a superficially recognized group a species and subdivide it into types, and to number the types. Why not work upward, call the numbered types species, and have more room for classification?

Bacteriologists, trained in pathological laboratories, have perhaps laid too little emphasis on the necessity of observing the laws of heredity in making classifications. It seems as though an application of these laws, with the same scale of nomenclature used by the classifiers of higher plants, might well be applied to the systematic arrangement of bacteria. Thus a tetanus strain of a pure biotype may give rise to many biotypes, as shown by colony formation. These derivative types are all typical tetanus bacilli. They represent elementary species, and are too many to catalogue, being of interest only to the student of heredity. They are no more deserving of specific names than are the commonly observed small mutations of higher plants and animals, and if named would require a trinomial nomenclature. There are some definite protein substances, however, differing radically in various tetanus bacilli, that probably are not subject to active mutation and are demonstrable by an immune reaction, agglutination. Four groups of tetanus bacilli have thus been distinguished by TULLOCH (**45, 46**), and four groups of vibrion septique bacilli by ROBERTSON (**42**). In the colon-typhoid group this reaction has long been considered specific, why not in these? To be sure, the details of the reaction

in these groups require more study, and other and better ways may be found to divide them. The following general rules will probably be found convenient for classifying bacteria.

BIOTYPE.—Strains that differ from each other in characters that are readily subject to mutation, and that breed true, may be termed biotypes. The word subspecies has so long been used in the higher groups with a geographical connotation that it will not be well to use it for subdivisions of bacterial species. The term type may then well be left as an independent unit of our vocabularies for non-specific use.

SPECIES.—Strains that behave alike in those characters that within their genus have not been found to mutate readily, may be grouped as species. The occasional derivation of one species from another is no more to be considered impossible than it is in higher groups. Bacteriologists have too long considered the species conception in higher groups as one of fixed immutable orms, which it is not. The recombination effects noted by DE VRIES (47), in which he showed the independent origin of three well defined types (*Oenothera nanella*, *O. eliptica*, and *O. lata*) from two others of quite widely divergent character (*O. Lamarckiana* and *O. laevifolia*) apparently cannot occur among bacteria. These recombination effects allowed the sudden appearance of groups of mutations that had occurred previously. Among bacteria, however, because of the rapidity with which vast numbers may be bred and the energy with which selection acts, several characters may be changed nearly simultaneously, and similar effects to those noted by DE VRIES may occasionally be observed, namely, the appearance of a number of new characters within a short space of time. On analogy, it would be perfectly reasonable to describe the strains that result from various changes as separate species. It is quibbling to define the word "species" so closely that no elasticity should be allowed in its application. Our knowledge is too meager and the possibilities too great to restrict closely the meanings of taxonomic words. Changes that may be considered specific may be discovered or perhaps even brought about by treatment of bacteria that is more radical than anything tried with plants, and there is no reason why

a bacteriologist who finds a new type that has originated in his own hands should not dignify it by the name of species, if he can show that such a change affects several characters.

GENUS.—Organisms that show the same general reactions on ordinary media and have the same general morphological habit may be grouped in genera. Such a scheme will compel the classification of most of the old and well recognized anaerobic species as genera, although it will unite some, such as NOVY's bacillus and *B. oedematiens*, and vibrion septique and the whale septicaemia bacillus into genera. It gives to the words species and genus approximately the same rank, in relation to mutational possibilities, as they possess among the higher forms. A vibrion septique strain can no more mutate to a sporogenes habit than can a pine tree mutate to an oak, but it can mutate in small detailed characters that may be of interest, and there are vibrion septique strains that differ in more fixed characters and that should be given specific differentiation.

A detailed plan (**27**) following these lines has already been presented. It is intended as a preliminary classification. Twenty-seven anaerobic genera have been defined, many more will have to be admitted in time, especially in the proteolytic group, and when large collections have been studied many emendations will have to be made. An organization (**26**) of these genera has been prepared, employing chemical criteria only; morphological criteria are found entirely inadequate for purposes of classification in this group. It has been thought premature to group the genera into tribes. Two subfamilies include all the anaerobic rods described in the genera.

Subfamily I. Clostridioideae.— Clostridiaceae that on meat medium produce after twenty days' incubation under oil at 37° a reaction of P_H 7.0 or a more acid reaction, the culture having been boiled after incubation. Type genus *Rivoltillus*, the vibrion septique type as described by HELLER (**25**).

Subfamily II. Putrificoideae.—Clostridiaceae that on meat medium produce after twenty days' incubation at 37° under oil a reaction of P_H 7.1 or a more alkaline reaction, the culture having been boiled after incubation. Type genus *Metchnikovillus*, the sporogenes type as defined in the description of *Bacillus sporogenes*,

described by the Medical Research Committee (3) under the name of Metchnikoff's type A. These subfamilies are united into the following family:

Clostridiaceae.—Eubacteriales that are rodlike, not spiral, that will not grow within 7 mm. of the surface of a shaft of clear tissue-free agar medium contained in a tube 12 mm. or more in diameter, incubated in air, in which they are able to grow in the depths. They may or may not possess peritrichial flagella; they may or may not form endospores. Most members of the group are characterized by their energetic catalytic action on proteins or on carbohydrates or on both of these types of substances.

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CURRENT LITERATURE

NOTES FOR STUDENTS

Forest geography of New Jersey.—HARPER subdivides New Jersey into nine forest regions, most of which form approximately parallel belts traversing the state from northeast to southwest.[1] Each of these is discussed in turn and the characteristic species noted. The most abundant tree in the state is *Pinus rigida*, but the most widely distributed tree probably is *Quercus alba*, which occurs in all of the nine forest regions; *Acer rubrum* is not far behind *Quercus alba* in this respect. Evergreen species probably make up about 40 per cent of the forests of the state.—H. C. COWLES.

Random assortment in inheritance of distinguishable homologous chromosomes.—Miss CAROTHERS,[2] from her work on Orthoptera, has already reported the occurrence of homologous chromosomes which could be identified one from the other by a size difference. Furthermore, since the form of a given homologue is constant for the individual, she has been able to demonstrate, from a study of a number of individuals of the population, that these heteromorphic homologous chromosomes (3 pairs) have a random segregation in relation to each other and to the sex chromosome. In the present paper, she actually follows these chromosomes from parent to offspring, making a cytological examination of the parents after they have been allowed to reproduce, and later an examination of the resulting progeny. Size, shape, and point of attachment of spindle fibers all seem to be practically constant heritable characters, by which the author identifies the individual chromosomes, and shows that their recombination in the progeny is according to the laws of chance. In the author's material one can say in regard to the chromosomes which enter the gametes, just as certainly as of a pair of contrasting unit characters which segregate in the F_2 generation, that this one was contributed by the father and that one by the mother. This amounts to a direct demonstration of those assumptions as to the behavior of the chromosomes in inheritance which have been necessary to account for the workings of Mendel's law. It is hoped that eventually such structural variations of the chromosomes will be correlated with the resulting somatic characters of the individual.—M. C. COULTER.

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SULPHUR AS A FACTOR IN SOIL FERTILITY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 289

JOHN WOODARD

Introduction

Although sulphur was recognized as an essential element in plant nutrition as early as the middle of the nineteenth century, the use of sulphur and sulphur compounds as fertilizers has never become general. Analyses for sulphur in soils have generally been low, yet when compared with the sulphur in the ash of plants, the amount present in the soil seemed sufficient for all the needs of the crop. The use of gypsum as a fertilizer, however, was quite extensive for a time, following the discovery of its beneficial effect on plants. BROWNE (13) credits this discovery to a clergyman in Germany in 1768. From there it spread to France and Great Britain, and was brought to the United States by BENJAMIN FRANKLIN, who used it on his farm near Philadelphia. For a time gypsum was extensively used as a fertilizer both in Europe and the United States and gave remarkable results. GRIFFITHS (25) reports experiments by SCHUBERT in Germany, and CROCKER (15) refers to the experiments of Judge PETERS, JOHN BINNS, and EDMUND RUFFIN in the United States. All these men obtained remarkable results with gypsum on legumes.

The use of gypsum alone, however, soon failed to increase crop yields, and investigators seeking for an explanation came to the conclusion that the gypsum acts chemically on the phosphorus or potassium compounds in the soil and liberates either phosphorus or

potassium or both. This view is presented by GRIFFITHS (25), VOORHEES (72), and HOPKINS (32). BROWNE (13) and BRUCKNER (14) consider the beneficial effect of gypsum due, in part at least, to the nutrient effect of the sulphur; while VENDELMANS (70) and HILGARD (31) mention its beneficial effects, particularly on the legumes, without giving any explanation.

In most fertilizer experiments sulphur has been added, together with phosphorus, in acid phosphate or basic slag, or with the potassium in potassium sulphate or kainit. When beneficial results have been obtained, the investigators have invariably ignored the possible effects of the sulphur. This may lead to erroneous conclusions, as was pointed out by LIEBIG (37) in 1855. He said that the sulphur or the calcium in the acid phosphate, or both, might have had a beneficial effect on the turnips in the Rothamsted experiments, as well as the phosphorus.

HOPKINS, MOSIER, PETTIT, and READHIMER (33) found that kainit increased the yields of corn, wheat, and oats on the waste hill land of Johnson County, Illinois, when used with bonemeal, ground limestone, and crop residues, over similarly treated plots without kainit. On the plots receiving no kainit, as well as on those receiving the kainit, cowpeas were grown once every three years and turned under as part of the crop residues. STEWART (66) compared potassium chloride and potassium sulphate as fertilizers for apple orchards in Pennsylvania. He found no appreciable difference in the effect of these salts. SMITH (65) found a greater yield of oat straw for potassium sulphate than potassium chloride in pots containing Hagerstown silt loam.

BROOKS (8) compared the effects of potassium sulphate and potassium chloride on alfalfa in field experiments at the Massachusetts Agricultural Experiment Station. Both plots received 600 pounds of bonemeal per annum, and both received 2 tons per acre of hydrated lime before planting the alfalfa. Both Grimm alfalfa and common alfalfa were used. Potassium sulphate gave increased yields of 0.50 tons of Grimm alfalfa and 0.75 tons of common alfalfa over potassium chloride. In every case the alfalfa on the plots receiving potassium sulphate was a darker green than on the plots receiving potassium chloride. The same difference in color

was reported for the same treatment on other crops. BROOKS (9) also made a comparison of different phosphate fertilizers. He found that acid phosphate and dissolved boneblack, which contain sulphur, gave greater increases in crop yields than raw bonemeal and rock phosphate, which contain little or no sulphur. A more rapid early growth of both tops and roots and earlier maturity were observed on the plots receiving the dissolved boneblack and acid phosphate than on the plots receiving raw bonemeal and rock phosphate.

The use of flowers of sulphur as a fertilizer was observed to have an influence aside from its effect in destroying the fungi which cause plant diseases. MARES (50) noticed a much greater vigor in vines that had been sulphured than in those which had not. He found that the sulphur was oxidized to sulphuric acid in the soil, and he thought that the sulphuric acid acted on the insoluble compounds containing potassium and made the potassium soluble. DEMOLON (16) found that heating the soil prevented the oxidation, and so he concluded that oxidation was caused by micro-organisms. PFEIFFER and BLANCK (56) obtained no increased yields of oats for the use of flowers of sulphur in field experiments. FEILITZEN (21) in Europe, and SHERBAKOFF (64) in the United States both obtained increased yields of potatoes from the use of flowers of sulphur.

BOULLANGER and DUGARDIN (3) found flowers of sulphur increased ammonification but decreased nitrification. The harmful effect on the nitrifying bacteria was probably due to the acidity, as LINT (38) found that the oxidation of sulphur in the soil increased the acidity very much. FRED and HART (23) report an increase in ammonification from the use of gypsum in peptone solutions, and WARINGTON (73) obtained an increase in nitrification when gypsum was applied to solutions of urea. GREAVES, CARTER, and GOLDTHORPE (24) studied the influence of calcium sulphate on production of nitrates and found it caused a great increase in all concentrations used. The increase was very high for the higher concentrations of calcium sulphate.

BRIOUX and GUERBET (7) found that flowers of sulphur increased availability of calcium and potassium in both calcareous

and noncalcareous soils, but had no effect on phosphorus. LIPMAN and MCLEAN (42) found that composting rock phosphate with sulphur increased the solubility of phosphorus. MCLEAN (48) found an increase of solubility of phosphorus in the sulphur-rock phosphate compost when compost was inoculated. The presence of soluble phosphates and sulphates did not inhibit the action. LIPMAN, MCLEAN, and LINT (43) found a great increase in acidity in the sulphur-floats mixture. LIPMAN and JOFFE (41) found no increased availability in phosphorus when acidity was increased by the addition of sulphuric acid. ELLETT and HARRIS (20) found greater availability of phosphorus in a manure-soil-floats-sulphur compost than in a soil-floats-sulphur compost. AMES and RICHMOND (2) found no increased availability of phosphorus in a compost to which calcium carbonate had been added. Acid conditions are necessary for the solution of the phosphorus. BROWN and GWINN (10) found an increased solubility of phosphorus in soil treated with sulphur as well as in composts. BROWN and WARNER (12) found no increased solubility of phosphorus in a manure-floats compost, but a great increase when flowers of sulphur were added to the compost.

The use of gypsum as a preservative of the nitrogen in manure has been investigated by HEINRICH (30), VIVIEN (71), NOLTE (53), and by AMES and RICHMOND (1). All these investigators report a saving of nitrogen from the use of gypsum on the manure.

Investigations on the effect of flowers of sulphur on the availability of potassium in greensands were conducted by MCCALL and SMITH (45). They found an increase in the availability of potassium in composts of sulphur, greensands, and manure, but no increase in availability of potassium in composts of sulphur, greensands, and soil.

Reports of investigators who studied the influence of gypsum on the availability of potassium do not agree. MCCOOL and MILLAR (46) found calcium sulphate applied to soil lowered the freezing point of the soil. No report was given as to the character of the compounds that lowered the freezing point. BRADLEY (4) found an increase in solubility of potassium but not of phosphorus in Oregon soils. BRIGGS and BREZEALE (6) found a decrease in

solubility of potassium in California soils when gypsum was added, and the solubility of potassium decreased as the amount of gypsum used was increased. BREZEALE and BRIGGS (5) grew wheat in water cultures, using extracts from orthoclase minerals with and without gypsum. The gypsum did not increase the availability of the potassium to the wheat. MORSE and CURRY (52) treated feldspars with gypsum for ten weeks in water, filtered off the solution and analyzed for potassium. Only slightly more potassium was found than when no gypsum was used. MCMILLAR (49) treated five different soils with gypsum for three months and analyzed for soluble potassium. Gypsum was used at the rate of ten tons per acre and resulted in an increase in soluble potassium in every case. TRESSLER (69) found an increase in soluble potassium in some soils, but no increase in others when treated with gypsum. LIPMAN and GERICKE (39) obtained an increase of available potassium in greenhouse soil, a slight increase in adobe soil, and no increase in sand. FRAPS (22) grew plants in pots of soil treated with gypsum and analyzed the plants for potassium. He found no increase in potassium in plants grown on the gypsum-treated soil above that on the soil without gypsum. He reports no analyses of the soils used, however, so it is not known whether these soils were deficient in potassium or not. If the soil has sufficient potassium in an available form to supply all the plants' needs, there would not likely be any increased absorption even if the soil treatment dissolved some of the insoluble potassium compounds in the soil. On the other hand, in a soil deficient in potassium and sulphur, the application of gypsum or any other fertilizer containing sulphur would stimulate the growth of roots, and the increased size of the root system would make it possible for the plant to absorb more potassium. This increased absorption would take place regardless of any possible effects on the solubility of the potassium compounds in the soil.

The experiments of MCMILLAR (49), TRESSLER (69), and LIPMAN (39) indicate a greater solubility of potassium in some soils when treated with gypsum, but other soils show no effect, while BRIGGS and BREZEALE (6) report a decrease in solubility when gypsum was used. It seems, therefore, that the beneficial

effects of gypsum can hardly be ascribed to its effect on the solubility of the potassium in the soil. It seems more likely that the soils that respond to the use of gypsum are deficient in some element that is supplied by the gypsum.

Recent studies of methods for the analysis of organic material for sulphur have shown that all the sulphur is not recovered in the ash when organic material is burned. HART and PETERSON (27, 28) found one hundred times as much SO_3 in the rice grain as in the ash of that grain, and forty times as much in the corn grain. Similar results were obtained with other grains, but the ratios were less in some cases. Onions, potatoes, crucifers, and legumes use large quantities of sulphur. Alfalfa removes twice as much sulphur as phosphorus from the soil. PETERSON (55) studied the sulphur compounds in plants and found proteins, volatile compounds, mustard oils, and sulphates. In ashing the plant material the sulphates remain, but at best part of the sulphur in other compounds is lost. Most soils are low in sulphur, which is present in the soil in the form of sulphates and organic matter. Sulphates are all soluble, and, like nitrates, they are not adsorbed to any great extent, and therefore are quickly leached out of the soil in the humid regions. The organic sulphur is insoluble but is readily oxidized to sulphates, so that it is gradually being lost unless taken up by the plant. LYON and BIZZELL (44) in their lysimeter studies at Cornell found that the loss of sulphur in the drainage from uncropped lysimeters was as great as the loss in drainage and in the crops from cropped soil. The oxidation of organic sulphur to sulphates seemed to continue at the same rate in cropped and uncropped soil, and that not taken up by plants was lost in the drainage.

Cultivation stimulates oxidation and consequently the loss of sulphur. SWANSON and MILLER (68) report a loss of 38.53 per cent of sulphur from the surface and 41.56 per cent from the subsoil of Kansas soils due to cropping. The surface soil of virgin land had 0.044 per cent sulphur, while adjoining cropped land had 0.027 per cent. The sulphur content of the subsoil was 0.062 per cent in the virgin land and 0.036 per cent in the cropped land. On the other hand, phosphorus was practically the same in the

cropped as in the virgin land in both surface and subsoil. The cultivated soils had been cropped for thirty to forty years.

LYON and BIZZELL (**44**) found an increased loss of sulphur in the drainage when burnt lime was used, while MACINTIRE, WILLIS, and HOLDING (**47**) found the loss greater for calcium carbonate than for calcium oxide. It seems the carbonate favors bacterial action much more than the oxide.

ROBINSON (**59, 60**) analyzed a large number of soil samples from different parts of the United States for sulphur and phosphorus. Most of them were low, some extremely low, in both phosphorus and sulphur. Many of the samples were much lower in sulphur than phosphorus. BROWN and KELLOGG (**11**) analyzed samples of Iowa soils and found the sulphur content varied from 719 to 938 pounds per acre in the surface soil, while the phosphorus content varied from 1289 to 1538 pounds per acre. SHEDD (**62**) analyzed samples of Kentucky soils and found the sulphur content in the surface soil varied from 213 to 1080 pounds per acre in virgin soil, and from 180 to 560 pounds per acre in cultivated soils. The phosphorus content in the surface soil ranged from 320 to 5860 pounds in virgin soil, and from 320 to 7240 pounds in cultivated soil.

Some sulphur is brought down from the air in rain water. The amount is probably greater during periods of heavy rainfall than when the precipitation is slight. Near cities, where a large amount of coal is burned, the amount is probably much greater than in country districts far from cities and railroads. The data, however, are too meager to form any definite conclusions. HALL (**26**) reports sulphur analyses of rain water at Rothamsted from 1881 to 1887 which give an annual average of seven pounds of sulphur in the rain water per acre per year. Analyses by HART and PETERSON (**27**) at the University of Wisconsin for part of a year led them to the conclusion that the amount in one year would be approximately the same as found at Rothamsted. STEWART (**67**) analyzed rain water at the University of Illinois and obtained as a seven-year average 45.1 pounds of sulphur per annum. All of these analyses are of rain water collected near cities. The water in the rain gauges is likely to be contaminated by dust and soot and by the droppings of birds which roost on the rain gauges.

LAWES and GILBERT (36) found, in their fertilizer experiments with red clover, that "the produce was considerably increased by the application of gypsum, and still more so by that of the sulphates of potash, soda, and magnesia, and superphosphate of lime." In four years the increased yield from the use of gypsum was 3.5 tons of dry hay, or an average of 0.9 ton per acre per year.

HUNT (35), at the Pennsylvania Agricultural Experiment Station, used gypsum in a rotation of corn, oats, wheat, and hay (timothy and clover). Gypsum was applied at the rate of 320 pounds per acre per rotation in two applications, 160 pounds to the corn and 160 pounds to the wheat. No other fertilizers were used, and no increases in yields were obtained from the use of gypsum. These experiments would be more valuable if the gypsum had been applied to the clover and other fertilizers had been used to remove the possibility of another limiting factor.

MILLER (51) grew clover in pots containing Oregon soils. Applications of sulphur were made in the form of flowers of sulphur, sodium sulphate, and gypsum. Gypsum and sodium sulphate gave increased yields, but the flowers of sulphur had little effect.

SCHREINER (61) studied the effect of different salts on oxidation in soil extracts in which wheat seedlings were grown. He reports increased oxidation from the use of calcium sulphate, potassium sulphate, and sodium sulphate.

DYMOND, HUGHES, and JUPE (18) compared the effect of ammonium sulphate and ammonium chloride on cabbages grown on non-calcareous soil. Greater yields were obtained with the ammonium sulphate than with the ammonium chloride. In their experiments with clover they obtained a 20 per cent increase in hay from the use of gypsum. In pastures they observed that legumes predominated where sulphates were applied, and grasses where no sulphates were used. Gypsum increased the yields of red clover, maize, and vetch in sand cultures, and of vetch in soil cultures. All the pots received applications of calcium and magnesium carbonates.

LIPMAN and GERICKE (40) compared the effects of different nitrogenous fertilizers on barley grown on Oakley's vitro sand, and found the greatest increase with ammonium sulphate. When

sulphur containing substances were added to the non-sulphur containing nitrogenous fertilizers, they produced yields equal to those from ammonium sulphate.

SHEDD (63) grew soy beans, oats, alfalfa, and wheat in pots containing Kentucky soils. Eight different soils were used, and flowers of sulphur added at the rate of 100 and 200 pounds per acre. Both controls and sulphur treated pots received tricalcium phosphate, potassium nitrate, and calcium carbonate. There were some increases but also some decreases.

EATON (19) grew sweet corn in pots containing sand. He compared the effect of gypsum, flowers of sulphur, and sodium sulphate. The controls as well as the different sulphur treatments were watered with a nutrient solution which contained no sulphur. Gypsum increased the yield, while flowers of sulphur and sodium sulphate gave increases for the smaller applications and decreases for the larger applications.

DULEY (17) reported a darker green in sweet clover and corn when fertilized with gypsum or sulphur. More nodules were also produced on the roots.

PITZ (57) grew clover in agar-agar containing dipotassium phosphate with and without calcium sulphate. Greater length of roots was obtained with the calcium sulphate. Clover was also grown in Miami silt loam with and without calcium sulphate. The calcium sulphate increased the root length.

HART and TOTTINGHAM (29) found a decided increase in development of beans, red clover, and peas when fertilized with either calcium sulphate or sodium sulphate. In beans and peas the increase was in the seed, in clover it was in the hay and roots. Sulphates increased the yields of both tops and roots in radishes. The yield of rape tops was increased by both calcium and sodium sulphates. Barley was not affected by the sulphates, and oats to only a slight extent.

OLSON (54) conducted field experiments with alfalfa at the Washington Agricultural Experiment Station and obtained increased yields from the use of acid phosphate and gypsum, but not from other forms of phosphorus. Two hundred pounds of gypsum per acre increased the yields of alfalfa from 100 to 500 per cent.

REIMER and TARTAR (58) conducted field experiments on several Oregon soils. Superphosphate, flowers of sulphur, rock phosphate, potassium chloride, potassium sulphate, iron sulphate, gypsum, monocalcium phosphate, sodium nitrate, ammonium sulphate, magnesium sulphate, sodium sulphate, iron pyrites, quick lime, and ground limestone were used as fertilizers. In almost every case enormous increases in yields (from two to ten times as much as the checks) were obtained for all the fertilizers containing sulphur, and no increase or only a small increase for the fertilizers which contained no sulphur. Acid phosphate was compared with gypsum and rock phosphate and with rock phosphate and flowers of sulphur. The yield on the plot receiving rock phosphate and gypsum was considerably greater, and that from the plot receiving rock phosphate and flowers of sulphur slightly greater, than the yield from the acid phosphate treated plot. The alfalfa on all the plots receiving sulphur in any form was a darker green than on the plots which received no sulphur.

Chemical analyses of soil samples from these experimental fields were made. The sulphur content varied from 0.015 to 0.038 per cent in the surface soil, and from 0.014 to 0.030 per cent in the subsoil. The phosphorus content varied from 0.048 to 0.076 per cent in the surface, and from 0.066 to 0.085 per cent in the subsoil. All were high in calcium, magnesium, and potassium.

Investigation

The analyses made by ROBINSON (**59, 60**) show wide variation in the sulphur content of different soil types. His investigations, although extensive, have included only a part of the numerous soil types found in the United States, so that other soil types should be analyzed to discover their sulphur as well as their phosphorus content. It is also necessary to conduct field experiments on the different soils, as analytical data alone are not sufficient evidence on which to base fertilizer practice. This investigation includes soil analyses and field experiments. Soil samples from Indiana, Kentucky, Michigan, Ohio, and Wisconsin were analyzed for phosphorus, sulphur, and volatile matter (loss on ignition). Field

experiments were conducted in Indiana and Kentucky on the fields from which the soil samples were taken.

Soil analysis

Methods of sampling.—The soil samples from Michigan and Ohio (nos. 1–9) were taken by Dr. William Crocker and those from Wisconsin (nos. 10–11) by Mr. E. H. Hall. The samples were taken in the usual way by means of a soil auger. The samples from Indiana and Kentucky were taken when the soil was very wet, and as only the surface soil was sampled, it was believed that more accurate sampling could be done by using a spade or shovel. Some soil was removed to a depth of seven inches, leaving one side of the hole vertical, then a thin slice of soil was cut with the spade to the full depth of seven inches. A narrow strip of this extending from top to bottom was removed for the sample. Three or four such samples from different parts of the field were taken and mixed to form a composite sample. The samples from Indiana were taken by John Woodard, except no. 18, which was taken by Mr. V. G. Mann, and those from Kentucky by John Woodard, except nos. 32–34, which were taken by Mr. J. C. Gentry. All the soil samples were air dried, sifted through a 2 mm. sieve, and thoroughly mixed.

Analytical methods.—Phosphorus was determined according to the official magnesium nitrate method of the Official Agricultural Chemists. A blank determination was run to determine the possible presence of phosphorus in the chemicals, but no phosphorus was found.

Sulphur was determined by a modification of the methods of Shedd and of Brown and Kellogg. In preliminary work it was found that higher results were obtained when the iron and aluminum were removed. In soils low in sulphur the barium sulphate precipitated very slowly, so, at the suggestion of Dr. Frederick Koch,[1] 10 cc. of approximately N/10 H_2SO_4 was added immediately before heating the solution and adding the barium chloride. This sulphuric acid was measured in a burette, and exactly the

[1] Unpublished work of Dr. Frederick Koch.

same quantity of the same acid was added to the blank determination, so that subtracting the blank subtracted the sulphur added in the sulphuric acid as well as that present in the reagents. In every case the 10 cc. was measured between the 10 and 20 marks on the burette. According to KOCH, barium sulphate does not precipitate readily when the concentration of the SO_4 ion is low. The addition of the sulphuric acid is then necessary to bring the concentration of the SO_4 ion up to the point where precipitation takes place readily. The method as finally adopted is as follows: The equivalent of 10 gm. of oven dry soil was weighed into a nickel crucible, moistened with a few drops of distilled water, and part of a weighed 20 gm. of sodium peroxide stirred in a little at a time with a nickel rod. (If the moisture was just right, reaction took place immediately without the application of heat, and the charge was fairly dry by the time most of the sodium peroxide had been stirred in. If too little water had been added, it was necessary to heat with an alcohol lamp to start the reaction. If too much water was added, it was necessary to heat with the alcohol lamp to bring to the desired degree of dryness before adding the last of the sodium peroxide.) After the charge was fairly dry, the rest of the sodium peroxide was placed over the charge, the crucible covered, and heated over a bunsen burner, raising the temperature gradually to a fairly high temperature which was maintained for an hour. After cooling, the fused mass was removed with hot distilled water to a 600 cc. beaker, neutralized with concentrated HCl, and then 10 cc. additional concentrated HCl added. The beaker was then heated for five or six hours on the steam bath with occasional stirring. It was then transferred to a 500 cc. flask, covered, and made up to the mark. The solution was shaken frequently for several hours and the 250 cc. filtered off. The 250 cc. of filtrate was transferred to a 600 cc. beaker, heated on the steam bath, and the iron, aluminium, and silica precipitated with ammonium hydroxide, allowed to stand a few minutes, and then filtered into a one liter beaker. The precipitate was washed with hot distilled water until the combined filtrate and washings had a volume of approximately 600 cc. Exactly 10 cc. of approximately N/10 H_2SO_4 was then added, heated to boiling, and 10 cc.

of hot 10 per cent $BaCl_2$ solution added a drop at a time from a pipette. The solution was boiled for ten minutes, placed on the steam bath for two or three hours, and then removed and allowed to stand over night. The barium sulphate precipitate was then filtered off, washed with cold distilled water, transferred to a weighed porcelain crucible, ignited to a dull red in a muffle furnace, cooled in a desiccator, and weighed. Blanks were determined using the same reagents and adding the same quality of the same sulphuric acid that was used in the determination.

The loss on ignition was determined on samples which had been used for determining moisture. The moisture was determined by heating 10 gm. of air dry soil in the oven for five or six hours. Part of the samples were heated to 100° C. in an ordinary oven and part of them to 35° C. in a vacuum oven. After weighing for the moisture determination, the sample was placed in the muffle furnace, heated to a dull red for an hour, cooled in a desiccator, and weighed. The loss on ignition was calculated as percentage of oven dry soil. Table I gives the results of the analytical work on all the soils analyzed. Phosphorus, sulphur, and volatile matter (loss on ignition) are reported as percentage of oven dry soil.

Sulphur is present in the soil either in the form of sulphates of calcium, magnesium, and iron, or in the form of organic matter. All the sulphates are quite soluble and are not readily adsorbed, so that they are leached out rapidly and only small amounts are present in the soil. On the other hand, the organic sulphur is insoluble and remains in the soil until oxidized to sulphates. One would expect, therefore, some sort of relation between the sulphur content of the soil and the volatile matter (loss on ignition), which is a rough method of determining the organic matter. The data in table I, however, indicate only a general relation, and that only when samples from the same soil type or closely related soil types are compared. The soil samples from Wisconsin are from the same soil type, but differ in amount of organic matter. There is also a difference in content of sulphur, and the higher sulphur content is found in the sample with the higher content of organic matter. This is true for both surface soil and subsoil. The Michigan

TABLE I

Sample no.	Soil strata (inches)	Name of farm or farm owner	Location	Percentage of volatile matter	Percentage of sulphur	Percentage of phosphorus
1 A. . .	0–6	Wah-Bee-Mee-Mee farm	Michigan	2.076	0.0158	0.0360
1 B. . .	7–14	Wah-Bee-Mee-Mee farm	Michigan	2.341	0.0157	0.0330
1 C. . .	15–24	Wah-Bee-Mee-Mee farm	Michigan	2.662	0.0216	0.0305
2 A. . .	0–6	Wah-Bee-Mee-Mee farm	Michigan	4.988	0.0486	0.0518
2 B. . .	7–14	Wah-Bee-Mee-Mee farm	Michigan	4.481	0.0405	0.0561
3 A. . .	0–6	Wah-Bee-Mee-Mee farm	Michigan	2.863	0.0183	0.0390
3 B. . .	7–14	Wah-Bee-Mee-Mee farm	Michigan	2.522	0.0159	0.0324
4 A. . .	0–6	Wah-Bee-Mee-Mee farm	Michigan	4.862	0.0361	Not determined
4 B. . .	7–14	Wah-Bee-Mee-Mee farm	Michigan	3.754	0.0263	Not determined
5 A. . .	0–6	Wah-Bee-Mee-Mee farm	Michigan	4.311	0.0319	0.0514
5 B. . .	7–14	Wah-Bee-Mee-Mee farm	Michigan	3.822	0.0283	0.0468
5 C. . .	15–24	Wah-Bee-Mee-Mee farm	Michigan	3.462	0.0177	0.0305
6 A. . .	0–6	Everett's farm	Ohio	3.631	0.0232	0.0788
6 B. . .	7–14	Everett's farm	Ohio	2.466	0.0140	0.0411
7 A. . .	0–6	Arnold's farm	Ohio	4.642	0.0334	0.0771
7 B. . .	7–	Arnold's farm	Ohio	2.984	0.0195	0.0423
8 A. . .	0–6	Jacoby's farm	Ohio	5.228	0.0281	0.0582
8 B. . .	7–	Jacoby's farm	Ohio	3.148	0.0050	0.0326
9 A. . .	0–6	Jacoby's farm	Ohio	14.327	0.0905	0.0939
9 B. . .	7–	Jacoby's farm	Ohio	5.969	0.0194	0.0343
10 A. . .	0–6	Wager's farm	Wisconsin	8.116	0.0351	0.0744
10 B. . .	7–	Wager's farm	Wisconsin	6.954	0.0202	0.0649
11 A. . .	0–6	Wager's farm	Wisconsin	6.836	0.0245	0.0795
11 B. . .	7–	Wager's farm	Wisconsin	4.043	0.0124	0.0457
12.	0–6	Ross's farm	Indiana	5.758	0.0172	0.1054
13.	0–6	Carr's farm	Indiana	4.721	0.0165	0.0628
14.	0–6	Reich's farm	Indiana	4.075	0.0118	0.0490
15.	0–6	Bentley's farm (cropped soil)	Indiana	4.809	0.0155	0.0566
16.	0–6	Bentley's farm (virgin soil)	Indiana	5.249	0.0233	0.0564
17.	0–6	Barnett's farm	Indiana	4.462	0.0183	0.0492
18.	0–6	McCulloch's farm	Indiana	4.807	0.0155	0.0578
19.	0–6	Adina farm	Kentucky	7.024	0.0258	0.1897
20.	0–6	Adina farm	Kentucky	4.526	0.0232	0.0799
21.	0–6	Adina farm	Kentucky	7.496	0.0131	0.1636
22.	0–6	Adina farm	Kentucky	4.884	0.0122	0.1298
23.	0–6	Adina farm	Kentucky	4.318	0.0206	0.0768
24.	0–6	Marshall's farm	Kentucky	5.517	0.0264	0.1377
25.	0–6	Downing's farm	Kentucky	5.466	0.0159	0.0977
26.	0–6	Downing's farm	Kentucky	5.229	0.0236	0.1765
27.	0–6	Downing's farm	Kentucky	5.327	0.0153	0.1370
28.	0–6	Gentry and Curry's farm	Kentucky	6.021	0.0245	0.2355
29.	0–6	Scott's farm	Kentucky	5.088	0.0235	0.1500
30.	0–6	Sharp's farm	Kentucky	6.540	0.0161	0.1779
31.	0–6	Moore's farm	Kentucky	4.723	0.0253	0.1007
32.	0–6	Fowler's farm	Kentucky	5.051	0.0250	0.1727
33.	0–6	Watt's farm	Kentucky	5.836	0.0163	0.1306
34.	0–6	Tuomey's farm	Kentucky	11.105	0.0313	0.3407

soil samples are also quite similar in texture. Here again we find a high sulphur content with a high organic matter content, and a low sulphur content with a low organic matter content. When we compare different soil types or samples from the same type but from fields which have been cropped differently, however, there is little evidence of any relation. Samples 7 B and 9 B have approximately the same sulphur content, yet the volatile matter in the latter is twice that in the former. Both these samples are subsoils from Ohio, and were taken from fields that were not far apart, but 7 B is on upland silt loam while 9 B is a muck soil. Again, the cropped soil (no. 15) and the virgin soil (no. 16) from Bentley's farm, Indiana, differ only slightly in volatile matter, but differ widely in sulphur content. Gentry and Curry's soil (no. 28) has slightly less volatile matter than Sharp's soil (no. 30), but considerably more sulphur. Sample 10 A from Wager's farm in Wisconsin is a fine sandy loam soil with very little clay but a large amount of organic matter, as may be recognized by its black color, yet it contains considerably less sulphur than sample 2 A from the Wah-Bee-Mee-Mee farm in Michigan, which is also a sandy loam soil, containing considerable coarse sand with sufficient organic matter to give a black color.

It seems, then, that from the sulphur standpoint, as well as the nitrogen standpoint, the character of the organic matter is of more importance than the amount. Sulphur, like nitrogen, is mainly present in the proteins, so that a small amount of high protein organic matter, such as one would obtain by plowing under legumes, would be more valuable than a larger quantity of organic matter from wheat or oat straw or cornstalks. It seems probable also that the proteins are more readily decomposed than the non-protein organic matter, so that the sulphur and nitrogen would be oxidized more rapidly than the carbon, and the sulphur and nitrogen content might become quite low when there was still a considerable amount of carbonaceous organic matter in the soil.

In all the samples analyzed, the sulphur content was less than the phosphorus content. One of the samples from Ohio which was taken in a low wet place was a muck, very high in organic matter. This soil had nearly as much sulphur as phosphorus in

the surface soil (no. 9 A), but the subsoil (no. 9 B) had only a little more than half as much sulphur as phosphorus. The difference between the sulphur and phosphorus contents in one of the Michigan soils was not great. The surface soil (no. 2 A) contained 0.0486 per cent sulphur and 0.0518 per cent phosphorus, while the subsoil (no. 2 B) contained 0.0405 per cent sulphur and 0.0561 per cent phosphorus. All the other samples were much higher in phosphorus than in sulphur. The difference was very great in one of the Indiana soils, which had over six times as much phosphorus as sulphur, and in the Kentucky soils, in most of which the phosphorus content was from five to eleven times as much as the sulphur. In two of the Kentucky soils the phosphorus content was only three times as much as the sulphur, and in one only four times as much.

The Michigan soils, samples 1–5, were taken on the Wah-Bee-Mee-Mee farm at White Pigeon, Michigan. Samples 1 and 5 were sampled to three depths and all the others to two depths. These soils are alluvial sandy loams, varying from light brown to dark brown on the surface and grading into a yellow sandy subsoil containing some gravel. The light colored samples contained more sand in both surface and subsoil and were lower in volatile matter, sulphur, and phosphorus, than the darker colored ones. All were low in both sulphur and phosphorus, but the sulphur is lower than phosphorus in all the samples. With the exception of sample 1, the sulphur was always lower in the subsoil than in the surface soil.

The Ohio soils, samples 6–9, were taken near Copley, Ohio. Nos. 6, 7, and 8 are upland silt loams containing some sand. The surface soil is a yellow brown grading into a uniformly light yellow subsoil, which indicates good underdrainage as well as good surface drainage. These soils apparently belong to the type mapped as the Wooster silt loam. The sulphur content was low in both surface and subsoil, while the phosphorus content was fairly good in the surface but low in the subsoil. In every sample the subsoil was lower in volatile matter, sulphur, and phosphorus than the corresponding surface soil.

Sample 9 is poorly drained, and the surface soil has a large amount of organic matter with some silt, sand, and a little clay.

The subsoil has much less organic matter, but the proportion of its other constituents is about the same as in the surface. The surface soil is very high in volatile matter, sulphur, and phosphorus, while the subsoil is very low in both sulphur and phosphorus.

The Wisconsin soils, samples 10 and 11, are from near Beloit, Wisconsin. They are fine sandy loams, dark brown on the surface and a lighter brown in the subsoil. In both samples the volatile matter, sulphur, and phosphorus are higher in the surface soil than in the subsoil. The sulphur content is low in both surface soil and subsoil in both samples, but the phosphorus is good in the surface soil of both samples, fair in the subsoil of sample 10, and poor in the subsoil of sample 11. Both sulphur and phosphorus are lower in the subsoil than the surface soil in both samples.

The Indiana soil samples (nos. 12–18) were taken near Charlestown, Clark County, Indiana. This region is underlain by limestone rock, but the rock has been covered by a thick layer of windblown material, from which most of the soils were formed. All the soils sampled were formed from this windblown material except no. 12, which was taken on the bluff of a small stream where there was considerable erosion. It seems that the erosion has removed the greater part of the windblown material, and to a large extent the soil is formed from the underlying limestone. This is probably the reason why this sample resembles in general appearance and in chemical composition the Kentucky soils rather than the adjacent soils from the windblown material or loess. Sample 12 has a light brown silt loam surface soil grading into a reddish yellow subsoil. Like the other Indiana soils, the volatile matter and sulphur are low, but the phosphorus is high like most of the Kentucky soils.

The loessal soils include two types, the one with good natural underdrainage and the other with poor drainage. The former, which includes samples 15–18, is a yellow gray silt loam in the surface soil and a yellow silt loam in the subsoil. The latter, which includes samples 13 and 14, has a gray or slightly yellowish gray silt loam surface soil underlain by a gray or gray and yellow mottled silt loam subsoil. Both are poorly drained, but sample 13 is more nearly level and has more gray color in both surface and subsoil. All the samples from both types are low in volatile matter, sulphur,

and phosphorus. Samples 15 and 16 were taken a few rods apart, the former from a field which had been in alfalfa for several years, and the latter from virgin land. Both have practically the same phosphorus content, but the sulphur is much higher in the virgin soil.

All the soil samples from Kentucky (nos. 19–34) are residual limestone soils, but no. 34 was derived from the Trenton limestone, which is high in phosphorus, while the others are all from the Cincinnati limestone, but no. 28 was taken from soil derived from Cincinnati limestone, but it was only a short distance from the division line between the Cincinnati and Trenton formations, and had probably received some material from the Trenton formation. Samples 19–27 are from Mason County, while samples 28–34 are from Mercer County. Samples 19 and 21 are clay loams, while 20 and 22–27 are silt loams. All are light brown to grayish brown in color. Sample 34 is a heavy clay loam, sample 28 is a heavy silt loam or light clay loam, while samples 29–33 are silt loams. Samples 31 and 33 are quite gray in color, and 33 contains iron concretions. No. 31 is known locally as white oak land, and both are recognized as poor soils. All the other samples are light brown except no. 34, which is a grayish brown. All the Kentucky soils are low in volatile matter except the clay loams, in which part of the volatile matter is probably water of combination. All are low in sulphur, no. 34 being the only one above 0.03 per cent. This sample is from the Trenton formation and contains many unweathered fragments of limestone. It is possible that the sulphur content as well as the phosphorus content of the Trenton limestone may be higher than in other formations. No. 34 contains 0.3407 per cent of phosphorus, which is eleven times as great as the sulphur content. This is much higher than any of the others, but all the others are high in phosphorus.

RELATION BETWEEN AMOUNTS OF SULPHUR AND PHOSPHORUS REMOVED BY CROPS AND SULPHUR AND PHOSPHORUS CONTENTS OF SOILS.—A better idea of the supply of sulphur and phosphorus in the soil can be obtained if the pounds per acre of these elements found in the surface soil is compared with the amounts removed by some of our common crops. Table II gives the amounts of sulphur

and phosphorus removed by some of the common crops. The yields per acre and the amounts of phosphorus removed by these yields are taken from HOPKINS and PETTIT's (34) table, while the amounts of sulphur removed are computed from HART and PETERSON's analyses.

As pointed out by HOPKINS and PETTIT (34), these yields are exceptionally large, but they have been obtained by some farmers, and others may obtain them under proper systems of farming. If, however, smaller yields are removed, it will not prevent soil depletion, but will only delay soil exhaustion if the elements removed

TABLE II

POUNDS PER ACRE REMOVED BY FARM CROPS

CROP	YIELD PER ACRE	POUNDS PER ACRE REMOVED ANNUALLY	
		Sulphur	Phosphorus
Corn, grain	100 bushels	7.8	17.0
Oats, grain	100 bushels	5.8	11.0
Wheat, grain	50 bushels	5.1	12.0
Timothy, hay	3 tons	11.4	9.0
Clover, hay	4 tons	13.0	20.0
Alfalfa, hay	8 tons	46.0	36.0
Potatoes	300 bushels	24.7	13.0

are not returned in some form. In actual practice, failure to return to the soil the elements of plant food which are removed in the crops will result in a gradual decrease in yields, so that the amounts of plant food removed will gradually become less. It is impossible to determine the time when complete exhaustion will take place, but a comparison of the amounts of plant food removed by large crops with the amounts present in the soil will emphasize the importance of renewing the supply in the soil before the soil supply is reduced below that necessary for satisfactory crop yields. Table III gives the pounds per acre of sulphur and phosphorus in the surface soils analyzed and the number of years' supply of each for several common farm crops, if maximum crops are removed, such as are given in table II.

Table III shows that all the soils are too low in sulphur to grow alfalfa for 40 years, while 22 of them have phosphorus enough to

grow alfalfa 40 years or longer, provided, of course, none of these elements is added in any way and none removed except in the crops. Sample 9A, which has the highest sulphur content, has sulphur

TABLE III

POUNDS PER ACRE OF SULPHUR AND PHOSPHORUS AND NUMBER OF YEARS' SUPPLY FOR VARIOUS CROPS IF MAXIMUM CROPS ARE REMOVED

SOIL NO.	SULPHUR						PHOSPHORUS					
	Pounds per acre in soil	No. of years' supply for					Pounds per acre in soil	No. of years' supply for				
		Corn	Wheat	Timothy	Clover	Alfalfa		Corn	Wheat	Timothy	Clover	Alfalfa
1 A.....	316	40	62	28	24	7	720	42	60	80	36	20
2 A.....	972	125	191	85	75	21	1036	61	86	115	52	29
3 A.....	366	47	72	30	28	8	780	46	63	83	39	22
4 A.....	722	93	142	63	56	16	.....	.....	.....	.....	.....	.....
5 A.....	638	82	125	56	49	14	1028	60	86	114	51	29
6 A.....	464	60	91	41	36	10	1576	93	131	175	79	44
7 A.....	668	86	131	60	51	14	1542	91	129	171	77	43
8 A.....	562	72	110	50	43	12	1164	68	97	129	58	32
9 A.....	1810	232	355	159	139	39	1878	110	156	209	94	52
10 A.....	702	90	138	62	54	15	1488	88	124	165	74	41
11 A.....	490	63	96	43	38	11	1590	94	133	177	80	44
12.....	344	44	67	30	26	7	2108	124	176	234	105	59
13.....	330	42	65	29	25	7	1256	74	105	139	63	35
14.....	236	30	46	21	18	5	980	58	82	109	49	27
15.....	310	40	61	28	24	7	1132	67	94	126	57	31
16.....	466	60	91	41	36	10	1128	66	94	125	56	31
17.....	366	47	72	32	28	8	984	58	82	109	49	27
18.....	310	40	61	28	24	7	1156	68	96	128	58	32
19.....	516	66	101	45	40	11	3794	223	316	422	190	105
20.....	464	60	91	41	36	10	1598	94	133	177	80	44
21.....	262	34	51	23	20	6	3272	192	273	364	164	91
22.....	244	31	48	22	19	5	2596	153	216	288	130	72
23.....	412	53	88	36	32	9	1536	90	128	171	77	43
24.....	528	68	104	46	41	11	2754	162	230	306	138	77
25.....	318	41	64	28	24	7	1954	115	163	216	98	54
26.....	472	61	93	41	36	10	3530	208	294	392	177	98
27.....	306	39	50	27	24	7	2740	161	228	304	137	76
28.....	490	63	96	43	38	11	4710	277	393	523	236	131
29.....	470	60	92	41	36	10	3000	176	250	333	150	83
30.....	322	41	63	28	25	7	3558	209	296	395	178	99
31.....	506	65	99	44	39	11	2014	118	168	224	101	56
32.....	500	64	98	44	38	11	3454	203	288	384	173	96
33.....	326	42	64	29	25	7	2612	154	218	290	131	73
34.....	626	80	122	55	48	14	6814	401	568	757	341	189

enough for 39 years of alfalfa and phosphorus enough for 52 years of alfalfa. Only one other soil, no. 2 A, had enough sulphur for 20 years of alfalfa, while three soils, nos. 19, 28, and 34, have enough

phosphorus for 100 or more years of alfalfa. No. 34 has phosphorus enough to grow alfalfa 189 years, but sulphur enough for only 14 years. The phosphorus content of no. 28 is sufficient to grow alfalfa for 131 years, but the same crop would deplete the sulphur in 11 years. All these soils have sufficient phosphorus to grow maximum yields of alfalfa for 20 years or longer, while all but two would be depleted of sulphur in less than 20 years.

Of the other crops mentioned, corn, wheat, and clover remove smaller amounts of sulphur than phosphorus; while timothy, like alfalfa, removes more sulphur than phosphorus. Timothy, however, removes only about one-fourth as much sulphur, and one-fourth as much phosphorus as alfalfa, so that the supply of each would last correspondingly longer, yet soil 9A is the only one that carries sufficient sulphur for 100 crops of timothy. Soil 9A has sulphur enough to grow timothy 159 years, clover 139 years, corn 232 years, and wheat 355 years. No. 34 has phosphorus enough for 401 corn crops, 568 wheat crops, and 341 clover crops; yet the sulphur would be depleted by 80 corn crops, 122 wheat crops, or 48 clover crops. The lowest phosphorus content is in soil 1A, a sandy loam soil, which has 720 pounds of phosphorus in the surface 7 inches of soil. The phosphorus in this soil would be depleted by growing corn 42 years, wheat 60 years, timothy 80 years, clover 36 years, or alfalfa 20 years. In the same soil the sulphur would be removed by 40 years of corn, 62 of wheat, 28 of timothy, 24 of clover, or 7 of alfalfa.

Table III shows the importance of both sulphur and phosphorus if maximum crops of legumes, particularly alfalfa, are to be grown. It also shows that, in most soils, sulphur is more likely to be deficient than phosphorus. It does not take into account the leaching of these elements from the soil, which is practically nil in the case of phosphorus and very high in the case of sulphur; nor the supply in the rain water, which is nil in the case of phosphorus and may be quite high in the case of sulphur near cities in the humid regions. Whether the amount of sulphur lost in the drainage water exceeds that gained in the rain water is still unknown. It is certain that the amount of leaching will vary with the character of the soil, the rainfall, and the character of the plant growth. The amount of

sulphur in the rain water will vary with the rainfall and the nearness to cities where large amounts of soft coal are used. It is possible that, in some places under certain conditions, the amount of sulphur brought down in the rain water will equal or exceed that lost in the drainage, but that in other places and under other conditions the loss will exceed the gain. Field experiments are needed to see whether the plants will respond to sulphur fertilization under field conditions. Remarkable responses were obtained by JUDGE PETERS, JOHN BINNS, and EDMUND RUFFIN in the Eastern United States (CROCKER, 15), and have recently been obtained on the Pacific Coast by REIMER and TARTAR (58) in Oregon, and by OLSON (54) in Washington. To secure further information along this line, cooperative experiments were conducted on some farms in Indiana and Kentucky from which some of the samples reported in table I were taken.

COOPERATIVE FIELD EXPERIMENTS WITH GYPSUM

The field experiments were conducted in cooperation with the farm owners. The farm owners were to apply gypsum and report on the effect on yields, if any. Some of the farmers failed to make any report, and those who did gave no weights, so that the results are not as satisfactory as could be desired. Results reported are as follows.

In the Indiana experiments, gypsum was applied to alfalfa, red clover, and tobacco. The only report received was with regard to the tobacco. This tobacco field was on the farm of Mr. Ross, southwest of Charlestown, Indiana. This is the field from which sample 12 was taken, and, as shown in tables I and III, is low in sulphur and high in phosphorus. Mr. Ross reports a marked increase in yield of tobacco from the use of gypsum on this field, but gives no quantitative data.

Gypsum was applied to alfalfa, red clover, sweet clover, and tobacco in Mason County, Kentucky. The crops were injured so badly by weather conditions, however, that no results were obtained.

In Mercer County, Kentucky, gypsum was applied to tobacco, clover, and alfalfa. Of the farmers responding, Mr. SHARP reported no increase in tobacco, while Mr. FOWLER reported an increase in

the second clover crop, and Mr. TUOMEY an increase in alfalfa. Neither of these men weighed the hay, so the results are not quantitative. Mr. SHARP's field, from which sample 30 was taken, is low in sulphur and high in phosphorus, but it showed evidences of being farmed hard, and was evidently low in nitrogen, which was probably the limiting element for a non-leguminous crop like tobacco. Mr. FOWLER's soil, no. 32, has 0.0250 per cent sulphur and 0.1727 per cent phosphorus, equivalent to 500 pounds of sulphur, and 3454 pounds of phosphorus, in the surface soil; so sulphur was probably the limiting element for clover. Mr. TUOMEY's field, sample 34, had 6814 pounds of phosphorus, the highest of the samples analyzed. This sample also contained small fragments of limestone, so that there was an abundance of lime. On the other hand, the sulphur content, 626 pounds, although higher than in many samples, is probably rather low for a plant like alfalfa, which uses such large quantities of sulphur.

These results are not conclusive, but it seems probable that sulphur may be a limiting element on some of these soils, and that gypsum is a satisfactory source of supply for this element. More field experiments are necessary in the humid part of the United States, and great care in conducting these experiments is necessary if satisfactory results are to be obtained. Experiments should be conducted through several years to avoid weather conditions, which may be the limiting factor in some years. On some soils drainage is necessary, and no fertilizer treatment will have any effect until this is done. Most soils in the humid part of the United States are acid. A large part of them are so acid that liming is necessary before any other treatment is effective, especially for leguminous crops. Table I shows a high phosphorus content in some of the soils reported in this paper, but those are exceptional soils. As a general rule soils are deficient in phosphorus, and farmers report increases in crop yields for the use of acid phosphate. It is impossible, however, to tell how much of the increase is due to the phosphorus and how much to the sulphur in the acid phosphate. A comparison of acid phosphate with rock phosphate and gypsum, and with gypsum alone, and rock phosphate alone would give some valuable results.

Many of the Illinois experiment fields include three check plots in each series. These check plots are all untreated and are only a short distance apart, yet some of them differ widely in crop yields. It is reasonable to assume that neighboring plots receiving the same fertilizer treatment would differ as widely. These differences due to factors not under the control of the investigators make the probable error large, and when only one plot of each treatment is used, the differences between plots with different treatments must be great before one can assume that the treatment has been effective. Where the differences are as great as in the work of REIMER and TARTAR (58) and of OLSON (54), there is no doubt that the treatment has been effective, but in many of the field experiments in different parts of the country the differences are too small to justify the conclusions drawn from them, as the probable error is so great. Where a number of plots of each treatment are used, the uncontrollable factors tend to neutralize each other and the probable error is reduced. As the number of plots of each treatment increases, smaller average differences are necessary to be significant. It seems probable that three plots of each treatment are necessary if satisfactory results are to be obtained. In the past investigators have had a tendency to scatter field experiments over a number of widely separated fields on the same soil type. It seems probable that more satisfactory results would be obtained if the work were confined to one field on each soil type, and each field had from three to five plots of each treatment.

Summary

1. Composite soil samples from Indiana, Kentucky, Michigan, Ohio, and Wisconsin were analyzed for total sulphur, total phosphorus, and volatile matter (loss on ignition), and cooperative fertilizer experiments with gypsum were conducted in fields in Indiana and Kentucky.

2. The analytical data show a general relation between the sulphur content and loss on ignition in soil samples from the same soil type or closely related soil types, but the relation is not apparent when different soil types are compared.

3. The sulphur contents in the surface soil vary from 0.0118 to 0.0905 per cent, while the phosphorus contents vary from 0.0360 to 0.3407 per cent. All the upland soils and most of the alluvial soils are low in sulphur. Most of the Kentucky soils and one of the Indiana soils are high in phosphorus. This is undoubtedly due to the influence of the rock from which the soils were formed, as all the Kentucky samples were from soils derived either from the Trenton limestone or the Cincinnati limestone, both of which are high in phosphorus content.

4. The sulphur and phosphorus contents were calculated to pounds per acre in the surface soil, and compared with the amounts of sulphur and phosphorus removed by maximum crops of corn, wheat, timothy, clover, and alfalfa. The highest sulphur content is sufficient for only 39 years of alfalfa, 139 of clover, 159 of timothy, 355 of wheat, or 232 of corn; while the lowest sulphur content is sufficient for only 5 years of alfalfa, 18 of clover, 21 of timothy, 46 of wheat, or 30 of corn. The lowest phosphorus content is equal to the amount removed by 42 years of corn, 60 of wheat, 80 of timothy, 36 of clover, or 20 of alfalfa. On the other hand, it would take 401 years of corn, 568 of wheat, 757 of timothy, 341 of clover, or 189 of alfalfa to remove as much phosphorus as is found in the soil with the highest phosphorus content.

5. On some of the soils tobacco, clover, and alfalfa have been benefited by the use of gypsum. The results, however, are not quantitative. More field experiments are needed and greater care should be taken to eliminate other factors as far as possible. Each treatment should be replicated to reduce the probable error.

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CYCLIC MANIFESTATION OF STERILITY IN BRASSICA PEKINENSIS AND B. CHINENSIS

A. B. STOUT

(WITH SEVEN FIGURES)

The transition from asexual or vegetative growth to the condition of flower and fruit production in hermaphrodite plants is to be recognized as a most fundamental aspect of sexuality. Furthermore, the inter-relations that exist between vegetative and reproductive vigor and the influence of the former on the latter are reflected and exhibited in certain phenomena of sterility.

It is now certain that vegetative vigor and the internal inter-relations incident to it may limit reproductive vigor and sexuality. The limitation from these causes may take place in two ways: (1) they may interfere with or influence the morphological development of flowers or other reproductive organs, and (2) they may affect the functioning powers of organs that are fully formed. If these influences are marked, one or more types of sterility may appear.

Only recently observations have indicated that, at least in some cases, the compatibilities and the fertility of the sex organs may vary rather definitely within the cycle of vegetative and reproductive development characteristic of the particular species. A phenomenon of this sort is reported by EAST and PARK (4), who found that in the few plants which are self-compatible in certain species and hybrids of *Nicotiana*, the self-compatibility develops only at the end of the flowering period. Cases of cross-compatibility appearing only at the end of the period of bloom are reported also. A very decided case of the development of self-compatibility only at the close of the period of bloom was observed by the writer, in a plant of *Lythrum Salicaria*, and reported at the annual meeting of the Botanical Society of America for 1917. These observations suggested that new evidence on the old problem of the relation between vegetative vigor and reproductive vigor, as expressed in the formation of flowers and the functioning of the

parts in seed formation, may be obtained by experimental means from a study of the fluctuations in fertility that are to be seen in those feebly self-compatible individuals which are to be found in species in which general sexual incompatibilities are strongly developed.

A subsequent report of a more detailed study on this problem (STOUT 11), however, showed that in *Verbascum phoeniceum*, *Eschscholtzia californica*, and *Cichorium Intybus* the various grades of self-compatibility operate very uniformly throughout the entire period of bloom, and that there are in the feebly self-compatible plants of these species no specially marked tendencies to self-compatibility at any definite phase of the blooming period. It was also found that in *Nicotiana Forgetiana* Hyb. Hort. and in *Lythrum Salicaria* end-bloom self-compatibility develops as an infrequent individual variation rather than as a phenomenon characteristic of the self-compatible plants. In these species there is no cyclic production of fruits and seeds which would indicate a general relation between vegetative vigor and the development of self-compatibility.

Such a cyclic occurrence of self-compatibility was found, however, and reported for *Brassica pekinensis*, and it was noted that the highest degree of self-compatibility attained by any given plant appeared very uniformly during the period of mid-bloom. Further studies with this species have since been made which show this to be the rule for all those individuals that are self-compatible in any degree. Similar behavior has also been found in cultures of *Brassica chinensis* and in hybrids between this species and *B. pekinensis*. So far as known to the writer, this is the most uniform and definite case of a general and definite periodicity in the modification of sexual compatibilities within a blooming period. In these species, also, flower abortion appears in the transition of vegetative to reproductive vigor, exhibiting an influence of vegetative vigor on the morphological development of flowers.

Material and methods

Several strains of the "head" sorts of *Brassica pekinensis*, commonly known as Chinese cabbage or Pe-tsai, and one strain of the loose-leaved or headless sort (the Nanking variety) were grown

from seeds furnished by the Office of Foreign Seed and Plant Introduction of the United States Department of Agriculture. The seeds of the strain of *B. chinensis* which have been grown were obtained from China by a Chinese student at Columbia University for the gardener in charge of the greenhouses belonging to the University, and the writer obtained seeds from the first lot of plants there grown. From controlled cross-pollinations between plants of the two species, seeds were obtained and plants of an F_1 hybrid progeny were grown.

The greater number of plants have been grown in pots in a greenhouse and brought into bloom during the winter and spring before they could suffer from the heat of summer. When thus grown, plants of the head varieties of *B. pekinensis* form a rather loose headlike rosette, much smaller and less compact than when grown under field culture, after which they shoot up into flower. Plants of the Nanking variety of *B. pekinensis* and plants of *B. chinensis* do not form a head even under the best of field culture; a very loose rosette of leaves develops, and this grades up into the leaves of the flowering stem. Pot grown plants of these species closely resembled field grown plants except that they were smaller.

A few plants of all strains have been grown to full maturity in the garden, both as spring and autumn crops. Such plants were larger than the pot grown plants and more flowers were produced, but their behavior in respect to fertility and sterility was identical with that of plants grown in the greenhouse. Special effort was taken to make controlled self-pollinations throughout the entire period of blooming.[1] Numerous plants have bloomed alone or in isolation from other species of *Brassica*, both in the greenhouses and in the field, and hand pollinations were made from one to four days apart as long as the plants bloomed. The plants not grown in isolation were "bagged." Flowering branches were inclosed in glassine paper bags on or within a day or two following the opening of the first flowers; the plants were visited at least twice a week (at first

[1] During the winter season of 1919–1920 the writer was greatly aided by the voluntary assistance of Mrs. MORTIMER J. FOX. Miss HESTER M. RUSK has assisted in the research and made many of the pollinations of the plants grown in 1920–1921. Through this efficient assistance and cooperation the many pollinations necessary to the research were accomplished.

many were visited daily), and pollen from fully dehiscent stamens in liberal amounts was placed on pistils of all freshly opened flowers. After the cyclic modification of self-compatibility was recognized, frequent cross-pollinations were made to test the functional power of pistils and stamens during the periods of self-incompatibility preceding and following the period of mid-bloom. The potency of the pollen has been studied by germination tests, and a cytological study of the phenomena of pollen tube growth and fertilization is under way.

Sterility in Brassica pekinensis and B. chinensis

Three distinct and quite different types of sterility are in evidence during the period of bloom in both these species.

I. One type is to be classed in general with impotence (STOUT 12), but here two very distinct types of impotence may be observed. These may be described as (1) flower abortion of the first flowers, and (2) arrested development of the last flowers that start to form.

II. In some plants of both species, axial proliferations develop from the pistils of many flowers, and the pistils of such flowers are functionless in respect to fruit production.

III. Among the flowers that open fully and are capable of functioning in certain relations, various grades of incompatibilities are in evidence, and self-compatibility whenever present is most strong during the period of mid-bloom.

I. IMPOTENCE

(1) FLOWER ABORTION.—Frequently in *Brassica pekinensis* the first flowers on the main stalk and often also the first flowers on laterals are completely aborted. The flower buds remain small and do not open, but soon become dead and black. A rather characteristic case of flower abortion is shown in fig. 1, which is of a pot grown plant of *B. pekinensis* blooming in February. Nearly forty of the first flowers of the main branch aborted, after which the flowers were completely formed, normal in appearance, and fully capable of functioning in certain fertilizations. The uppermost lateral branch coming into bloom later than the main axis had only five flowers that aborted, and the next lower lateral had no aborted

flowers. On such plants the lateral branches which come into bloom at the time when the main branch is producing normal flowers as a rule have normal flowers from the first (figs. 1–3). This coordination between flowers opening simultaneously on different branches as to kind of development is very marked. At first view, this abortion of flowers appears to resemble the blasting of flowers which frequently occurs in all sorts of plants as the direct effect of unfavorable environmental influences, but here the phenomenon is due primarily to internal conditions. As grown in the various cultures, the plants came into bloom at various times, some were producing mid-bloom and potent flowers, while other plants by them and just coming into bloom showed flower abortion. The abortion, therefore, is essentially self-induced.

FIG. 1.—Typical case of flower abortion in plant of *Brassica pekinensis;* about forty of first flowers on main axis aborted; there are five such flowers on uppermost lateral and none on second lateral, showing correlation in morphological character according to time of blooming.

Flower abortion of the first flowers is the rule among plants of the varieties of *B. pekinensis* which form leafy heads and which are grown in the field under conditions which favor the development of heads. In such plants, if left to bloom, the flowering branches are at first inclosed within the head. At the time when the flowering branches first come to the light,

they are somewhat blanched and tender, and the first flowers are already aborted. This condition of itself suggests that, in this

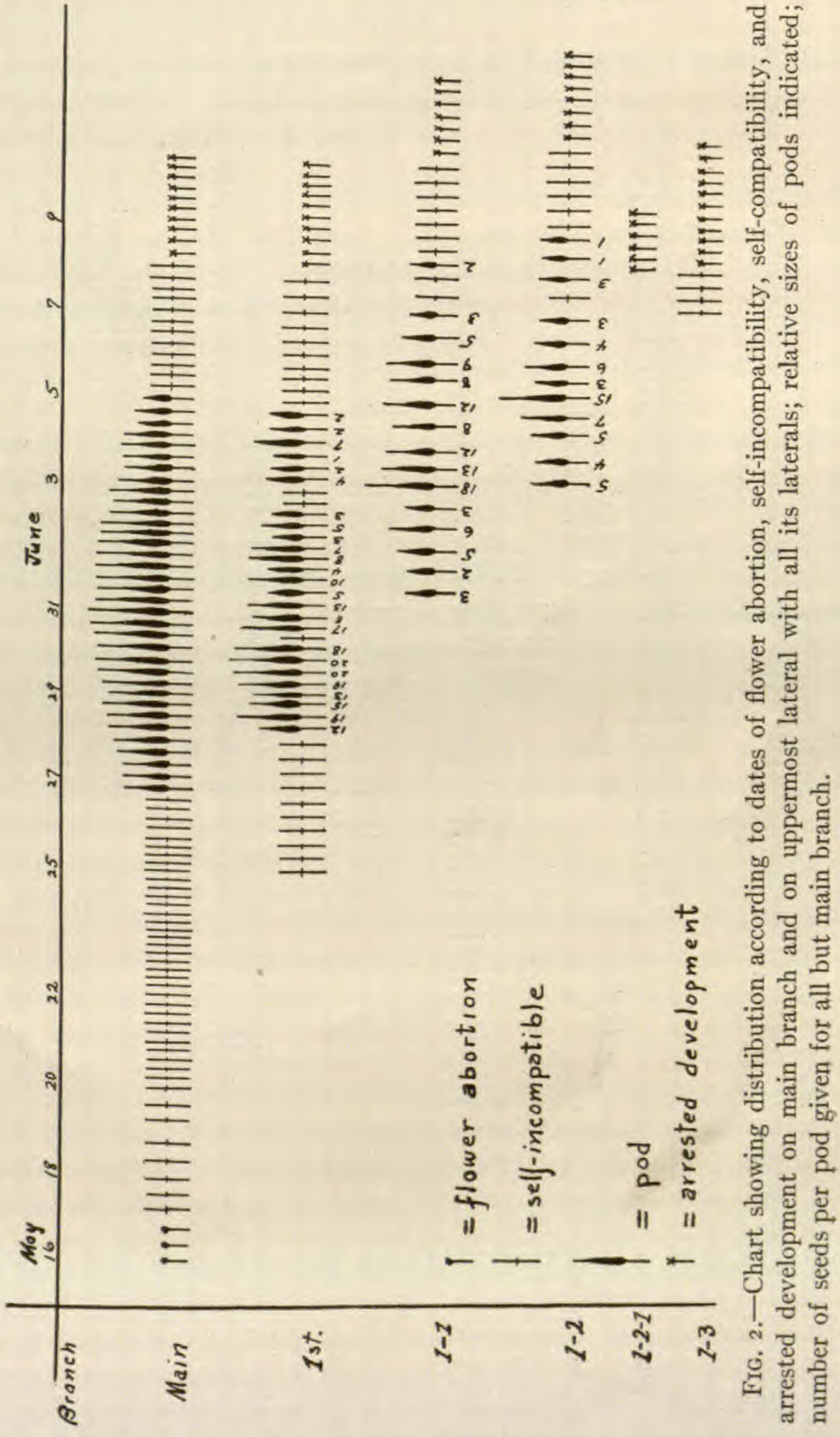

FIG. 2.—Chart showing distribution according to dates of flower abortion, self-incompatibility, and arrested development on main branch and on uppermost lateral with all its laterals; relative sizes of pods indicated; number of seeds per pod given for all but main branch.

case, temperature and light conditions are important factors which are concerned in the abortion of the first flowers.

Flower abortion, however, is quite pronounced in many plants of *B. pekinensis* grown in pots and in which the head is scarcely

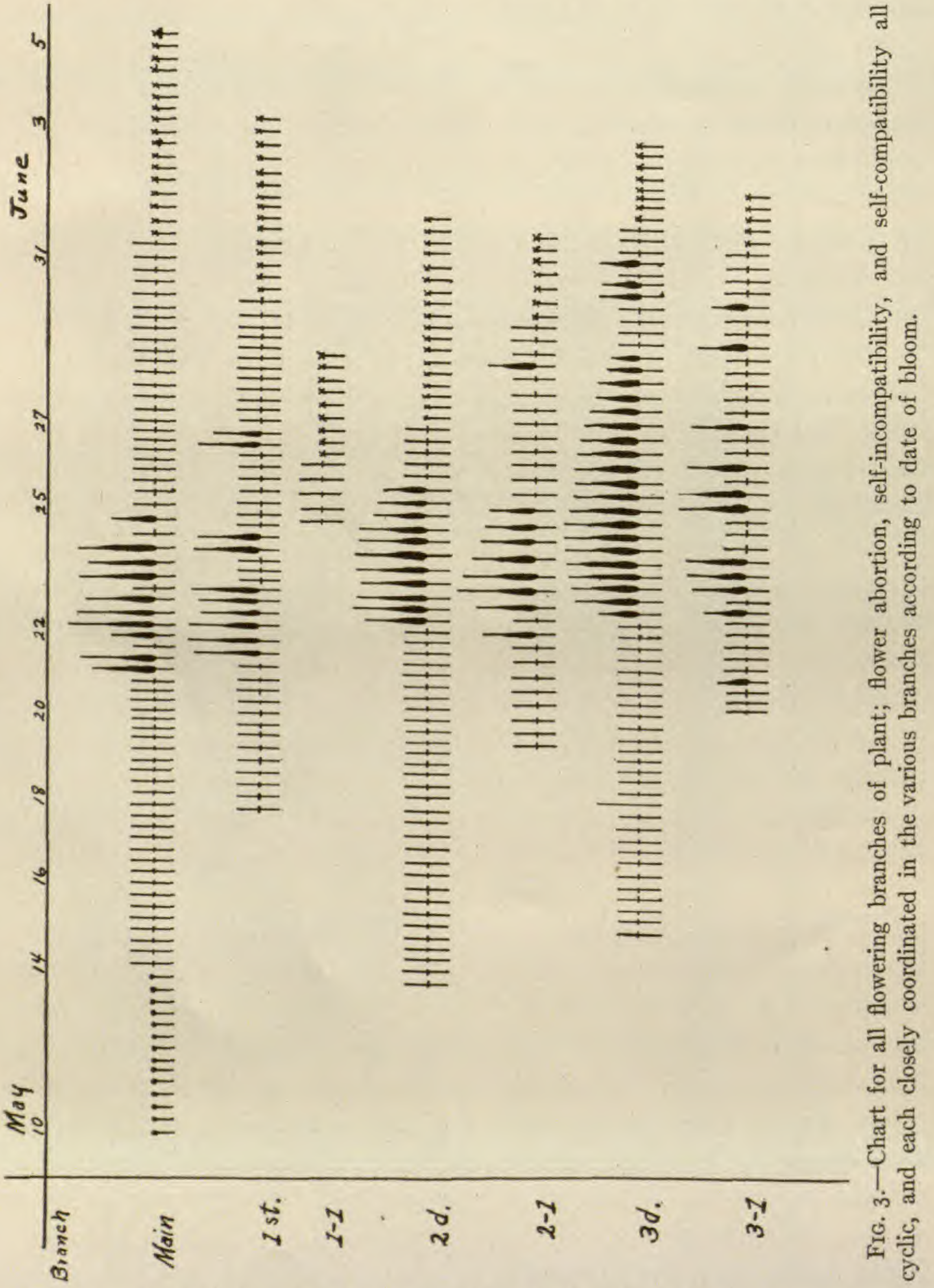

FIG. 3.—Chart for all flowering branches of plant; flower abortion, self-incompatibility, and self-compatibility all cyclic, and each closely coordinated in the various branches according to date of bloom.

developed. In the Nanking variety in which the leaves form only a loose rosette, about one-third of the plants grown have had some

aborted flowers, but usually only a few of the first flowers abort. Flower abortion also appears in many plants of *B. chinensis* which have very loose rosettes of leaves.

In these species flower abortion occurs as a transitional stage between a period of vigorous vegetative vigor and a period of flower formation and seed production. The plants which exhibit abortion are not able to pass at once into complete reproductive activity in producing potent flowers. The amount of abortion is greatest in the varieties of *B. pekinensis* in which vegetative vigor is most marked and in which excessive vegetative growth can readily be induced by good cultural conditions and which have been selected and bred for this feature. Flower abortion occurs in numerous plants of these sorts that are grown in pots, as it does in many plants of the loose-leaved kinds, but it apparently tends to be less marked in these.

Flower abortion is here undoubtedly correlated with the degree of vegetative vigor. It is not merely due to a stifling of flowers from simple direct injury because of inclosure within a head, however, but to a constitutional feature of which the formation of a leafy head or rosette is an extreme expression. In this sense the abortion of flowers is self-induced and to some degree hereditary. Usually the transition from aborted flowers to apparently normal flowers is sudden and complete (fig. 1). Sometimes, however, the first flowers to appear after the aborted ones, or the first flowers when there are no aborted ones, are poorly developed, are plainly immature and undersized, and especially in *B. chinensis* there may be premature opening.

(2) ARRESTED DEVELOPMENT OF LAST FLOWERS.—At least some of the last flowers which begin to form remain immature and functionless. In the first of such flowers the corollas wither quickly and may become dry and papery without falling. Then the flowers become smaller in size and more incomplete in development until at last they are mere stubs of tissue. Usually from six to ten flowers in these various stages of arrested development may be counted at the tip of each branch. On short, lower, lateral branches and on secondary or later laterals all of the flowers may fail to develop fully. This condition is shown in the illustrations. The distribution of flowers that fail to develop is indicated in figs. 2 and 3.

This type of sterility, of course, is very common in all sorts of flowering plants, and is clearly associated with old age and death of the entire plants or of the individual flowering branches. In these species of *Brassica* it is unusually conspicuous, and begins to develop when growth has ceased and parts of the plant, especially the basal leaves, are dying or even dead and falling from the plant. Flowers that have aborted or developed poorly at the beginning of the period of bloom, and those in which development is arrested, are all functionless. Their failure to produce fruit is entirely independent of any sort of fertilization. It is clearly due to impotence.

II. Proliferation

In a few plants of several strains of both *Brassica chinensis* and *B. pekinensis*, noticeable axial proliferations develop. The axis anlage inclosed within the carpels of the pistil grows and branches until it bursts open the pistil. The pedicel of the flower enlarges; the proliferated branch may become several inches long and bear as many as twenty-five flowers, many of which are able to function in seed production. Proliferation may be regarded as the sterilization of a pistil by vegetative growth of the tissue beneath and within it. In the end it is the expression of a tendency to vegetative vigor which culminates in the production of many more pistils and stamens.

Although proliferation is often irregular in its distribution, it is most frequent during the earlier portion of the period of bloom. Frequently it is most highly developed in the first flowers of plants which show little or no flower abortion, but it often does appear later. The last flowers of those which open normally as a rule are free from proliferations. This abnormality is certainly to be regarded as an expression of excess vegetative vigor, as a result of which the axis about which flower parts are grouped resumes active vegetative growth. The stamens in many of the flowers whose axes proliferate seem to be normal, but the pistils are not productive of fruit.

Another type of excessive vegetative vigor is seen in the development of green leaves at the base of each flower, giving a leafy inflorescence. This has only been observed in a few plants, and

its possible relation to the production of flowers and to their impotence has not been determined.

III. Physiological incompatibility

During the phase when the flowers are completely developed, many flowers are produced that are capable of producing pods and seeds. In general the plants produce such flowers in abundance, in succession for a period of about twenty days, and with continuous and rather rapid elongation of branches (cf. fig. 1 with 4 and 6). A free and indiscriminate functioning of the organs in seed production, however, is decidedly limited by incompatibilities in fertilization.

Self-incompatible plants.—Plants may be completely self-incompatible throughout, as was the case for the plant shown in fig. 4. The first six flowers on the main branch aborted, but the very first flowers on the three uppermost laterals were normal. A few flowers at the ends of the branches failed to develop. In all, about seventy flowers on the main branch, forty on each of the first and second laterals, and fifty on the third lateral were capable of functioning. Three lower branches, which bore together about one hundred normal flowers and were like the third lateral in general appearance, were not included in the photograph. This plant grew in isolation in a greenhouse, and self-pollinations were made by hand at least three times a week throughout the period of bloom. At least two hundred flowers were carefully self-pollinated, but not a pod resulted. The pods which were formed on this plant were all from compatible cross-pollinations. Six fine large pods near the base of the main stem were all from flowers that opened rather early; the two first flowers on the first lateral yielded fine pods to a cross; and large pods containing viable seeds were obtained by crossing some of the very last flowers to open normally on the main stem and on the first and second laterals. The stamens were apparently normal throughout the time when flowers opened normally; pollen from many stamens examined at different times was found to be plump and normal in appearance, and the use of pollen in certain crosses covering the entire period of normal bloom resulted in seeds. Such tests have been made repeatedly on

numerous plants with results as noted, which show that the failure to set seed to self-pollination is due to a sexual incompatibility between reproductive elements that are capable of functioning in certain other relations.

A total of 1371 plants of these two species of *Brassica* and hybrids between them have been tested at the time this is

FIG. 4.—Plant of *B. pekinensis*, completely self-incompatible but producing good pods containing viable seeds to compatible crosses at any time while flowers are fully developed.

FIG. 5.—Feebly self-compatible plant of *B. chinensis;* first two flowers and last to open normally on main axis highly fertile in compatible cross.

written, and of these 653 were found to be completely self-incompatible. Plants were thus classed when no pods developed to selfing at any time throughout the entire period of bloom. There were, however, several grades to be seen in respect to the length of time the pistils remained attached to the plant. In the plant shown in fig. 4, with few exceptions, the pistils of flowers

selfed fell soon after the petals had fallen. In other plants, and especially plants of *B. chinensis*, nearly all the pistils of selfed flowers remained attached only during the period of mid-bloom.

SELF-COMPATIBLE PLANTS.—A total of 718 plants of the various cultures grown were self-compatible in some degree. For the

FIG. 6.—Plant of *B. pekinensis*, highly self-compatible during period of mid-bloom; no flower abortion; first flowers to bloom on laterals were self-compatible, showing correlation with main branch in physiological character according to time of bloom.

purpose of a general classification the self-compatibility was judged as *feeble*, *medium*, and *strong*, but there were many grades within each class with no sharp distinctions between them. The weakest grade includes the cases in which, most typically, a few small pods containing only aborted seeds developed. In some cases pods of good size were formed, as is shown in fig. 5, but the seeds were all

shriveled and not viable. Such plants were classed as feebly self-compatible. Plants whose self-compatibility was classed as medium produced some viable seeds. The number of pods, the number of shriveled seeds, and to some extent the number of viable seeds varied greatly among plants thus grouped. The plants classed as strongly self-compatible produced numerous pods, and the total number of viable seeds was high. In these also the number of pods, their size, and the numbers of viable and of shriveled seeds varied greatly. The various grades of self-compatibility were seen among sister plants that were as nearly identical as is possible in regard to vegetative vigor, number of branches and flowers produced, and as to calendar dates for period of blooming.

FIG. 7.—Plant of *B. chinensis;* no flower abortion; plant highly self-compatible; showing cycle of self-compatibility with climax at time of mid-bloom.

It was readily recognized that the self-compatibility of such plants was most strong during the period of mid-bloom, and that previous to and following this period there was complete self-incompatibility. A highly self-compatible plant grown in isolation and carefully self-pollinated from day to day appeared at the end of the fruiting period, as shown in figs. 6 and 7. Repeated tests by crossings showed that in these, as in the case of the self-incompatible

plants, the flowers that opened normally during the time of self-incompatibility were functional in compatible crosses. The results of a test of this sort are shown in fig. 5. The cyclic development of self-compatibility with its coordination among the various branches of an individual according to time of bloom is shown in figs. 2 and 3.

There is evidence from other species (SIRKS 10) that various grades of cross-compatibility may exist between the individuals of the same race or species, that the group relations may be variable in different cultures of the same species or race (EAST and PARK 4), and that in general cross-incompatibilities appear with much the same irregularity in heredity and in expression as do self-incompatibilities.

The writer's studies with these plants have been chiefly concerned with self-compatibility. In the species of *Brassica* studied the cross-relations have not been studied sufficiently to state with certainty whether the grades of cross-incompatibility undergo cyclic changes like those of self-incompatibility, but perhaps it may be assumed that certain of the weaker grades of cross-incompatibility do thus operate.

Heredity of mid-bloom self-compatibility in pedigreed lines of descent

In the first or "parent" series grown of both *Brassica pekinensis* and *B. chinensis*, of a total of 253 plants there were 21 plants that produced viable seeds to self-pollination during the period of mid-bloom. From such seeds pedigreed progenies were grown through two generations, to test the inheritance of self-compatibility and to determine the result of repeated selection for this character. A summary of the records for the various series and families grown to date is presented in table I. In these records the first series of plants grown are given arbitrary numbers. The number of a series with that of the self-compatible plant used as a seed parent is employed in designating the series of succeeding generations. Thus the line of descent and the relationship of the various series of sister plants are fully indicated.

In one series of the selfed F_1 generation of *B. pekinensis* (series 1–2) comprising 88 plants, 24 were strongly self-compatible. In

TABLE I

RECORDS OF SELF-COMPATIBILITY FOR FAMILIES OF *Brassica pekinensis*, OF *B. chinensis*, AND OF HYBRIDS BETWEEN THESE SPECIES

ANCESTRY, GENERATION, AND SELF-COMPATIBILITY OF PARENT	RECORD FOR PROGENY				
	Total number of plants	Number self-incompatible	Self-compatible		
			Feeble	Medium	Strong
Brassica pekinensis					
P series 1, seeds of S.P.I. no. 44892	9	7	0	1	1
P series 2, seeds of S.P.I. no. 44935	10	6	2	1	1
P series 3, seeds of S.P.I. no. 44291	20	19	0	1	0
P series 4, seeds of S.P.I. no. 44312	12	9	1	0	2
P series 5, seeds of S.P.I. no. 44292	8	8	0	0	0
P series 34, seeds of S.P.I. no. 38783	114	67	45	2	0
P series 15, seeds of S.P.I. no. 45187	61	55	5	1	0
F_1 series 1–2, parent strongly self-compatible	88	25	19	20	24
F_1 series 2–1, parent strongly self-compatible	7	7	0	0	0
F_1 series 3–1, parent medium self-compatible	80	43	27	7	3
F_2 series 1–2–9, parent strongly self-compatible	36	5	19	9	3
F_2 series 1–2–18, parent strongly self-compatible	133	14	99	18	2
F_2 series 1–2–29, parent strongly self-compatible	46	24	21	1	0
F_2 series 1–2–38, parent strongly self-compatible	60	1	43	15	1
F_2 series 3–1–1, parent strongly self-compatible	19	15	2	2	0
F_2 series 3–1–9, parent strongly self-compatible	16	10	6	0	0
F_2 series 3–1–32, parent strongly self-compatible	18	12	6	0	0
Brassica chinensis					
P series 8	19	4	4	8	3
F_1 series 8–1, parent strongly self-compatible	111	42	56	12	1
F_1 series 8–5, parent medium self-compatible	48	43	3	2	0
F_1 series 8–6, parent strongly self-compatible	57	28	26	2	1
F_1 series 8–15, parent strongly self-compatible	122	40	74	7	1
F_2 series 8–1–13, parent strongly self-compatible	13	7	5	1	0
F_2 series 8–5–32, parent medium self-compatible	18	7	10	1	0
F_2 series 8–5–39, parent medium self-compatible	9	7	1	1	0
F_2 series 8–15–5, parent strongly self-compatible	48	30	16	2	0
F_2 series 8–1, no. 39×8–1 no. 32 (medium×medium)	30	23	7	0	0
F_2 series 8–5, no. 39×8–5 no. 41 (medium×self-sterile)	32	15	17	0	0
F_2 series 8–5, no. 30×8–5 no. 17 (both self-sterile)	16	15	1	0	0
F_2 series 8–5, no. 27×8–5 no. 32 (self-sterile×medium)	8	7	0	1	0
Brassica chinensis×B. pekinensis					
F_1 8–5, no. 23×2 no. 3 (both self-incompatible)	19	19	0	0	0
F_1 8–5, no. 19×3 no. 16 (both self-incompatible)	34	27	5	2	0
F_1 8–5, no. 18×3–1 no. 63 (medium×self-sterile)	50	12	14	19	5

the next generation of this family, the progeny of four strongly self-compatible plants, 275 plants were grown. Of these only 44 were completely self-incompatible. There were, however, only 6 plants as highly self-compatible as the immediate seed parents. The majority of the plants (182 in number) were feebly self-compatible and did not produce any viable seeds to selfing. This family, however, was somewhat more highly self-compatible than was the family derived from plant no. 1 of series 3, in the second generation of which only 2 plants out of 53 produced viable seeds to selfing.

Selection for the highest grades of self-compatibility in *B. chinensis* was also carried through the second generation. In the F_1 generation, 26 out of 338 plants produced viable seeds to selfing. In the F_2, 5 out of 88 plants produced such seeds. The proportion of self-compatible plants was low and remained about the same, not being appreciably increased or decreased in the second generation. No plants classed as highly self-compatible were found in the F_2, but this may have been due to the proportionally smaller number of plants grown in this generation.

Four series comprising 86 plants were grown from seeds obtained by crossing certain plants of the F_1. Of these, 25 were feebly self-compatible and one plant produced viable seeds. The F_1 hybrids between the two species exhibited the three types of sterility characteristic of the parent species. There was no indication of a general impotence of both sex organs (pistils and stamens) such as often results from hybridity. During the time when flowers opened normally, branches left to open pollination produced pods and viable seeds, and about 100 plants of this generation grown in the field and left to open cross-pollination produced pods in abundance.

As to mid-bloom self-compatibility, the F_1 hybrids were like the pure bred parents. Relatively few were highly self-compatible. In one series, derived from crossing a plant of a medium grade self-compatibility and one completely self-incompatible, of 50 plants, 12 were fully self-incompatible, 14 were feebly self-compatible, 19 were self-compatible of medium grade, and 5 were

highly self-compatible. Two series were grown from parents that were self-incompatible. In one of these all of the 19 progeny were self-incompatible; in the other series of 34 plants 27 were fully self-incompatible, 5 were feebly self-compatible, and 2 were self-compatible of medium grade during the period of mid-bloom.

SUMMARY.—The results obtained in these various pedigreed cultures show that self-compatibility is a character which is not directly hereditary. Self-compatibility occurs sporadically in a few members of these prevailing self-incompatible species. This character does not breed true. Selection for self-compatibility does not immediately lead to the establishment of self-compatible races. Neither is self-compatibility nor self-incompatibility dominant in crosses. There is some indication, however, that certain races may be secured in which the mode of distribution in respect to self-compatibility is higher than in others.

Discussion and conclusion

The strains of *Brassica pekinensis* and *B. chinensis* studied were previously selected and bred for excessive leafy growth rather than for fruit and seed production, yet they are reproduced exclusively by seeds. The vegetative vigor is not in the least utilized in the development of parts which may propagate the plants vegetatively. In their habit of growth and bloom, the stage of sexual reproduction in these plants quickly follows a period of remarkably vigorous vegetative development, hence these species are favorable material in which to study the correlative relations of the asexual or the vegetative phase to the sexual or reproductive (by seeds) phase in the complete life cycle.

The two types of sterility, impotence (including flower abortion and arrested development), and proliferation, or the destruction of a pistil by vegetative growth, as they occur in *B. pekinensis* and *B. chinensis*, are both phenomena associated with the formation of floral organs. The other type of sterility, physiological incompatibility or relative sterility which is present, is concerned with the physiological inter-relations of the sex organs in the various processes of fertilization.

These three types of sterility develop and operate in these two species and in their hybrids in intimate correlation with the cyclic

alternation of vegetative and reproductive vigor. Flower abortion occurs normally as a transitional stage between the formation of green leaves and the production of functional sporophylls. Those plants which exhibit flower abortion are not able to pass directly from producing green leaves or leaves with branches at the nodes to the production of flowers, and flower abortion occurs as a transitional stage. The abortion of flowers appears in the phase where vegetative vigor is waning, but before reproductive vigor is fully in evidence. There is also a marked agreement among the various branches of a plant as to the grade of development reached at any one date of blooming (figs. 1–3), which indicates a definite relation between the condition causing flower abortion (and also normal flower formation) and a condition of the plant as a whole. These phenomena, therefore, have many aspects characteristic of physiological correlation.

The arrested development of flowers at the ends of branches after a period of vigorous blooming of the plant is obviously due to an extreme waning of vigor and the approaching death of the plant as a whole, and of course is a phenomenon prevalent in all sorts of plants. Axial proliferation from the pistils is to be considered as a resumption of vegetative growth after the differentiation of the pistils has been accomplished.

Turning to the functional relations of the sex organs in these two species of *Brassica*, at least to the compatibility in self-fertilization, it is seen that they also exhibit a periodicity on their occurrence which forms a very definite cycle. A total of 718 plants that were self-compatible to some degree have now been observed in these two species and in hybrids between them. With the exception of a few individuals in which pods developed irregularly, the maximum of self-compatibility was reached during the mid-bloom of the plant (figs. 2, 3, 5–7). Previous to and following this period, the self-compatibility grades into complete self-incompatibility or into a much weaker grade of self-compatibility. Furthermore, the climax of self-compatibility is remarkably coordinated among the different branches according to the time of blooming quite as is the earlier development of flower abortion.

The remarkably uniform development of self-compatibility during the time of mid-bloom in *Brassica chinensis*, *B. pekinensis*,

and hybrids between them, is convincing evidence that the functions of fertilization are here operating in a cycle of intensities. The period of mid-bloom may be considered as the time when conditions are most favorable for fertilization. The cross-fertilizations which are highly effective both previous to and following the maximum for self-compatibility are hence to be considered as indicating a different and possibly a stronger grade of sexual relation. It seems conclusive that, judged by the functional relations in fertilization, the physiological properties of the sex organs in these plants vary in a rather definite cycle.

It is clear that self-compatibility as contrasted with certain grades of cross-compatibility in these species of *Brassica* is limited to a specific period following the transition from vegetative to reproductive activity and limited by the waning senility of the plant as a whole. Self-compatibility appears coincidently with the climax of the reproductive activity.

Sexual reproduction itself is generally characterized as a phenomenon of maturity (COULTER 3). The differentiations of sex as indicated by anatomical features and by the physiological compatibilities are perhaps to be considered as a smaller cycle operating within the larger alternation of vegetative and reproductive phases and subject to the same biogenetic regulation.

In the flowering plants especially, there is great diversity among species in the relative development of their vegetative and reproductive habits and in the inter-relations between these two phases. Perhaps the most universal of the biogenetic conditions incident to the transition from the vegetative to the reproductive phase is that change in nutrition which leads to the accumulation of carbon compounds. This is an internal condition that arises in the plant as a whole in the course of maturity, in contrast with relative excess of nitrogenous material that is characteristic of the vegetative stage. The decided influence of nutritive relations in regulating development and in influencing fruitfulness has recently been discussed by KRAUS and KRAYBILL (7), who have emphasized the fact that a well-balanced development, especially in regard to fruitfulness of fruit-bearing plants, is associated with a proper balance between nitrogen and carbohydrate metabolism.

It is not to be considered, however, that a single simple change in nutrition is the sole biogenetic factor regulating the appearance of maturity and its attending morphogenesis of flowers. In flowering plants, such as the species of *Brassica* whose sterilities are reported in this paper, there is progressive differentiation of parts in reference to metabolic activities which is most obvious in respect to the manufacture, distribution, and consumption of food. It has been shown that there are also special stimulating and inhibiting influences which in a decided manner regulate and correlate development. That these influences may be substantive and special (but metabolic) and different from food materials was postulated by SACHS (8) in one of the latest of his papers; that some influences are stimulative and correlative in the sense of nervelike impulses or even electrical stimuli have repeatedly been shown in studies of the nature of transmission and excitation in phenomena of dominance and control in correlative growth and development (CHILD 1).

It is to be noted that the complete life cycle of flowering plants involves two periods of vegetative vigor and maturity; one for the sporophyte and one for the gametophyte. The former culminates in the production of spores and the latter in the production of gametes. The generations are antithetic. In its length of life, vigor of vegetative growth, and reproductive power (number of gametes), the gametophytic phase has become relatively weak and highly specialized. In the sporophyte great vegetative vigor is correlated with great reproductive vigor in the production of spores (which are, however, in themselves asexual) and in the nurture of the gametophyte and the embryo. Sex differentiation in the great group of flowering plants has been pushed back during the progress of evolution into the sporophytic stage of the entire cycle, and here sexuality now culminates in seed formation in which the nutrition of the embryo is a most important factor. Sexual reproduction in these higher plants has become more and more inter-related with the vegetative phase of the sporophyte and subject to its internal and biogenetic regulation.

The decided influence of such regulation is seen in the fact that in the great group of hermaphrodite plants, the whole trend of the

morphological and physiological differentiations constituting sexuality is initiated in the morphogenesis of flowers. The cells of pistils and stamens are not only alike in their preformed genetic composition, but they are identical in this particular with the cells that entered into the preceding vegetative structures. CORRENS (2) has noted that the regeneration from sister cells of the egg and sperm (the archegonial and antheridial cells) in certain monoecious mosses shows that, at least in hermaphrodites and monoecious plants, maleness and femaleness are carried equally by both male and female gametes. The male gametophytes and their most highly specialized male cells are male only because of a temporary suppression of femaleness. Likewise the femaleness of egg cells is a temporary and one-sided expression of cells carrying both sex potencies. The various expressions of maleness and femaleness even in the sex generation, at least in hermaphrodite plants, according to CORRENS, are "phenotypic" or biogenetic expressions independent of any qualitative differentiation in the component units of the germ plasm. The expressions of the so-called factors for sex or the so-called inhibitors of one or the other sex are hence independent of corresponding differentiations in germ plasm which may have arisen during sporogenesis. The expression of sex, therefore, is on the same basis as are the somatic differentiations that arise among the various parts of the individual. It hence becomes a most fundamental biological problem to consider and to determine as far as possible what conditions determine these differences in the level of the so-called "physiological gradient."

Maturity, with its transition from the vegetative to the reproductive phase, whether giving homologous or antithetic alternation or a continuation of either, occurs in cycle after cycle with remarkable uniformity. This emphasizes the phylogenetic or hereditary aspect of particular phases of the development. One may assume a "gene" or a "factor" for maturity, and assume that it is gradually awakened from a dormant condition to the exercise of its influence at a particular time and in a particular group of cells. One may further assume that the loss of such a gene would throw a line of progeny into a condition of perpetual immaturity, so that flowers or other reproductive organs could never be formed. The evidence

is decidedly against such a view. The loss of maturity, as seen especially in the complete failure of flower formation, has very universally been shown to be due to the indirect influence of such external factors as light, heat, and nutrition on the metabolism and attending correlations in the organism (see numerous papers by MÖBIUS, VÖCHTING, KLEBS, SACHS, and GOEBEL, and recent papers by GARNER and ALLARD **5** and **6**, and by SETCHELL **9**).

That species or strains showing flower abortion and physiological incompatibility are different genetically from others that do not show such sterilities is obvious. That these types of sterility are more completely hereditary in some species than in others is clear. That these characteristics are not definitely and directly represented as such in the germ plasm by hereditary units is very evident from the results of genetical studies. Self-compatibility and self-incompatibility especially are not found to be alternative conditions in tests by crossing or in line breeding; the heredity is decidedly irregular and sporadic even when compatibilities are not cyclic in their appearance as they are in *Brassica chinensis* and *B. pekinensis*.

The various types of sterility seen in these species of *Brassica* decidedly indicate a mutually limiting relationship between vegetative and reproductive vigor. Their irregular inheritance, their appearance at definite periods in the cycle of development of the plant as a whole, and especially the cyclic manifestation of self-compatibility, indicate that the morphological and physiological differentiations of sex are regulated and determined by those internal and biogenetic processes which in general determine the cycle of growth, development, and maturity in the life of the individual.

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THE PELTATE PEPEROMIAS OF NORTH AMERICA[1]

WILLIAM TRELEASE

(WITH PLATES I–IV)

A herbaceous pepper from Santo Domingo, with large slightly peltate leaves, was called *Piper maculosum* by LINNAEUS in 1753, the year conventionally agreed upon as the starting point in phanerogamic nomenclature. This species was transferred to *Peperomia* by the elder HOOKER in 1825. When RUIZ and PAVON established the genus *Peperomia* in 1794, they included in it three Peruvian species with peltate leaves; one of these they named *P. umbilicata* and one *P. variegata*. In 1804 VAHL described, as *Piper hernandifolium*, a fifth species, from the West Indies, with such foliage. DIETRICH recognized this as a *Peperomia* in 1831. Another South American *Peperomia* was described in 1815 by HUMBOLDT, BONPLAND, and KUNTH, who also found in Mexico what they took for the *Peperomia umbilicata* of RUIZ and PAVON.

Following these determinations, most of the Mexican specimens with centrally peltate foliage have been called *P. umbilicata;* but MARTENS and GALEOTTI differentiated a close counterpart of *P. umbilicata* in 1843, as well as a diminutive species with barely peltate leaves. On the latter, before the Belgian botanists had secured printing of their account of GALEOTTI'S numerous discoveries in the Mexican flora, MIQUEL, who was engaged on a monographic study of the Piperaceae, based the name *Tildenia mexicana*, and transferred it to *Peperomia* under the same specific name in 1843 in his classic monograph of the Piperaceae, where their other peltate species appears as *P. monticola*, and which also added the Brazilian peltate species *P. arifolia* now so much cultivated in a silvery-striped variety. A Guatemalan species rather closely comparable in some respects with the original *P. umbilicata* was named *P. claytonioides* in 1847 by KUNTH, to whom are com-

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monly attributed the names used in the joint publication of HUMBOLDT, BONPLAND, and KUNTH.

As a result of studies preliminary to his own monograph of the Piperaceae, in 1866 DE CANDOLLE added two South American species to the *P. umbilicata* group, as well as one, *P. ovato-peltata*, questionably based on both Mexican and Costa Rican material and scarcely distinguishable today, and a caulescent subpeltate species, *P. cordulata*, from Panama. Shortly after the publication of DE CANDOLLE'S monograph in 1869, he gave publicity to a diminutive Mexican species, *P. fugax*, which LIEBMANN had discovered and named in manuscript, in addition to two scarcely differentiable larger peltate species, *P. Muelleri* and *P. Bourgeaui*, from eastern Mexico. Slightly antedating the publication of these, BAKER described another, *P. puberula*, very closely related to them, from Gautemalan plants cultivated in England.

It was not until 1887 that another addition was made to the *P. umbilicata* group, when WATSON distinguished in what he called *P. gracillima* a west Mexican counterpart of the original Peruvian species and its representatives in eastern Mexico and Guatemala. Four years later, DE CANDOLLE described *P. scutellata*, of Costa Rica, and in the course of the following decade further named an additional peltate species, *P. macrandra* from Mexico; and from Central America added five others, *P. peltata*, *P. sciaphila*, *P. Tuerckheimii*, *P. tecticola*, and *P. podocarpa;* and in 1902 *P. Bakerii* was described by him from Cuba.

At the end of the century all of the available material was gone over critically by DAHLSTEDT in an exhaustive revision; but an even more fruitful comparative study of the corm-producing group, centering about *P. umbilicata*, was made nearly a decade later by A. W. HILL, whose Andean field observations and seedling studies of the geophilous species led to a very satisfactory morphological distinction between North American and South American groups, centering respectively about *P. umbilicata*, and the Mexican forms that had been mistaken for or too closely connected with that Peruvian plant. In addition to describing several new peltate species from the southern continent, he differentiated under the name *P. campylotropa* what appeared to be the most frequently

encountered northern surrogate of the true *P. umbilicata*, and added a new Guatemalan species, *P. bracteata*.

The purpose of the present paper is to outline succinctly the result of a preliminary study of the peltate-leaved species of *Peperomia* of North America, inclusive of the West Indies, based chiefly on the extensive collections of the United States National Herbarium and the New York Botanical Garden. For the privilege of examining these, I am greatly indebted to Mr. MAXON and Dr. BRITTON. No effort is made here to give full descriptions or citations; but the admitted species are keyed apart sufficiently for their present characterization, and reference is made to all publications affecting their nomenclature.

Although they agree in possessing more or less strikingly peltate foliage, the plants here accounted for constitute four natural groups of species, of which the first three are interrelated, while the fourth has little in common with the others except that it falls within the generic limits of *Peperomia* as these are now drawn. Each of the first two groups is subdivisible into minor groups on floral as well as vegetative characters. These distinctions are sufficiently evident from the synopsis without separate discussion. Except for the very distinct fourth group, Hernandifoliae, all are continental.

SYNOPSIS OF GROUPS

Acaulescent; pistil not in a pit; stigma apical
- From a round or lens-shaped corm; glabrous ..... CAMPYLOTROPAE
- Subrhizomatous; glabrous; diminutive ..... MEXICANAE
- Subrhizomatous; glabrous; rather large ..... MACRANDRAE
- Substoloniferous; pubescent; diminutive ..... TUERCKHEIMIEAE

Shortly caulescent; pistil not in a pit; stigma subapical
- Small and delicate; somewhat pubescent ..... FUGACES
- Moderately large ..... ARIFOLIAE

Repent; moderately large; pistil not in a pit; stigma apical .. CORDULATIFORMES

Repent; rather large; pistil in a pit, beaked; stigma at base of beak ..... HERNANDIFOLIAE

CAMPYLOTROPAE

Rather small, acaulescent from a smooth corm, glabrous; leaves peltate near the middle or well above the base; spikes usually very

slender and loosely flowered; bracts oblong-ovate, pointed; pistil not immersed in the rachis; stigma apical.

Leaves orbicular, rarely subacuminate; scapes unbranched
  Filaments scarcely protruding beyond the bract; berries essentially sessile
    Stigma sessile
      Ovary blunt
        Berries ellipsoid ..... *P. monticola*
        Berries globose ..... *P. Painteri*
      Ovary subacute ..... *P. astyla*
    Stigma on a more or less evident style
      Style or stylopodium broadly conic ..... *P. Parryana*
      Style slender-conic ..... *P. tenuimucronata*
      Style rather abruptly cylindric ..... *P. campylotropa*
  Filaments conspicuously exserted
    Anther cells at length divergent ..... *P. schizandra*
    Anther cells not divergent
      Berries flask-shaped, sessile ..... *P. amphoricarpa*
      Berries ovoid, substipitate ..... *P. gracillima*
Leaves subrhombic-orbicular; scapes unbranched ..... *P. bracteata*
Leaves orbicular-acuminate; scapes often branched;
  berries sessile
  Berries flask-shaped with tapering style ..... *P. schizostachya*
  Berries subglobose with abrupt style ..... *P. claytonioides*
Leaves ovate
  Scapes often branched; berries sessile
    Scapes characteristically branched ..... *P. sciaphila*
    Many scapes unbranched ..... *P. ovato-peltata*
  Scapes unbranched; berries stipitate, with slender style ..... *P. peltata*

PEPEROMIA MONTICOLA Miquel, Syst. Piper. 71. 1843.—*Piper* (§*Peperomia*) *hydrocotylifolium* Martens and Galeotti, Bull. Acad. Bruxelles **10**:129. 1843.—*?Peperomia umbilicata macrophylla* C.DC. in DC. Prodr. **16**[1]:394. 1869.—Acaulescent; glabrous throughout; leaves round, centrally peltate, 3.5–5.5 cm. in diameter; berries sessile, ellipsoid; stigma sessile at the apex.

Southern Cordillera of Mexico, the type from near Tehuacan, Puebl. (*Galeotti* 6023, which is also the type of *Piper hydrocotylifolium*).

As with *P. mexicana*, this was distinguished and described by its discoverer, but antedated in publication by another, as was true also of two of GALEOTTI'S Mexican oaks.

Peperomia Painteri, n. sp.—*?Peperomia umbilicata subacutifolia* C.DC. in DC. Prodr. **16**[1]:394. 1869.—Aspect and general

characters of the preceding; leaves 1.5–2 cm. in diameter; berries globose; stigma sessile at the apex.—Fig. 5.

Table-land and adjacent Cordillera of Mexico, the type from Ixmiquilpan, Hidalgo (*Rose*, *Painter*, and *Rose* 8966).

Peperomia astyla, n. sp.—Aspect of the preceding; leaves 1.5–2.5 cm. in diameter, sometimes blunt-acuminate; ovary ovoid-flask-shaped, subacute; stigma apical.—Fig. 4.

Western Sierra Madre of Mexico, the type from Lake Patzcuaro, Michoacan (*Pringle* 4124).

Peperomia Parryana, n. sp.—Aspect of the preceding but the scapes sometimes 20 cm. long; ovary round-ovoid, tapering into a short broadly conic disk or style; stigma apical.—Fig. 7.

Eastern Sierra Madre and adjacent table-land of Mexico, the type from about San Luis Potosi, S.L.P. (*Parry* and *Palmer* 802).

With more filiform spikes and larger broadly conic style, it is var. **borealis,** n. var.

Type from La Silla, Monterrey, N.L. (*Pringle* 3018).

Peperomia tenuimucronata, n. sp.—Aspect of the preceding species; ovary flask-shaped, slenderly attenuate; stigma apical.—Fig. 12.

Western Sierra Madre of Mexico, and Cape region of Baja California, the type from near Santa Teresa, Tepic (*Rose* 3432).

PEPEROMIA CAMPYLOTROPA Hill, Ann. Botany **21**:156. 1907.—Aspect of the preceding species and taken as typical of the group which they constitute; leaves 1.5–3.5 cm. in diameter; berries round-ovoid, with a short cylindric style; stigma apical.—Fig. 10.

Mexican table-land and adjacent mountains; taken to be *P. umbilicata* HBK. (not RUIZ and PAVON), the type of which was from Santa Rosa de la Sierra (*Humboldt*).

Peperomia schizandra, n. sp.—Aspect of the preceding; leaves 4–5.5 cm. in diameter; stamens somewhat exserted, their anther cells at length widely spreading; berries ovoid, somewhat acuminately pointed; stigma apical.—Fig. 1.

Western Sierra Madre of Mexico, the type from Bolaños, Jal. (*Rose* 2883).

Peperomia amphoricarpa, n. sp.—Aspect of the preceding; stamens exserted; ovary oblong; berries ovoid-flask-shaped, gradually attenuate; stigma apical, for a time white-fimbriate.—Fig. 11.

Southern Cordillera of Mexico, the type from the Cerro San Felipe, Oax. (*Nelson* 1139). Presumably it was this which MARTENS and GALEOTTI took for *P. umbilicata*.

PEPEROMIA GRACILLIMA S. Wats.—*Peperomia gracillima* S. Wats., Proc. Amer. Acad. **22**:448. 1887.—Aspect of the preceding; leaves 1.5–2 or even 4 cm. in diameter; filaments exserted beyond the bract; ovary ovoid, (?normally) stipitately contracted, with a stout style; stigma apical.—Fig. 6.

Western Sierra Madre of Mexico, the type from the Rio Blanco, near Guadalajara, Jalisco (*Palmer* 585).

PEPEROMIA BRACTEATA Hill, Ann. Botany **21**:155. 1907.—Aspect of the preceding; leaves subrhombic-orbicular, slightly blunt-pointed, 2–3 cm. in diameter; berries subfusiform-globose, with a thick terminal disk or stylopodium; stigma apical.

Guatemala, the type from Sactos, Huehuetenango (*Seler* 2743).

Peperomia schizostachya, n. sp.—Aspect of the preceding; leaves subangularly ovate, more or less cuspidate or deltoid-pointed, 2×2.5–5×7 cm.; scapes simple or forked; berries flask-shaped, attenuate; stigma apical.—Fig. 2.

Costa Rica, the type from the Rio Virilla, near San Juan (*Tonduz* 10106=7273).

PEPEROMIA CLAYTONIOIDES Kunth, Index Sem. Hort. Berol. **1847**:11.—Aspect of the preceding; leaves ovate or round-ovate, more or less acuminate, 2–4 cm. in diameter; spikes characteristically 2 or 3 at end of the branching scape; berries subglobose-cylindric, with an abrupt style; stigma apical.

Guatemala, the type cultivated from an unrecorded Guatemalan locality.

With scapes much longer than the leaves, and bearing 4–6 alternate branches near the end, it is var. **longiscapa** C.DC., MS. n. var.—Fig. 13.

Guatemala, the type (*Bernhardi*) from an unspecified locality.

With scapes scarcely surpassing the leaves, bearing 4–6 subapical spikes scarcely 3 cm. long, it is var. **pinulana** C.DC., MS. n. var. (*P. pinulana* C.DC., Bot. Jahrb. **10**:289. 1889).

Guatemala, the type from Pinula, near Xalapa (*Lehmann* 1693).

PEPEROMIA SCIAPHILA C.DC., An. Inst. Fis.-Geogr. Costa Rica **9**:175. (1898?).—*?Peperomia ovato-peltata* Auct., as to Costa Rica.—Acaulescent; leaves broadly ovate, blunt-acuminate, rounded at base, peltate below the middle; spikes scarcely 2 cm. long, 2–7 nearly sessile at end of a filiform scape surpassing the leaves; ovary ovoid, subacute; stigma apical.—Fig. 14.

Costa Rica, the type from the Rio Virilla near San Juan (*Tonduz* 9630).

PEPEROMIA OVATO-PELTATA C.DC., Jour. Botany **4**:132. 1866.—Aspect of the preceding; leaves broadly ovate, acuminate, peltate one-third above the rounded or subcordate base; spikes solitary or 2 or 3 near the end of a filiform scape equaling or surpassing the leaves; ovary round-ovoid, contracted into an equilong fleshy style; stigma apical.

A problematic species, perhaps representing a depauperate form of the preceding and if so to replace it in name. Two specimens (New Spain, *Pavon;* Costa Rica, *Hoffmann* 521) are mentioned with the original description, but both are questioned there.

PEPEROMIA PELTATA C.DC., Ann. Conserv. and Jard. Bot. Genève. **2**:277. 1898.—*P. pedicellata* Dahlst., Svensk. Vet.-Akad. Handl. **32²**:35. 1900.—*P. ovato-peltata* Auct., as to Guatemala.—*?P. mexicana* Auct., as to Guatemala.—Acaulescent; leaves broadly ovate, subacuminate, truncately cordate, peltate toward the base, 2×3–3×4 or 5 cm.; scape unbranched; berries long and slender, tapering into an extremely slender stipe; stigma apical.—Fig. 15.

Guatemala, the type from Santa Rosa (*J. D. Smith* 3829, which is also the type collection of *P. pedicellata*).

MEXICANAE

Diminutive, subacaulescent from a small, finally branching rhizome; glabrous; leaves peltate at the very base; peduncles

unbranched; spikes filiform, loosely flowered; bracts ovate, pointed; pistil not immersed in the rachis; stigma apical.

Leaves ovate-oblong, style slender.....*P. mexicana*

PEPEROMIA MEXICANA (Miq.) Miquel, Syst. Piper. 75. 1843.—*Tildenia mexicana* Miquel, Diar. Inst. Reg. Nederl. 83. 1842.—*Piper* (§*Peperomia*) *parvulum* Martens and Galeotti, Bull. Acad. Roy. Bruxelles **10**:130. 1843.—*Peperomia Galeottiana* Hooker, Icon. Plant. **4**. *pl. 327*.—Essentially acaulescent, from a short rhizome, very small, glabrous throughout; leaves ovate and obtuse or characteristically lance-ovate or elliptic-ovate and attenuate, peltate extremely close to the base, 8×12–10×25–30 mm.; spikes solitary at end of filiform scapes twice as long as the petioles; berries oblong, pointed; stigma apical.—Fig. 3.

Eastern Sierra Madre of Mexico, the type from Mirador, Vera Cruz (*Galeotti* 7111, which is also the type of *Piper parvulum*).

MACRANDRAE

Moderately large, subacaulescent from a short branching rhizome, glabrous; leaves peltate below the middle; scapes unbranched; spikes loosely flowered; bracts ovate, pointed; pistil not immersed in the rachis, stigma apical.

Leaves elongated-ovate; berries oblong.....*P. macrandra*

PEPEROMIA MACRANDRA C.DC., Ann. Conserv. and Jard. Bot. Genève **2**:276. 1898.—Essentially acaulescent, from a short thick polycephalous rhizome, glabrous throughout; leaves ovate, acuminate, peltate toward the base, 2.5×4–4.5×7 cm.; spikes solitary at end of scapes rather surpassing the petioles; filaments exserted; berries ellipsoid-oblong, (teratologically?) gradually tapering into an equilong style; stigma apical.

Southern Cordillera, Mexico, the type from San Felipe, Oax. (*Pringle* 4654).

A large form, presumably from the same geographic region, with round-ovate leaves as much as 8×10 cm. and shorter beak on the fruit, is var. **ampla,** n. var.

Type, without other data, occurring in the United States National Herbarium as *Pringle* 13282.—Fig. 8.

Tuerckheimieae

Diminutive, subacaulescent from a short rhizome, sparsely villous; leaves peltate toward the base; peduncles unbranched; spikes filiform, loosely flowered; bracts round-ovate, scarcely pointed; pistil not immersed in the rachis; stigma apical.

Leaves ovate, small ..... *P. Tuerckheimii*

Peperomia Tuerckheimii C.DC., Ann. Conserv. and Jard. Bot. Genève **2**:279. 1898.—Nearly acaulescent, small, dingy-villous; leaves alternate, ovate, acute, peltate toward the base, 2×2.5 cm. with equilong petiole; spikes axillary, filiform, short (20–40 mm.); berries ovoid, subacute; stigma apical.—Fig. 9.

Guatemala, the type from Pansamala (*von Tuerckheim* 433).

Fugaces

Diminutive, caulescent, slightly pubescent; leaves peltate toward the base; peduncles unbranched; spikes filiform; pistil not immersed in the rachis; stigma subapical.

Leaves ovate; spikes pubescent ..... *P. fugax*

Peperomia fugax Liebmann, in C.DC., Linnaea **37**:370. "1871–3."—Shortly caulescent (3 cm. high), locally hairy; leaves alternate, ovate, acute, peltate toward the base, ciliolate, scarcely 10×13 mm., on an even shorter hairy petiole; spikes axillary; ovary ovoid; stigma suboblique.

Eastern Sierra Madre, Mexico, the type from Mirador, Vera Cruz (*Liebmann*).

Arifoliae

Moderately large, with a usually evident thick erect stem from a rhizome; leaves peltate below the middle; peduncles unbranched; spikes moderately slender and closely flowered; bracts orbicular; pistil not immersed in the rachis; stigma subapical.

Glabrous ..... *P. arifolia*
Pubescent
  Spikes rather thick
    Stigma obliquely subsessile ..... *P. puberula*
    Stigma on a short style ..... *P. Muelleri*
  Spikes scarcely 1 mm. thick ..... *P. tecticola*
  Spikes 1-2 mm. thick ..... *P Killipi*

PEPEROMIA ARIFOLIA Miquel, Syst. Piper. 72. 1843.—Shortly caulescent, succulent, glabrous; leaves alternate, round-ovate, subacute or short-pointed, peltate toward the truncately subcordate base, 5–6×6–9 cm., the petiole nearly as long; spikes terminal, solitary, about 100 mm. long, on nearly equilong peduncles; berries ovoid; stigma essentially sessile at or very near the end.

A Brazilian species, the type cultivated in Europe; common everywhere in conservatories in the nearly acaulescent silver-striped var. *argyreia* (or *P. Saundersii*); reported, doubtless in cultivation, for Bermuda; and represented by a var. **acutifolia** C.DC., n. var., with acute smaller leaves 3.5×5.5 cm., on petioles 7.5 cm. long, cultivated in Switzerland from Costa Rican (?cultivated) material.

PEPEROMIA PUBERULA J. G. Baker, in Saunders, Ref. Bot. **5**. *pl. 302*. 1871.—Shortly caulescent, rather succulent, somewhat velvety; leaves alternate, broadly ovate, subacute or abruptly short-pointed, peltate toward the rounded or subcordate base, 3×4–6×7 cm., the petiole of more or less equal length; spikes terminal and axillary, 50–100 mm. long, on somewhat shorter peduncles; berries obovoid, the subsessile stigma slightly oblique.—Fig. 17.

Guatemala, the type cultivated in England from Alta Vera Paz.

PEPEROMIA MUELLERI C.DC., Linnaea **37**:366. "1871–3."—*Peperomia Bourgeaui* C.DC., Linnaea **37**:370. "1871–3."—Shortly caulescent, rather succulent, gray-pilose; leaves alternate, broadly ovate, more or less acuminate, peltate toward the subcordate base, 4–6×6–7.5 cm., the petiole slightly shorter; spikes terminal and axillary, slender, equaling or surpassing the leaves, the peduncle shorter than the petiole; berries round-ovoid, obliquely short-mucronate, terminated by the stigma.

Eastern Sierra Madre, Mexico, the type from Orizaba, Vera Cruz (*Mueller* 653); that of *P. Bourgeaui* from the Rio Blanco near Orizaba (*Bourgeau* 3230).

PEPEROMIA TECTICOLA C.DC., An. Inst. Fis.-Geogr. Costa Rica **9**:175. (1898?).—Shortly caulescent, rather succulent, loosely short-hairy; leaves alternate, ovate or elongate-ovate, somewhat acuminate, peltate toward the rounded or shallowly subcordate base, 2×4 cm., the petiole about equilong; spikes terminal or

axillary, solitary, 80–120 mm. long, densely flowered, on peduncles about as long as the petioles; bracts round-peltate; berries round-ellipsoid; stigma oblique.

Costa Rica, the type from San José (*Tonduz* 7262, in a roof gutter).

A form with larger leaves 3–3.5×6–7 cm. and spikes fully 150 mm. long, is var. **muricola,** n. var., Costa Rica, the type from San Juan (*Tonduz* 10146, on a wall).—Fig. 16.

Peperomia Killipi, n. sp.—Shortly caulescent, rather succulent, glabrate; leaves alternate, round-ovate, somewhat blunt-attenuate, barely subpeltate at the rounded or slightly cordate base, ciliolate, 3–3.5 cm. long and wide, the petiole about equilong; spikes terminal or axillary, solitary, 60 mm. long, densely flowered, on elongated peduncles; bracts round-peltate; berries round-ovoid; stigma oblique.

Panama, the type from Alhajuela, on the Chágres River (*Killip 3218*).

CORDULATIFORMES

Moderately large, repent or ascending, rooting from some nodes; leaves in part barely subpeltate at the very base, alternate; spikes solitary, terminal or opposite the leaves, closely flowered; bracts orbicular; pistils not immersed in the rachis; stigma apical.

Glabrous; berries sessile
- Spikes rather thick ..... *P. cordulata*
- Spikes scarcely 1 mm. thick ..... *P. cordulatiformis*

Somewhat hairy; berries stipitate ..... *P. podocarpa*

PEPEROMIA CORDULATA C.DC., Jour. Botany 4:137. 1866.—Ascending, glabrous, slender; leaves alternate, round-ovate or ovate, subacute, obscurely subpeltate at the subcordate base, 5.5×7 cm., the petiole about 1 cm. long; spikes terminal, solitary, 150 mm. long; berries ovoid-acute; stigma oblique.

Panama, the type (*Fendler* 265) from an unrecorded locality.

Peperomia cordulatiformis, n. sp.—Ascending, glabrous, rather succulent; leaves alternate, round-ovate, bluntly short-acuminate, obscurely subpeltate at the cordate base, 3.5×5–5×6 cm., the petiole about 1 cm. long; spikes opposite the leaves, solitary,

filiform, scarcely 50 mm. long; berries round-ovoid; stigma oblique.—Fig. 19.

Panama, the type from Mamei Hill in the Canal Zone (*Pittier* 3806).

PEPEROMIA PODOCARPA C.DC., An. Inst. Fis.-Geogr. Costa Rica **9**:175. (1898?).—Repent, more or less pilose, delicate, the stem scarcely over 1 mm. thick; leaves alternate, broadly ovate, cuspidate, peltate near the rounded base, 4×6 mm., the petiole shorter than the blade; spikes paired on short axillary or terminal stalks, very slender and short (0.5×15 mm.) and short-peduncled; berries oblong-ellipsoid, slender-stipitate, obtuse.

Costa Rica, the type from El General (*Pittier* 10595).

HERNANDIFOLIAE[2]

Rather large, repent and rooting from the nodes; leaves variously peltate, alternate; spikes solitary, or paired on a common stalk, closely flowered; bracts orbicular; pistils sessile, immersed in ovoid pits in the rachis, beaked, the stigma at base of the beak and exposed at the level of the rachis; the glutinous berries finally broken loose and extruded at right angles to the contracting rachis.

Usually puberulous, berries subovoid ..... *P. hernandifolia*
Leaves ciliate; berries cylindric ..... *P. Bakerii*
Glabrous
  Leaves round-ovate ..... *P. peltilimba*
  Leaves lance-ovate, barely subpeltate ..... *P. scutellata*

PEPEROMIA HERNANDIFOLIA (Vahl) A. Dietr., Sp. **1**:157. 1831.—*Piper hernandifolium* Vahl, Enum. **1**:344. 1804.—*Peperomia Ponthieui* Miq., Syst. Piper. 186. 1843.—Repent, somewhat succulent, usually puberulous, the stem 2 mm. thick with elongated internodes; leaves alternate, broadly ovate, somewhat obliquely acuminate, rounded at base or subcordate, peltate below the middle, 4–5×6–8 or even 8×12 cm., the petiole 4–10 cm. long; spikes 1 or 2 at end of an axillary 1-bracted stalk 3–4 cm. long, 30–50 mm. long with an equilong peduncle; berries oblong-ovoid,

[2]The Antillean *P. distachya*, representative of a related group of species, sometimes has leaves barely subpeltate.

with a slender flexuous beak; stigma anterior at base of the beak.—Fig. 18.

Through the West Indies (to which the type is ascribed) and reaching Venezuela.

In the Eastern Sierra Madre of Mexico it is represented by an almost entirely glabrescent form, var. **calva,** n. var., the type of which is from Orizaba (*Botteri* 1158).

A Costa Rican form, glabrescent except that the rather large leaves are ciliate, is var. **ciliifera,** n. var., the type from Estrella, Cartago (*Cooper* 5917).

A glabrate Costa Rican form with filiform peduncle is var. **filipes,** n. var., the type from La Palma (*Tonduz* 12539).—Fig. 18, habit.

With even the upper face of the coriaceous, abruptly short-acuminate leaves puberulent, it is var. **cryptocarpa,** n. var., the type from near the Finca Sepacuite, Alta Verapaz, Guatemala (*Cook* and *Griggs* 533).

PEPEROMIA BAKERII C.DC. in Urban, Symbolae Antillanae **5**:296. 1902.—Of the aspect of *P. hernandifolia* and scarcely separable, but with ciliate leaves and more cylindrical slender-beaked berries.

Cuba, the type from Lomas de Taro-Taro, Pinar del Rio (*Baker* 3833).

Peperomia peltilimba C.DC., n. sp.—Repent, glabrous, rather thick-stemmed; leaves alternate, round-ovate, sharply acuminate, peltate toward the rounded base, 3.5–5×4–5.5 cm., the petiole scarcely as long as the blade; spikes paired on bracted stalks, relatively thick and short (3×2.5 mm.); ovary ovoid, obliquely beaked, the stigma anterior on the beak.

Costa Rica, the type from San Ramon (*Brenes* 14178).

PEPEROMIA SCUTELLATA C.DC., Bull. Soc. Roy. Bot. Belg. **30**:230. 1891.—Repent, glabrous, rather slender-stemmed; leaves alternate, lance-ovate, barely subpeltate at the very base, 3.5×7–8 cm., the petiole 4–8 cm. long; spikes paired on a short terminal stalk, shorter than the leaves; berries cylindrical, slender-beaked; stigma anterior on the beak.

Costa Rica, the type from near Division (*Pittier* 3611).

PEPEROMIA MACULOSA (L.) Hook., Exot. Fl. *pl. 92.* 1825.—*Piper maculosum* L., Sp. Pl. 30. 1753.—*Peperomia septuplinervia* C.DC., Jour. Botany **4**:142. 1866.—*Peperomia monsterifolia* Grisebach, Cat. Pl. Cub. 64. 1866.—Ascending, sparingly hairy, succulent, the rather thick stem mottled; leaves alternate, elliptic-ovate, subcuspidate, peltate near the rounded or truncately subcordate base, 8–11×12–17 cm., the mottled petiole 6–15 cm. long; spikes solitary or paired at the end, large (6×250 mm.), caudately tapering; berries subsessile, ovoid, tapering into an equilong beak; stigma anterior on the beak.

Through the Antilles, the type from Haiti (*Plumier*).

PEPEROMIA VARIEGATA Ruiz and Pavon, Fl. Peru. **1**:33. *pl. 52.* 1794.—Aspect of and not considered by MIQUEL separable from the preceding, but glabrous and with broader leaves 12–15 cm. long.

Peru to Demarara, and reported for Costa Rica and Guatemala possibly on forms of the preceding; the type from Peru.

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EXPLANATION OF PLATES I–IV

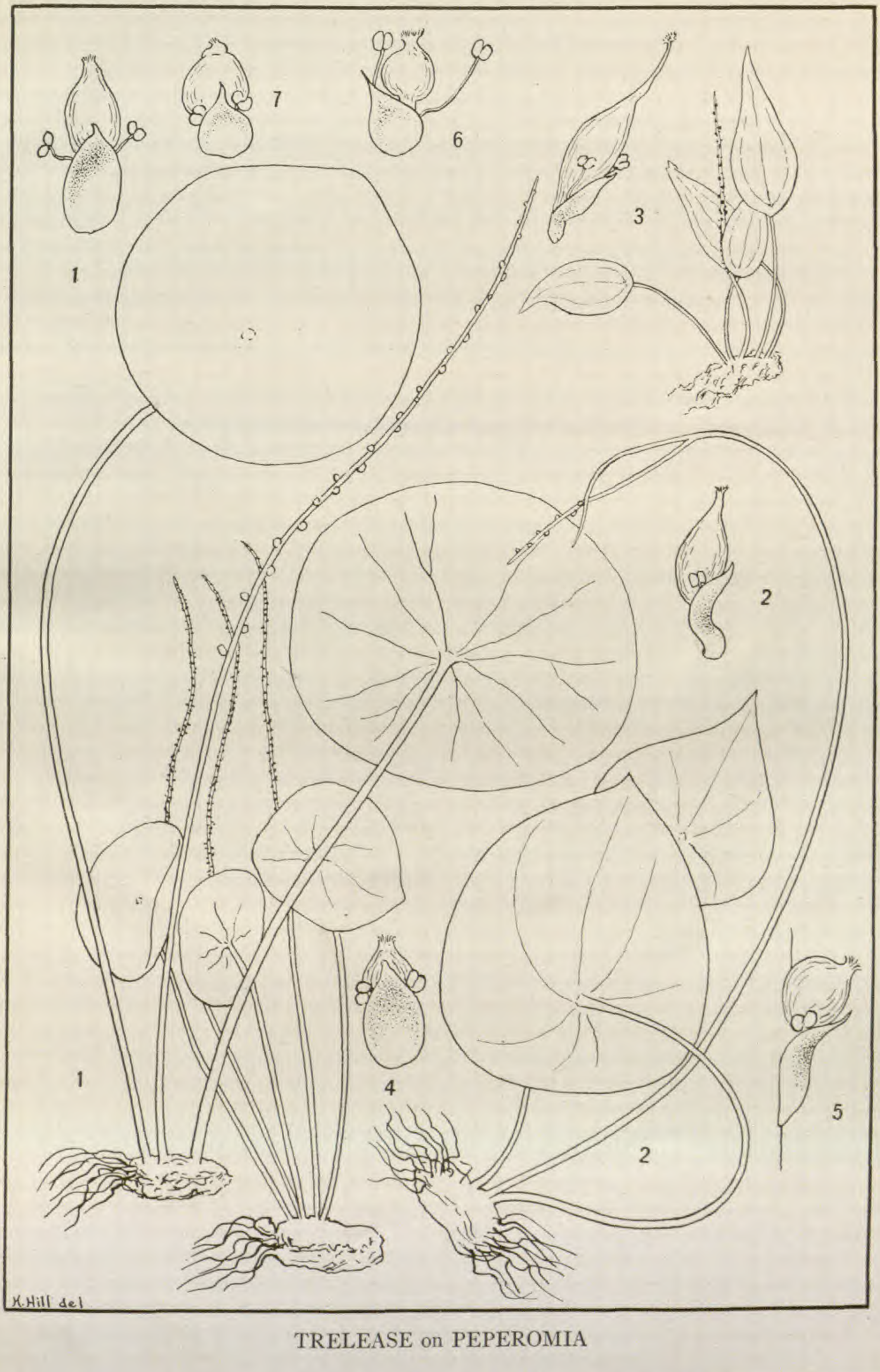
Habit illustrations are of natural size; inflorescence or fruit details are enlarged 20 diameters.

PLATE I

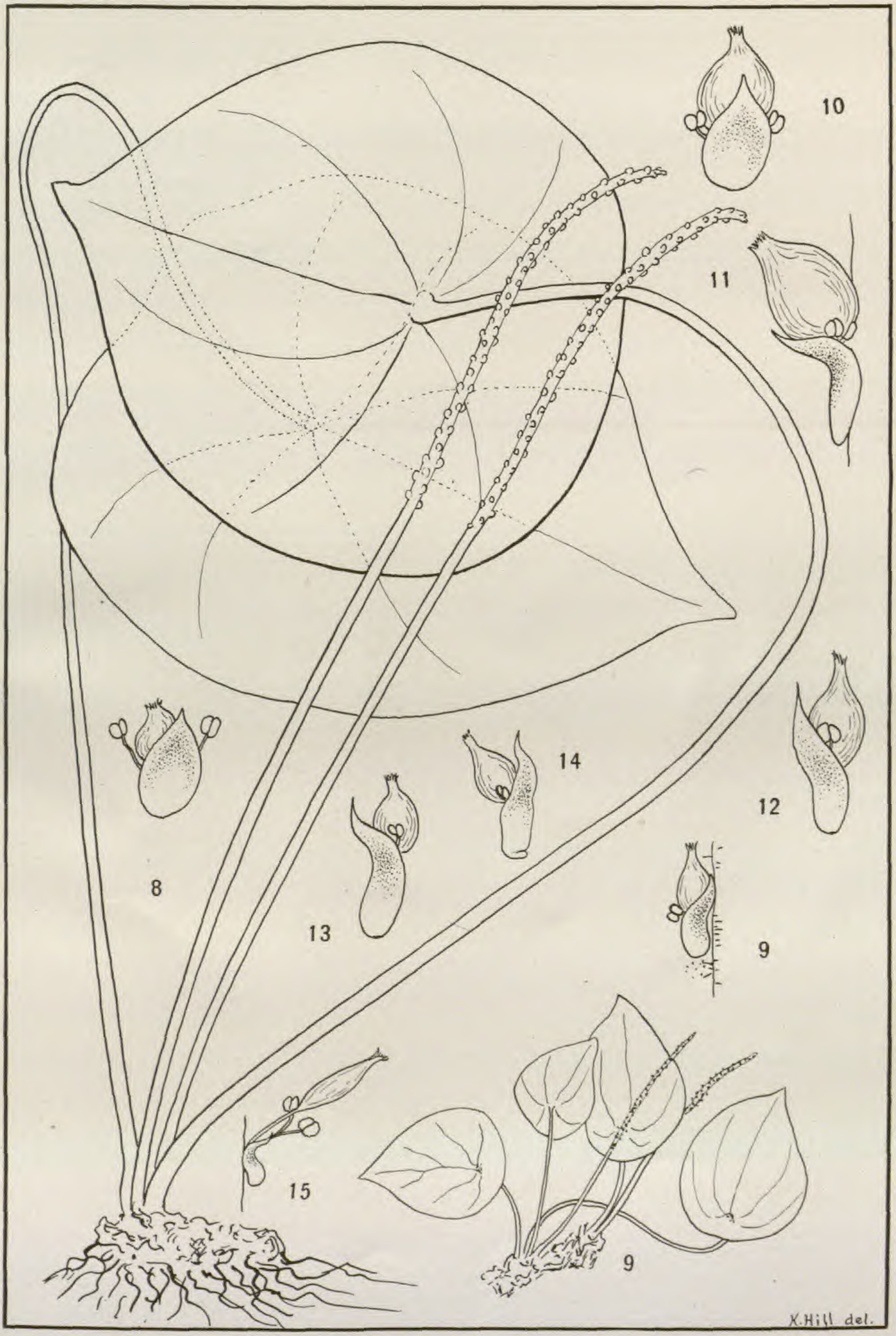
FIG. 1.—*Peperomia schizandra*, from type.
FIG. 2.—*Peperomia schizostachya*, from type.
FIG. 3.—*Peperomia mexicana*, from type number, also cotype of *Piper parvulum*.
FIG. 4.—*Peperomia astyla*, from type.
FIG. 5.—*Peperomia Painteri*, from type.
FIG. 6.—*Peperomia gracillima*, from cotype.
FIG. 7.—*Peperomia Parryana*, from type.

PLATE II

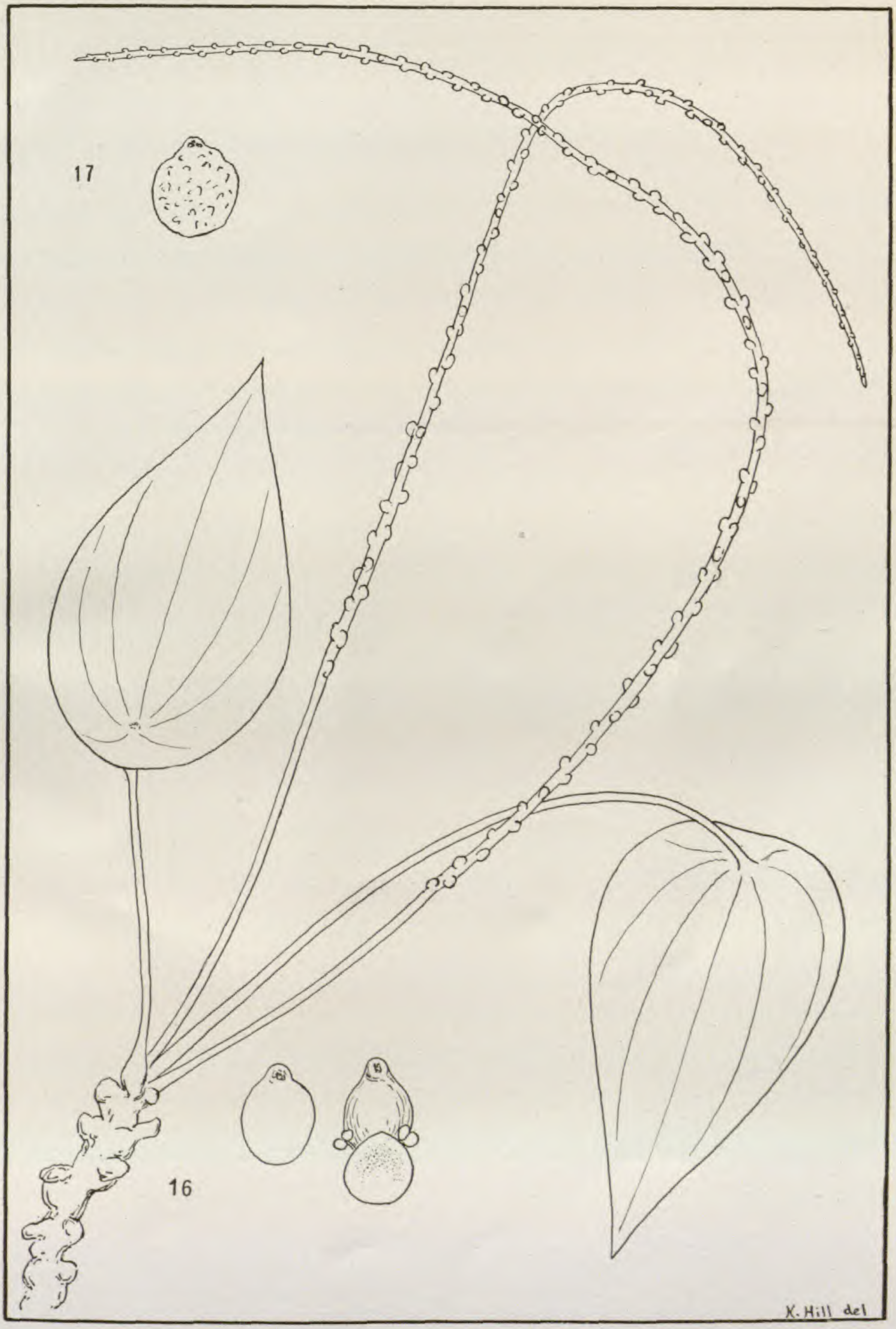
FIG. 8.—*Peperomia macrandra ampla*, from type.
FIG. 9.—*Peperomia Tuerckheimii*, from type collection.
FIG. 10.—*Peperomia campylotropa*, from sheet in United States National Herbarium (?erroneously) as *Bourgeau* 3020.



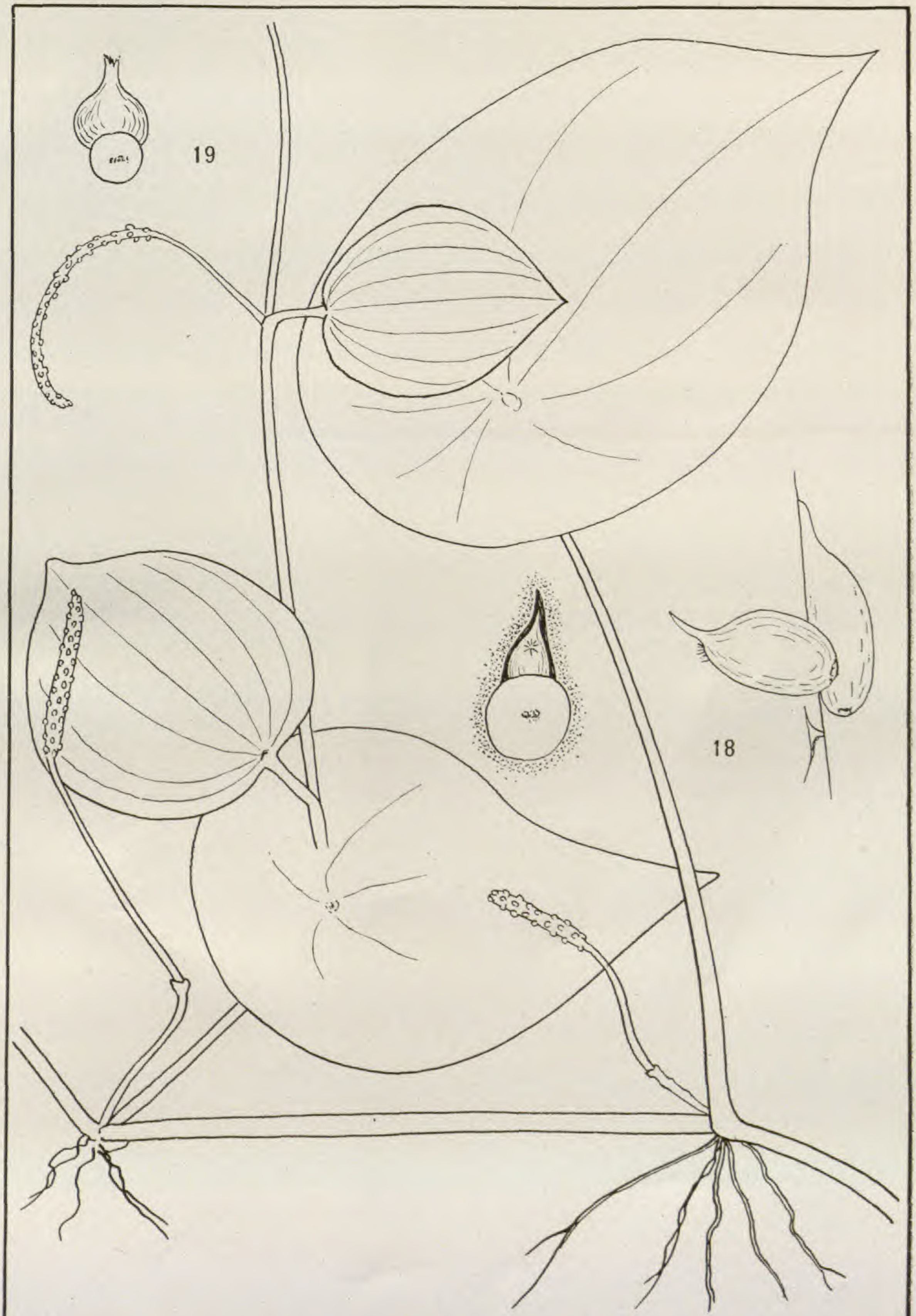
TRELEASE on PEPEROMIA



TRELEASE on PEPEROMIA



TRELEASE on PEPEROMIA



TRELEASE on PEPEROMIA

FIG. 11.—*Peperomia amphoricarpa*, from type.

FIG. 12.—*Peperomia tenuimucronata*, from type collection.

FIG. 13.—*P. claytonioides longiscapa*, from a Santa Rosa specimen (*von Tuerckheim* II, 2319).

FIG. 14.—*Peperomia sciaphila*, from type collection.

FIG. 15.—*Peperomia peltata*, from type collection.

PLATE III

FIG. 16.—*Peperomia tecticola muricola*, from type.

FIG. 17.—*Peperomia puberula*, from Santa Rosa specimen (*Heyde* and *Lux* 3826).

PLATE IV

FIG. 18.—*Peperomia hernandifolia:* habit of var. *filipes*, from type; fruit of insular form, from Dominica (*Eggers*).

FIG. 19.—*Peperomia cordulatiformis*, from type.

FLOWERS AND INSECTS. XXI

DATA OF ANTHECOLOGY

CHARLES ROBERTSON

In KNUTH'S *Blütenbiologie* is found a mixture of the botanical subject of insect visitors of flowers, the entomological subject of flower visits of insects, and collectors' notes which have no definite relation to either subject but rather to the personal movements of collectors. He states that his work is based upon MÜLLER'S, but he fails to include data from works which follow MÜLLER'S method (MÜLLER'S *Alpenblumen* and MACLEOD'S works on flowers of the Pyrenees and Flanders), and includes collectors' notes which suppress it, although such notes from French and Italian records are excluded as perhaps not justifying the labor of compilation. In a work which repeats MÜLLER'S lists for Low Germany for the third time American anthecological lists are suppressed.

ANTHECOLOGICAL DATA.—These are lists of insect visitors made to show the species, their frequency, their efficiency as pollinators, and the possibility of their having some influence in determining the characters of the flowers. MÜLLER'S lists show these details. In the case of the bees he indicated the sexes, and whether they were sucking nectar or collecting pollen. To note the sexes is important because female bees fly longer than males and are more likely to make repeated visits. To note the fact of pollen-collecting is also important. A female bee will carry pollen all day from flowers on which the male rarely occurs. From observations at Carlinville the females of nest-making bees average 20.6 visits to the males 10.3. The inquiline bees show females 8.8 to males 8.0. In anthecology MÜLLER'S lists are valuable as regards species and visits, but they fail to indicate the frequency. In 1908 I rejected MÜLLER'S method and adopted the practice of capturing the visitors as they came, noting species, and counting individuals. It is impossible to indicate the importance of insects to flowers by lists of species, because efforts to increase the lists involve an exaggeration of the importance of rare and exceptional cases.

ENTOMOLOGICAL DATA.—If lists of visitors of flowers are broken up and the visits are redistributed under each insect, the subject changes from a botanical to an entomological one. It would require as much space and as many entries to make the second set of lists. If these insect visits are distributed under the different flower classes and colors, they may still hold an indirect relation to anthecology. To be correctly arranged as entomological data

TABLE I

Data	Lists	Bees	Diptera	Other Hymenoptera	Lepidoptera	Coleoptera, Hemiptera	Total
1. First 62							
Entomological	122	82.5	4.2	6.3	4.6	2.3	476
Anthecological	175	33.3	41.8	4.8	10.1	9.7	1647
Next 38							
Entomological	108	90.5	1.4	2.1	4.8	1.0	475
Anthecological	141	60.6	9.5	1.6	25.6	2.5	1077
Total 100							
Entomological	230	86.5	2.8	4.2	4.7	1.6	951
Anthecological	316	44.1	29.1	3.5	16.2	6.8	2724
2. 65 Compositae							
Entomological	213	79.2	10.2	5.8	3.0	1.5	985
Anthecological	255	35.6	37.0	5.1	14.7	7.3	3309
59, Germany, Müller	.....	48.1	27.1	7.7	9.8	7.0	1103
85, Illinois	.....	40.3	27.1	16.9	11.2	4.4	4030
3. 51 plants							
Flowers and insects	.....	40.4	25.1	15.5	13.8	4.9	1386
Anthecological, Europe	.....	42.5	34.7	6.3	8.9	7.5	887
Entomological, Europe	.....	88.6	4.2	2.3	1.5	3.1	256
4. Visits in Knuth	.....	44.6	25.4	12.4	11.7	5.7	6127
Other observers	.....	53.2	17.8	11.0	10.6	7.2	3289
Flowers and insects	.....	43.4	26.5	12.2	12.3	5.3	6163

they should be distributed under the natural families of plants. The visits of each nest-making bee should be distributed under three headings: one showing the pollen visits of the female, another showing the nectar visits of the female, and a third showing the nectar visits of the male. If they are arranged under alphabetical lists of plants, and the sexes and pollen and nectar visits of the bees suppressed, they have little use as entomological data.

MIXING ANTHECOLOGICAL LISTS AND COLLECTORS' NOTES.—In his *Blütenbiologie* KNUTH gives a list of 37 entomologists whose notes are included as if they furnished anthecological data. The

difference is quite definitely shown in the first 100 cases in which collectors' notes and anthecological lists are given for the same flowers (table I, 1). The latter show for visits: bees 44.1 per cent and flies 29.1, while the former show bees 86.5 per cent and flies 2.8. One indicates a rather miscellaneous assemblage, while the other shows such a strong preponderance of bees as to reveal the fact that most of the entomologists were collecting bees particularly. In the first 62 cases the bee visits stand 82.5 per cent, flies 4.2, against bees 33.3, flies 41.8, the maximum changing from bees to flies. In the next 38 cases the bee visits are 90.5 and 60.6, a difference of 29.9. These are Papilionaceae and rather melittophilous, so that the bee collectors' notes give a more correct approximation. In many cases they did not say whether the bees were collecting pollen or not, and often did not mention the sexes at all.

In the case of 65 species of Compositae for which there were both collectors' notes and anthecological lists (table I, 2) the percentages of visits stand: for the former, bees 79.2, flies 10.2; for the latter, bees 35.6, flies 37.0, the maximum again shifting from bees to flies. Comparing 59 species observed by MÜLLER (*Fertilisation of flowers*), 65 observed by anthecologists in Europe, and 85 observed by me in Illinois, there is more resemblance between the European and Illinois lists than between collectors' notes and anthecological lists based on the same 65 species observed in Europe.

In the case of 51 plants observed both in Europe and Illinois (table I, 3) the percentages of bee visits show: for the collectors 88.6, for the anthecologists, Europe 42.5, Illinois 40.4. Here again there is more resemblance between anthecological observations for Europe and Illinois than between anthecological lists and collectors' notes for Europe. As entomological data the collectors' notes are just as unsatisfactory. In the three cases the percentages of visits of flies range: for the collectors, 1.4 to 10.2, with an average of 4.5; for the anthecologists, 9.5 to 41.8, with an average of 29.5.

The insect visits to flowers recorded in my *Flowers and insects* are not included in KNUTH's work, but are broken up in a final list and redistributed under the insects, making a quasi-entomological subject of them, but they are vitiated by being mixed with collectors' notes. These visits recorded in KNUTH (table I, 4) show for bees 44.6 per cent of the total. The visits recorded for other

observers show bees as 53.2 per cent. This is a higher percentage than is shown in anthecological observations of any region, and indicates that observations were introduced which discriminated in favor of bees to the exclusion of other insects. My *Flowers and insects* shows for bee visits 43.4 per cent instead of 44.6. The difference comes from using some entomological notes which were separated from my anthecological lists and were never intended to be used as anthecological data.

DYSTROPIC VISITS.—Useless visits of insects should be considered from the standpoint of the flowers and regarded as marks of imperfect adaptation. LOEW has called insects which make such visits "dystropic," and MÜLLER has called *Bombus mastrucatus* a "dysteleologue," as if these insects were under some teleological obligation to make useful visits. So far as the flower is concerned, a bee collecting pollen from it without effecting pollination cannot count among the useful visitors. So far as the bee is concerned, however, the visit is legitimate and the flower must be counted among its pollen visits. In the case of a family of small bees (Halictidae) 165 visits, mostly for pollen, were observed to flowers which they failed to pollinate. KNUTH, while omitting American visits under the plants to which they relate and redistributing them under the insects which make them, has indicated the so-called dystropic visits under conditions which make them entomologically irrelevant.

EXAGGERATION OF FRAGMENTARY OBSERVATIONS.—Probably anyone who contemplates methods of publication will notice this as a common characteristic. Fragmentary observations, as regards publication, reviewing, or abstracting, get more consideration than they are entitled to, for the reason that it is easier and cheaper to do so. When it was complained that KNUTH suppressed anthecological lists, the statement did not apply to the most fragmentary, the most worthless ones. His work gives 55 of my lists averaging 2.8 visits, and excludes 207 lists averaging 28.9 visits. There is only one reason: the short lists are easier to copy and to print.

One list containing 18 Syrphidae and 128 other insects is omitted, while another list for the same plant, consisting exclusively of 5 Syrphidae, is given. Special mention is made of the occurrence

of one Syrphid on a flower of which a list containing 10 Syrphidae and 21 other insects is not included. In *Blütenbiologie* **2**:254, KNUTH specially mentions the hive-bee and *Syritta pipiens* as visitors of *Ptelea trifoliata*, but in **3**:444 a list containing these two insects together with 49 others is merely grouped. In **2**:476, *Andrena combinata* (sex?), observed by SCHMIEDEKNECHT, is specially mentioned, while MACLEOD'S list containing 6 bees (sexes given) and 70 other insects is combined. In the third volume of *Blütenbiologie* the seeming discrimination in favor of collectors' notes as against anthecological lists perhaps may be explained partly from the fact that they are usually short. Why long lists are usually published in the second volume and always omitted in the third requires another explanation.

GENERAL RESULTS.—While one might hold that a general work should treat all of the data alike, and that, when it repeats one set of lists for the third time, it should collect another set once, it is not certain that it should give local lists at all. As regards details, local lists decline in value as the distance increases from the place where they were made. As regards species, the lists from Illinois and Germany are quite different. A student in one place does not need to know the specific name of every insect taken on flowers in the other, but only the different kinds. Of course in giving the general groups errors may easily be made. "Hymenoptera" is used for the three groups, long-tongued bees, short-tongued bees, and other Hymenoptera, for any two of them, or for any one of them exclusively. Bees and the other Hymenoptera do not belong to the same ecological class. Of 437 entomophilous flowers bees were found on 95.4 per cent, while flies were found on 60.4 per cent, and the other Hymenoptera on only 43.0 per cent. Table I shows that the visits of bees range from 33.3 to 60.6 per cent, while the visits of other Hymenoptera range from 1.6 to 16.9. In DAVIS' *Handbook of flower pollination* (**1**:165), "hymenopterid flowers" is a translation of "Immenblumen," used by KNUTH in referring to a statement in which LOEW wrote "Bienenblumen."

CARLINVILLE, ILL.

CURRENT LITERATURE

BOOK REVIEWS

Soil conditions and plant growth

The rapid advancement of our knowledge of the soil as related to plant production is reflected by the necessity for a new revision of RUSSELL'S *Soil conditions and plant growth*.[1] The fourth edition appears in a slightly different size and binding, marking its separation from the series of *Monographs on Biochemistry* edited by PLIMMER and HOPKINS, and the inauguration of a new series, *The Rothamsted Monographs on Agricultural Science*, edited by E. J. RUSSELL. Students of soil problems and plant physiology will welcome this departure, and await with keen interest the other five volumes announced as in preparation.

The present volume covers the entire field, and the others are to deal with more restricted phases, in more complete and more critical fashion than would be possible without undue expansion of the original work. The chapter headings remain about as they were. Chapter ii becomes "Soil conditions affecting plant growth" instead of "The requirements of plants"; and a second appendix is added, which shows in tabular form the amounts of minerals absorbed from the soil by agricultural crops in England.

Nearly every chapter shows enlargement, especially those which deal with the soil conditions affecting plant growth, the biological conditions of the soil, and the relation between the microorganic population of the soil and plant growth. The literature citations are increased from 18 to 28 pages, and the whole work from 240 to 355 pages of text. More figures have been added, with a frontispiece of BOUSSINGAULT, the founder of modern agricultural chemistry, which adds to the attractiveness of the volume.

The literature mentioned by WALSTER[2] in the review of the third edition has nearly all been given consideration in the revision, and the discussion is brought up to date as nearly as possible. The book is an excellent general introduction to the field, and will be indispensable to students of plant physiology, ecology, and agriculture. With this new departure in publication of monographs, the Rothamsted Station, already justly famous for its splendid contributions to agricultural practice, is certain to extend very greatly its influence as a center of scientific investigation.—C. A. SHULL.

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[1] RUSSELL, E. J., Soil conditions and plant growth. 4th ed. pp. xii+406. *figs.* 32. New York: Longmans Green & Co. 1921.

[2] BOT. GAZ. **67**:171–173. 1919.

NOTES FOR STUDENTS

Species hybrids.—According to one definition of species, which maintains that crossing is impossible beyond the species boundary, there can be no such thing as a species hybrid. The experience of many breeders, however, shows numerous cases of successful crossing between forms that are commonly regarded as distinct species. As might well be expected, many of these species crosses exhibit peculiarities, and it is of considerable theoretical interest to analyze them. In this connection it is enlightening to consider some of the ideas which were suggested to EAST and HAYES[3] by their experiments on hybrid vigor, and which may be freely interpreted as follows. As the width of a cross increases, four characteristic stages are encountered. (1) Very narrow crosses commonly bring little more than the redistribution of a few Mendelian characters. (2) Wider crosses, involving a considerable number of Mendelian factors, in many plants will produce a first hybrid generation which is characterized by a certain amount of hybrid vigor, the amount increasing with the width of the cross. (3) A point is reached where the fertility of the hybrid falls off, and at a certain width of cross perfectly sterile hybrids may be expected. It is interesting that hybrid vigor commonly continues to increase even though fertility decreases. Here loss in "efficiency" in the reproductive system is distinctly not accompanied by loss in efficiency in vegetative development. This peculiarity is clarified by the following idea. Wide crosses involve the fusion of relatively "inharmonious" gametes, which might be expected to produce disturbances in the ontogeny of the resulting individual. The grosser mechanism which regulates vegetative development can evidently weather such disturbances, while the more finely balanced mechanism of gamete formation is upset. (4) Finally, in the widest crosses, even vegetative development is impaired, and poorly developed hybrids result, a point eventually being reached where crosses are entirely unsuccesful.

Species crosses in *Nicotiana*, made by GOODSPEED and CLAUSEN,[4] and by EAST,[5] and the wheat-rye crosses of JESENKO[6] gave results which, although not identical, were significantly similar. All seem to be of the third type, the first generation hybrids being vigorous but almost sterile. The nature of this sterility is pictured in the following theory, which was proposed by BABCOCK and CLAUSEN, and supported and somewhat enlarged by EAST. Two *Nicotiana* species, *A* and *B*, each having a haploid chromosome number of 24, are crossed

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[3] EAST, E. M., and HAYES, H. K., Heterozygosis in evolution and in plant breeding. U.S. Dept. Agric. Bur. Plant Ind. Bull. 243. pp. 58. 1912.

[4] GOODSPEED, T. H., and CLAUSEN, R. E., Mendelian factor differences versus reaction-system contrasts in heredity. Amer. Nat. **51**:31–46, 92–101. 1916.

[5] EAST, E. M., A study of partial sterility in certain hybrids. Genetics **6**:311–365. *figs.* 17. 1921.

[6] See BABCOCK, E. B., and CLAUSEN, R. E., Genetics in relation to agriculture New York. 1918 (p. 238).

to produce a hybrid which contains 24 chromosomes from parent *A* and 24 from parent *B*. Reduction division by this hybrid should produce gametes of numerous types, ranging from those which would possess the 24 chromosomes of the *A* parent and none of the *B* chromosomes, to the other extreme in which the reverse would be true. It is assumed that gametes in the intermediate condition, with a liberal mixture of *A* and *B* chromosomes, fail to develop. "Any gamete containing elements derived from both systems would give a reaction system subject to profound disturbances incident upon the inharmonious relations set up" between the *A* and *B* elements. In consequence the only gametes which successfully develop are such as contain all or almost all of the *A* chromosomes, or all or almost all of the *B* chromosomes. This hypothesis of selective survival of the gametes produced by the F_1 species hybrid is supported by the following very striking facts. Back crosses with either parent result in the production of a relatively much higher number of forms identical or almost identical with that parent than would otherwise be expected. Also, in the scanty F_2 generation which it is sometimes possible to obtain, many more individuals appear which are identical or almost identical with the original grandparents (*A* and *B*) than would otherwise be expected.

SAX[7] arranges the species of *Triticum* in three groups, characterized by haploid chromosome numbers of 7, 14, and 21 respectively. Crossing within the groups produces fertile hybrids, but crossing between the groups results in more or less sterile hybrids. It is noteworthy that the F_1 endosperms are well developed in the fertile crosses, but shriveled in those crosses which are to produce sterile or partially sterile F_1 plants. In all cases, however, hybrid vigor appears in the vegetative parts of the F_1 plants. Evidently endosperm development as well as gametogenesis is sensitive to the disturbances resulting from the union of "inharmonious" gametes. The F_2 results of SAX,[8] however, appear not to agree with the ideas of the other investigators, since in this generation no greater sterility appears in the intermediates than in the segregates resembling the grandparents. Ecologists as well as geneticists will be interested in the natural law which is suggested by this work of SAX. In a group of species of which the chromosome numbers vary in multiples of an original basic number, adaptability varies directly with the chromosome count. Thus the 21 chromosome wheats are the most adaptable, and the 7 chromosome wheats are the least adaptable.—M. C. COULTER.

Fusarium resistant cabbage.—The selection of *Fusarium* resistant strains of cabbage is being continued at the Wisconsin Experiment Station by JONES[9]

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[7] SAX, KARL, Sterility in wheat hybrids. I. Sterility relationships and endosperm development. Genetics **6**:399–416. 1921.

[8] SAX, KARL, Chromosome relations in wheat. Science **54**:413–415. 1921.

[9] JONES, L. R., WALKER, J. C., and TISDALE, W. B., *Fusarium* resistant cabbage. Wis. Agric. Exp. Sta. Res. Bull. 48. 1–34. *figs. 10.* 1920.

and others. In an earlier report[10] the symptoms of the yellows disease of cabbage are described. It is caused by a vascular parasite (*Fusarium conglutinans*) which persists indefinitely in the soil, eventually forcing abandonment of large areas of fertile soil for cabbage culture. Soil disinfection having proved ineffective or impracticable, the only hope for control lies in the selection of disease resistant strains. Selections have been continued since 1910 with very effective results.

The success of this work rests largely on the fact that the cabbage is nearly self-sterile and is normally cross-pollinated. As a result all standard varieties of cabbage are variable in type to a greater or less degree. Fortunately, moreover, wherever cabbage was grown on soil badly infested with yellows, although a large majority of the plants were killed, always a few showed a high degree of resistance and developed normally. By selection of such heads for seed growing the next season, and saving the seed from each plant separately, individual head strains were secured which were planted on badly infected soil the second season. Thus by continual selection and elimination of weaklings, high resistant strains were quite readily secured.

It should be pointed out that there are numerous horticultural varieties of cabbage adapted to various localities and uses. In making selections for resistance, therefore, the importance of adhering as strictly as possible to the horticultural characteristics of the original type was recognized. Consequently the earlier efforts were directed upon the late or winter type of cabbage, known as Hollander or Danish Ball Head, which is grown most generally in Wisconsin. A highly resistant strain of this variety was secured to which the name Wisconsin Hollander has been given. The latter differs slightly from the original in having a more vigorous plant with a longer stem and flatter head, and in maturing somewhat later. These objectionable features have now been overcome by reselecting from the Wisconsin Hollander an earlier strain with the more desirable qualities of shorter stem and spherical head. For purposes of distinction the former strain is now designated as Late Wisconsin Hollander and the latter as Early Wisconsin Hollander.

Following similar methods, selections from two late summer varieties of the flat head type, the All Seasons and the Brunswick, have yielded highly resistant strains of each. These new strains are known as the Wisconsin All Seasons and Wisconsin Brunswick. To meet further demands of the trade, selections are now under way on three still earlier maturing varieties, the All Head Early, the Glory of Enkhuizen, and the Copenhagen Market.

In general, throughout this work, a number of closely similar seed heads from the same source were grown in mixed plantation to insure sufficient setting of seed. The seed from individual plants was then saved separately and tried out in head to row tests. In certain cases, however, attention was

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[10]JONES, L. R., and GILMAN, J. C., The control of cabbage yellows through disease resistance. Wis. Agric. Exp. Sta. Res. Bull. 38. 1–70. *figs.* 23. 1915.

given to selfing of individual plants with some success. In order to hasten the work, winter seed growing in the greenhouse or out-of-doors in the southern states was practiced with moderate success.

Seedsmen and growers are interested in the possibility of producing seed of these resistant strains in the commercial cabbage seed growing sections. The difficulty encountered in this procedure lies in the fact that yellows does not occur generally in our seed growing sections. The small percentage of susceptible plants would thus not be eliminated, and tendency toward reversion would be expected. The investigators have studied this question by having a resistant strain grown for one generation in the Puget Sound seed growing section, and then testing it on diseased soil together with Wisconsin grown strains. Little or no reversion was noted when this was carried on for only one generation. The practice, therefore, is being approved for the present provided precautions are taken to supply stock seed each year from plants selected on diseased soil, and to so isolate seed fields as to avoid all possibility of cross-pollination with other varieties.—J. C. WALKER.

Abnormal behavior in corn endosperm.—If pollen from red grained corn be applied to the silks of a colorless grained variety, the resulting grains will be red. This familiar phenomenon of xenia is explained by the known facts of double fertilization. This cross, however, may produce a very few aberrant grains; of these, only a part of the surface is red and the rest colorless. Such grains are commonly spoken of as "mosaics," while the terms "mottled," "spotted," and "variegated" usually refer to different phenomena. WEBBER[11] observed this phenomenon, and suggested two possible explanations: (1) the second male nucleus fails to fuse with the female fusion nucleus, and these two elements divide independently in producing endosperm; (2) the second male nucleus fuses with one of the female polars, the other polar dividing independently in the production of endosperm.

The first explanation was disproved by EAST[12] in the following manner. Factors *R* and *C* must be present simultaneously for the production of red endosperm. A cross between the two colorless grained types, *CCrr* and *ccRR*, therefore, will produce a red grained ear. Even here, however, aberrant grains sometimes appear, part of the grain being white and the rest colorless. Failure of the second male nucleus to fuse with the female polar nucleus in such a case would result in a grain which was entirely colorless, a thing which never occurred. It is only by fusion of male and female nuclei that any part of the endosperm can be red.

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[11] WEBBER, H. J., Xenia, or the immediate effect of pollen in maize. U.S. Dept. Agric., Div. Veg. Phys. Path. Bull. **22**:1–44. 1900.

[12] EAST, E. M., Xenia and the endosperm of angiosperms. BOT. GAZ. **56**:217–224. 1913.

WEBBER'S second explanation was disproved by EMERSON[13] as follows. A colorless, sugary type, *CCrrsusu*, was used as female parent in a cross with a colorless, starchy type, *ccRRSuSu*. The resulting grains were red, starchy, save for a few aberrant grains which were red in part and colorless in part but starchy throughout. WEBBER'S second explanation fails here, since fusion of the second male nucleus with only one of the polars would produce grains which were red, starchy in part (from male nucleus fused with one polar) and colorless sweet in part (from independent polar).

These two critical experiments serve to disprove WEBBER'S explanations and demonstrate that the normal program of double fertilization is invariable in corn. The next thing which was invoked to explain these aberrant grains was "somatic mutation" in the endosperm, but for several reasons this was unsatisfactory as an explanation.

EMERSON[14] has finally obtained critical evidence which indicates a very satisfactory explanation of the phenomenon. The factor *wx* for waxy endosperm (*Wx*, corneous endosperm) is known to be carried on the same chromosome with the *C* factor. A cross was made between a colorless, waxy female parent, *c-wx c-wx* and a red corneous male parent, *C-Wx C-Wx* (the *R* factor being present in both parents). The resulting triploid endosperm was of the formula *c-wx C-wx C-Wx*. If non-disjunction (passing of both halves of a divided chromosome to one pole) occurred in connection with the third of these chromosomes, one of the resulting nuclei would be diploid for this chromosome set. *c-wx c-wx*, and the other tetraploid, *c-wx c-wx C-Wx C-Wx*. Endosperm produced by the former should be colorless, waxy; endosperm produced by the latter should be red, corneous. Emerson obtained aberrant grains which were of exactly this constitution, the colorless areas being at the same time waxy, and the red areas corneous. This experiment, considered together with the previous ones, indicates that occasional non-disjunction is the explanation of these aberrant grains. The frequency of these particular aberrant grains is one in 423, and one may expect non-disjunction to take place in connection with some one chromosome in the corn endosperm in about one of every fourteen grains. Direct cytological demonstration is to be hoped for. Non-disjunction is known to occur at times elsewhere in the plant and animal kingdoms. Possibly the triploid nature of endosperm furnishes an especially favorable condition for its occurrence.—M. C. COULTER.

Prairie vegetation.—The prairies of Illinois, occurring as they do on the tension line between great forest and and grassland formations of North America, afford peculiar advantages in the study of the development of this

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[13] EMERSON, R. A., Anomalous endosperm development and the phenomenon of bud sports. Zeit. Induk. Abstamm. Vererb. **14**:241–259. 1915.

[14] EMERSON, R. A., Genetic evidence of aberrant chromosome behavior in maize endosperm. Amer. Jour. Bot. **8**:411–424. *fig. 1*. 1921.

type of vegetation. In a recent article SAMPSON[15] has made an excellent contribution to our knowledge of these rapidly disappearing grasslands. He found, in various parts of the state, remnants of the original prairie grasslands varying in size from strips along roadways and railways to tracts of hundreds or even thousands of acres in extent. Some of the largest areas were on the floodplain of the Mississippi River and occur even within the city limits of Chicago. The principal virgin areas were visited during the summers of 1915–18 and carefully studied.

The most notable contribution appears in a very complete explanation of the dynamics of these grasslands. Two main lines of succession are recognized, the hydrarch and xerarch, with a common climax association type in which *Andropogon furcatus* is dominant. The hydrarch succession commonly begins with an association dominated by *Scirpus fluviatilis*, succeeded by others in which *Spartina Michauxiana* or *Calamagrostis canadensis* is abundant. In the subclimax *Panicum virgatum* or *Agrostis alba* may be most conspicuous. Variations in the intermediate stages occur and are illustrated by examples.

Owing to the agricultural value of the upland prairie areas the xerarch succession is not so easily solved, although there is abundant evidence of the nature of the climax association. Mixtures of herbaceous species with few grasses seem to be the probable pioneer forms, with a mixed aggregation of grasses or a comparatively pure stand of *Andropogon scoparius* as the intermediate stage.

The present abundance of *Poa*, appearing both as the dominance of *P. pratensis* in the climax association and of *P. compressa* in the subclimax of the xerarch succession, is shown to be due to man's influence in cutting and grazing. The retrogressions due to grazing, as well as the various types of succession, are made clear by numerous diagrams, by floristic analyses of the various associations, and by an annotated list of the principal species.

A very commendable feature of the report is a non-technical summary in which the main results of the study, including the principal successions, are stated in terms intelligible to the ordinary citizen acquainted with the prairies but without botanical training. A series of excellent plates also add to the interest and value of the report.—G. D. FULLER.

Taxonomic notes.—The collection of plants made by COMPTON in New Caledonia and the Isle of Pines in 1914 is being published by various taxonomists, the first part containing the Angiosperms by RENDLE, BAKER, and MOORE.[16] It includes 830 species, 230 of which are new. The ten new genera

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[15] SAMPSON, H. C., An ecological survey of the prairie vegetation of Illinois. Ill. Dept. Regist. and Educ. Div. Nat. Hist. Surv. Bull. **13**:523–577. *pls. 48–77. figs. 9.* 1921.

[16] A systematic account of the plants collected in New Caledonia and the Isle of Pines by Professor R. H. COMPTON in 1914. Part I. Flowering plants (Angiosperms), by RENDLE, A. B., BAKER, E. G., and MOORE, S. LEM. Jour. Linn. Soc. **45**:245–417. *pls. 13–24.* 1921.

are as follows: *Comptonella* (Rutaceae), *Salaciopsis* (Celastrineae), *Montagueia* (Anacardiaceae), *Paracryphia* (Eucryphiaceae), *Enochoria* (Araliaceae), *Merismostigma* (Rubiaceae), *Tropalanthe* (Sapotaceae), *Depanthus* (Gesneraceae), *Adenodaphne* (Lauraceae), and *Dendrophyllanthus* (Euphorbiaceae). The largest families are Orchidaceae (60 spp.), Euphorbiaceae (60 spp.), Rubiaceae (60 spp.), and Myrtaceae (56 spp.), and they also furnish a large proportion of the novelties described.

LESTER-GARLAND[17] has published a revision of the African genus *Baphia* (Leguminosae), recognizing 58 species, 3 of which are described as new. As an illustration of the recent development of our knowledge of the African flora, it may be stated that this genus is represented by 6 species in the *Genera Plantarum*.

BLATTER and HALL[18] have published a new genus (*Bonnayodes*) of Scrophulariaceae from India, related to *Bonnaya*, *Ilysanthus*, and *Limnophila*, but differing from each in a decisive character.

MOORE[19] has described a new genus (*Phanerocalyx*) of Oleaceae from Africa. It is represented by two species, and is most nearly related to *Strombosia*.

HUTCHINSON and PEARCE[20] have published a revision of the African genus *Tryphostemma* (Passifloraceae), recognizing 25 species, 6 of which are described as new. In the second volume of the *Flora of Tropical Africa* the genus was represented by a single species.

SPRAGUE[21] has published a revision of *Belotia*, a Central American genus of Tiliaceae, recognizing 11 species, 6 of which are described as new.

GLEASON[22] has presented a much needed rearrangement of the Bolivian species of *Centropogon* and *Siphocampylus* (Lobeliaceae), genera which have been very much confused, recognizing 14 species of the former genus and 26 of the latter.

WAINIO[23] has published two papers describing a collection of Japanese lichens by YASUDA. Thus far the published list includes 182 species, 94 of which are described as new.—J. M. C.

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[17] LESTER-GARLAND, L. V., A revision of the genus *Baphia* DC. (Leguminosae). Jour. Linn. Soc. **45**:221–243. 1921.

[18] Species novae Indiae orientalis. Decas I. Jour. Indian Bot. **21**:44–54. 1921.

[19] MOORE, S. LEM., *Alabastra diversa*. XXXIV. Jour. Botany **59**:244–249. 1921.

[20] HUTCHINSON, J., and PEARCE, K., Revision of the genus *Tryphostemma*. Kew Bull. no. 7. 257–266. 1921.

[21] SPRAGUE, T. A., A revision of the genus *Belotia*. Kew Bull. no. 7. 270–278. 1921.

[22] GLEASON, H. A., A rearrangement of the Bolivian species of *Centropogon* and *Siphocampylus*. Bull. Torr. Bot. Club **48**:189–201. 1921.

[23] WAINIO, E. A., Lichens ab A. YASUDA in Japonia collecti. Bot. Mag. Tokyo **35**:45–62; 63–80. 1921.

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INFLUENCE OF SALTS ON BACTERIAL ACTIVITIES OF SOIL

J. E. Greaves

Salts occurring naturally in soils and those applied to them in various operations influence the number, species, and activity of the soil microflora. These factors in turn are reflected by yields obtained. Some substances applied to a soil may serve as food for the growing plant; others increase plant growth indirectly. This latter effect may be due to the changing of the physical, chemical, or bacterial properties of the soil. The substance may alter the physical properties of the soil to such an extent that the bacterial flora is modified. This in turn may increase or decrease the available plant food of the soil. Other substances may react chemically with constituents within the soil, and in so doing liberate nutrients which may be utilized directly by the growing plant (24). Again, they may directly modify the microflora and microfauna of the soil both as to numbers and physiological efficiency. In some cases all three changes may be wrought by the same salt. In each case the soil is so changed that its crop-producing power is modified. The question arises, therefore, as to what effect this or that fertilizer or soil amendment is going to have upon the bacterial activity of the soil. Furthermore, there are millions of acres of land which contain varying amounts of soluble salts. Some of these soils contain such large quantities of the so-called "alkalis" that no vegetation grows upon them.

Other soils contain only a medium amount of soluble salts, and the vegetation consists chiefly of alkali-resisting plants. Still other soils contain much smaller quantities of soluble salts, and they become injurious only when the soil is improperly handled. The reclaiming of the heavily charged soils and the maintaining of the others in a productive condition are problems confronting the soil chemist. These problems can be solved more successfully when the laws governing the influence of salts upon the growing plant and their action upon the chemical, physical, and biological properties of the soil are understood. This study was undertaken, therefore, with the hope of obtaining light on some of these laws. It was carried on with soils which naturally were productive, but

TABLE I

MOLES OF THE VARIOUS SALTS PER 100 GM. OF SOIL REQUIRED TO RETARD AMMONIFICATION IN THE SOIL IN UNIT TIME

	Chloride	Sulphate	Nitrate	Carbonate
Calcium	156×10^{-7}	5×10^{-3}	78×10^{-7}	11×10^{-3}*
Potassium	625×10^{-7}	125×10^{-6}	1×10^{-3}	6×10^{-3}
Iron	125×10^{-6}	625×10^{-7}	25×10^{-5}	6×10^{-3}
Manganese	125×10^{-6}	25×10^{-5}	5×10^{-4}	11×10^{-3}
Sodium	125×10^{-6}	5×10^{-4}	5×10^{-4}	6×10^{-3}
Magnesium	5×10^{-4}	5×10^{-4}	156×10^{-7}	1×10^{-3}

* Not toxic at 11×10^{-3}, highest concentration tested.

became unproductive through the addition of known quantities of various salts. This would give a soil which at first had the same physical, chemical, and biological properties, hence any difference found must be due to the salt added.

Using as a measure of toxicity of the various salts that quantity which if applied to a definite weight of soil reduces the ammonia produced in the soil as compared with a similar untreated soil kept under similar conditions, the values reported in table I were obtained. The quantity recovered from the soil by leaching was also determined, but for this study the quantity applied is used for the reason that the direct or indirect action of the addition of a specific salt is being determined and not the absolute point of toxicity, which undoubtedly will vary with different soils and conditions.

The soil used was a calcareous loam (8), rich in potassium, phosphorus, and all the essential elements except nitrogen, which was low, as was also the organic matter of the soil. The results as reported are the average of a great number of determinations, and represent rather accurately the toxic point of the various salts in this specific soil, hence the results represent the relative toxicity for several salts in this specific medium. This may or may not vary in a different medium. The results, therefore, are not to be taken as absolute but as relative, which can only justly be comparable with other results obtained under like conditions.

It is quite evident from these results that the toxicity of a compound is governed by both anion and cation. Without exception the chlorides are more toxic than the corresponding nitrates. The sulphate varies, depending upon the cation with which it is combined, whereas in every instance the carbonate is less toxic than any of the other salts. The relative toxicity for the anions, therefore, can be written in the order $Cl > NO_3 > SO_4 > CO_3$, thus indicating that the monovalent anions are more toxic than the divalent anions. It must be borne in mind, however, that there would be more anions in unit volume of the monovalent than of the divalent, where a divalent cation is combined with the monovalent anion, as these salts were tested on the basis of mole concentrations. When re-examined with equivalent ionic concentration, the difference, although small, still maintained the same order.

Excluding the difficultly soluble salts, and averaging the results for the cation, we obtain the series $Mn > Mg > Fe > Ca > Na > K$, or the divalent cation is found to be more toxic than the monovalent. Here also the number of ions would enter, and if the toxicity be due either to an osmotic or precipitant reaction the order is what would be expected.

Examining the results obtained with nitrification (13) on the same basis as has been done with ammonification, we obtain as the points of toxicity the results given in table II.

The most important conclusion to be drawn from these results as compared with the preceding is the greater sensitiveness of the nitrifying organisms to the different salts as compared with the

ammonifying organisms. There are three chlorides, one nitrate, and one sulphate which were slightly more toxic for nitrifiers than for ammonifiers. In most cases the resistance of the ammonifiers to salts is sufficiently greater that nitrification in a soil may be materially decreased without seriously interfering with ammonification. Moreover, the toxicity of salt increases with concentration much more rapidly toward nitrifiers than toward ammonifiers.

TABLE II

MOLES OF THE VARIOUS SALTS PER 100 GM. OF SOIL REQUIRED TO RETARD NITRIFICATION IN SOIL IN UNIT TIME

	Chloride	Nitrate	Sulphate	Carbonate
Calcium	78×10^{-7}	156×10^{-7}	3×10^{-3}*	625×10^{-7}
Potassium	156×10^{-7}	312×10^{-7}	156×10^{-7}	156×10^{-7}
Iron	1×10^{-3}	312×10^{-7}	156×10^{-7}	625×10^{-7}
Manganese	312×10^{-7}	25×10^{-5}	1×10^{-3}	78×10^{-7}
Sodium	4×10^{-3}	156×10^{-7}	156×10^{-7}	156×10^{-7}
Magnesium	78×10^{-7}	78×10^{-7}	625×10^{-7}	78×10^{-7}

* Not toxic at 3×10^{-3}, highest concentration tested.

TABLE III

MOLES OF THE VARIOUS SALTS PER 100 GM. OF SOIL REQUIRED TO RETARD AZOFICATION IN SOIL

	Chloride	Sulphate	Nitrate	Carbonate
Sodium	2×10^{-3}*	2×10^{-3}*	2×10^{-3}*	2×10^{-3}*
Potassium	2×10^{-3}*	1×10^{-3}	2×10^{-3}*	125×10^{-6}
Calcium	1×10^{-3}*	1×10^{-3}*	1×10^{-3}*	1×10^{-3}*
Magnesium	1×10^{-3}*	1×10^{-3}*	1×10^{-3}*	1×10^{-3}
Manganese	1×10^{-3}*	1×10^{-3}	1×10^{-3}*	1×10^{-3}*
Ferric	5×10^{-3}*	5×10^{-3}*	5×10^{-3}	5×10^{-3}

* Not toxic at this concentration, the highest tested.

The order of toxicity of the anion in the case of the nitrifiers is nearly the reverse of that for the ammonifiers. The order for nitrification is $CO_3 > NO_3 > SO_4 > Cl$, whereas the order of toxicity of the cation as measured in terms of nitrification becomes $K > Mg > Fe > Mn > Ca > Na$. It is evident, therefore, that both the anions and cations exert an influence in determining the toxicity of the salts.

Examining nitrogen fixation (12) in the same light the results given in table III are obtained.

Possibly potassium carbonate is more toxic to the nitrifiers of the soil than it is to the nitrogen-fixing organisms. It is certain that the azofiers in soil are much more resistant to these salts than are the other classes of microorganisms. The concentration of the salts in the soil was not high enough to permit conclusion as to the relative toxicity of the various anions and cations. The tests, however, make it certain that alkali soils which have a vigorous nitrifying or ammonifying microflora will maintain a vigorous azofying flora, even though they contain considerable quantities of soluble salts.

The influence of an anion upon the internal friction of colloids varies with concentration and reaction of medium, and it is interesting to note that the series for the ammonifiers is the same as that for acid solutions of proteins, whereas that for the nitrifiers is the order for these ions upon a neutral or alkaline solution of the protein. If, therefore, toxicity of these salts is due in a measure to their changing the internal viscosity of the protoplasm, we should have to assume a slight difference in the protoplasm making up the cell of the two groups of microorganisms, the one being electro-positive and the other electro-negative.

The order of toxicity of the cation to the ammonifiers is not far different from what would be expected if toxicity were due to a precipitation of the protoplasmic colloids. When the nitrifying series are examined in the light of the HOFMEISTER series, however, the potassium ion is found on the opposite extreme of the series from where it should be.

Whether this is due to analytical error or to the potassium ion being especially poisonous because of its changing the state of turgescence of the organic colloid is not clear. It would appear, however, that if the latter were the correct explanation, we might expect potassium to change positions in the ammonifying series. This would appear more reasonable, for both the ammonifiers and nitrifiers function normally in the same medium, and the same evolutionary forces have been at work bringing these organisms to their present condition.

The relative toxicity of the anions toward ammonifiers and the relative toxicity of the cation as measured in terms of both ammoni-

fication and nitrification point to the conclusion that toxicity is due in a large measure to osmotic influences. This conclusion is based on the assumption that the salts on passing into solution behave as they would in pure water, an assumption which is not warranted, for it is a well known fact that the addition of a salt to a soil causes an exchange of ions. This may either increase or decrease the total number of soluble particles in the soil solution, and hence correspondingly change the osmotic pressure of the soil solution. The logical procedure, therefore, is to determine the osmotic pressure (14) of the soil at the concentrations at which the various salts become toxic to the different classes of microorganisms. The results for such tests for the ammonifying series are given in table IV.

TABLE IV

OSMOTIC PRESSURE OF SALT-TREATED SOIL TOXIC TO AMMONIFYING ORGANISMS

	Chloride	Nitrate	Sulphate	Carbonate	Average
Sodium	1.62	2.77*	1.96	8.41*	1.79
Potassium	1.53	1.89	1.75	9.05*	1.72
Calcium	1.43	1.53	2.45	1.61	1.75
Magnesium	1.62	1.68	1.88	1.80	1.75
Manganese	1.56	3.53*	1.78	2.04	1.79
Iron	2.34	2.71*	1.84	1.84	2.00
Average	1.68	1.70	1.94	1.84	.....

* Not counted in the average.

Sixteen out of the twenty-four salts become toxic when the osmotic pressure ranges between 1.43 and 1.96 atmospheres. The average for the cation shows a variation of from 1.72 to 2.00 atmospheres, and in all except the iron ranges between 1.72 and 1.79, a difference of only 0.05 of an atmosphere. The anion shows a variation from 1.68 to 1.94. The small variation in osmotic pressure among the various salts at which they become toxic indicates that the osmotic pressure is an important factor in determining toxicity.

We find that when the ammonia produced had been reduced by about 10 per cent, the average osmotic pressure of the soil solution was 2.55 atmospheres; when the ammonia was reduced 25 per cent, the osmotic pressure was 5.49 atmospheres; and when ammonia

produced was 50 per cent of normal, the osmotic pressure was 9.5 atmospheres. Table VI gives these data for both ammonification and nitrification.

The nitrifiers, therefore, are more sensitive to osmotic changes than are the ammonifying organisms, but in both cases the toxicity increases more rapidly than does the osmotic pressure. This is very rapid in the case of the nitrifiers.

TABLE V

OSMOTIC PRESSURE OF SALT-TREATED SOIL TOXIC TO NITRIFYING ORGANISMS

	Chloride	Nitrate	Sulphate	Carbonate	Average
Sodium	4.25*	1.81	1.80	1.84	1.82
Potassium	1.53	1.67	1.75	1.49	1.61
Calcium	1.60	1.54	.....	1.54	1.56
Magnesium	1.81	1.59	1.88	1.79	1.78
Manganese	1.65	2.73*	2.22	2.01	1.96
Iron	4.94*	1.89	1.85	1.86	1.87
Average	1.65	1.70	1.90	1.85	.....

* Not counted in the average.

TABLE VI

REDUCTION OF PRODUCT	OSMOTIC PRESSURE TO REDUCE	
	Ammonification 1 per cent	Nitrification 1 per cent
10 per cent	0.255 atmospheres	0.197 atmospheres
25 per cent	0.199 atmospheres	0.117 atmospheres
50 per cent	0.190 atmospheres	0.088 atmospheres

The average for the anions in the case of the nitrifiers is within experimental error, the same as that for the ammonifiers. The average for the cation is slightly lower for the nitrifiers than for the ammonifiers, but quite similar in both series, thus indicating that the toxicity in both sets is governed by the same factor, possible osmotic pressure plus a physiological effect produced by the salt. The physiological influence may be due to the replacing of ion in the living protoplasm, thus changing its physical, chemical, and electrical properties so that they are incompatible with life. It is thus assumed with LOEB (18) that the toxicity of sodium salts on entering the cell is due to the formation of sodium proteinates,

which, if present in too great a proportion in the cell, confer upon the protoplasm properties which are incompatible with the maintenance of normal functioning. The toxicity of calcium salts is likewise attributed to an undue predominance of calcium proteinate in the cell. An admixture of several types of protein salts is required to give the protoplasm of the cell the exact qualities essential to the maximal furtherance of its vital activities. Two factors, therefore, may enter in the toxicity of a salt: (1) the permeability of the salt for the living protoplasm, and (2) the chemical, physical, or electrical influence of the salt upon the protoplasm after entering the cell.

Those cases in which the osmotic influence is the predominating factor should show a marked decrease in toxicity when the solution is diluted (11); whereas in those cases in which the physiological effect predominates, the addition of another salt which would increase osmotic pressure may show a decrease in toxicity, due to a physiological balancing of the solution.

Numerous experiments have shown that the relative toxicity of sodium chloride, sodium carbonate, potassium carbonate, and calcium carbonate, as measured in terms of ammonification, decreases as the water added to a soil increases. All the other salts become relatively more toxic, thus indicating that some factor in addition to osmotic pressure is entering. In this regard the nitrifying organisms act quite differently, and toxicity is neutralized with potassium chloride, potassium sulphate, magnesium nitrate, and magnesium chloride, but not with the remaining salts when water is added. Were osmotic pressure alone the disturbing factor, it would be impossible to neutralize the toxic action of one salt by the addition of another, thus increasing osmotic pressure and at the same time decreasing toxicity.

This conception of antagonism and balanced solutions was first applied to a study of bacteria by LIPMAN in 1909. In his experiments (20) on the rate of ammonification of *Bacillus subtilis*, he showed that there is some antagonism between sodium and magnesium. On the other hand, he (21) found no antagonism, but increasing toxicity, when magnesium and calcium were com-

bined. Later he (**22**) demonstrated that there exists, as measured by ammonification, a true antagonism between sodium chloride and sodium carbonate, and between sodium sulphate and sodium carbonate, thus indicating that the anions as well as the cations at times may play a part in antagonism.

KELLEY (**16**), in studying the ammonification and nitrification of certain soils, found no antagonism between magnesium and sodium. LIPMAN and BURGESS (**23**), however, observed in the case of nitrogen fixation by *Azotobacter chroococcum* an antagonism between sodium and magnesium.

WINSLOW and FALK (**39**) have observed antagonistic effects in experiments on *Bacillus coli*. They found that cultures suspended in solutions of sodium chloride or calcium chloride were decreased in number, that higher concentrations produced sterilization of the culture, and that a combination of sodium chloride and calcium chloride in the molecular proportions of 5 to 1 was favorable to the growth of the organisms.

SHEARER (**29, 30**) also demonstrated similar effects of salts upon the viability of *Meningococcus* and *Bacillus coli*. He found that a combination of sodium chloride and calcium chloride was favorable to growth, whereas each salt used separately retarded growth.

BROOKS (**1**) found that, as measured by the rate of respiration of *Bacillus subtilis*, there is a marked antagonism between sodium chloride and calcium chloride, and between potassium chloride and calcium chloride. The antagonism between sodium chloride and potassium chloride is slight, and the antagonism curve shows two maxima. Later, using the same method and organism, he (**2**) found a well marked antagonism between magnesium chloride and sodium chloride, and, contrary to the findings of LIPMAN (**21**), a very slight antagonism between magnesium and calcium. This is in keeping with my own (**9**) experience, which showed that a true antagonism exists between calcium sulphate *vs.* sodium carbonate, calcium sulphate *vs.* sodium nitrate, calcium sulphate *vs.* sodium sulphate, calcium sulphate *vs.* calcium chloride, calcium sulphate *vs.* magnesium sulphate, as measured in terms of ammonification.

This clearly indicates that the anion as well as the cation plays a part in antagonism. A similar antagonism exists between these salts as measured in terms of nitrification.

Furthermore, using the ammonia produced as the criterion, an antagonism is seen to exist between sodium sulphate *vs.* iron sulphate, calcium chloride *vs.* iron sulphate, sodium chloride *vs.* iron chloride, sodium chloride *vs.* iron sulphate, magnesium chloride *vs.* iron nitrate, sodium chloride *vs.* iron carbonate, calcium chloride *vs.* iron carbonate, calcium chloride *vs.* iron nitrate, sodium nitrate *vs.* iron chloride, calcium chloride *vs.* iron chloride, sodium carbonate *vs.* iron nitrate, sodium carbonate *vs.* iron carbonate, sodium sulphate *vs.* iron nitrate, sodium chloride *vs.* iron nitrate, magnesium sulphate *vs.* iron nitrate, sodium carbonate *vs.* iron sulphate, sodium nitrate *vs.* iron nitrate, sodium nitrate *vs.* iron sulphate, magnesium sulphate *vs.* iron chloride, and magnesium sulphate *vs.* iron carbonate. This was small in the case of the first pair, and increased in the order named until the last, which neutralized 75 per cent of the toxic effect of magnesium sulphate.

As measured in terms of nitrification, a true antagonism was found to exist between sodium carbonate *vs.* iron carbonate, sodium chloride *vs.* iron chloride, magnesium sulphate *vs.* iron nitrate, sodium carbonate *vs.* iron sulphate, sodium nitrate *vs.* iron sulphate, sodium sulphate *vs.* iron carbonate, calcium chloride *vs.* iron carbonate, sodium nitrate *vs.* iron carbonate, sodium chloride *vs.* iron nitrate, magnesium sulphate *vs.* iron carbonate, sodium nitrate *vs.* iron chloride, sodium sulphate *vs.* iron nitrate, sodium sulphate *vs.* iron chloride, magnesium chloride *vs.* iron carbonate, calcium chloride *vs.* iron nitrate, magnesium sulphate *vs.* iron chloride, sodium chloride *vs.* iron sulphate, magnesium chloride *vs.* iron chloride, sodium carbonate *vs.* iron chloride, and magnesium chloride *vs.* iron nitrate. This was low in the case of the first pair, and increased progressively in the order named up to the last named pair, in which the iron nitrate increased the nitrification 420.7 per cent over that soil treated with magnesium chloride alone.

The results, therefore, indicate the toxicity of soluble salts toward soil microorganisms to be due to an osmotic effect which makes it impossible for the cell to take up its normal nutrients,

but permits foreign or unbalanced constituents to enter. These foreign or unbalanced salts on entering the cell protoplasm interact with the proteins thereof, forming within the living protoplasm foreign proteinates, the physical, chemical, and electrical properties of which are different from those of the normal protoplasm. Hence we have the protoplasm rendered incapable of normal functioning. The first effect is governed to a marked extent by the osmotic pressure of the medium in which the organism is functioning, and the second by the specific salt, acid, or base which comes in contact with the protoplasm.

STIMULATING ACTION OF SALTS.—Many salts when added to a medium in which bacteria are functioning first stimulate, and as the concentration is increased the specific salt becomes toxic. The

TABLE VII

PERCENTAGES OF AMMONIA PRODUCED IN SOIL TO WHICH VARIOUS SALTS WERE ADDED, THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate
Sodium	106.0	107.8	100.0	110.1
Potassium	100.0	102.2	100.0	108.9
Calcium	100.0	100.0	103.2	114.6
Magnesium	100.7	100.0	104.5	103.5
Manganese	100.6	116.0	123.8	111.2
Iron	118.6	102.9	103.9	107.9

extent of this stimulation varies with the salt, the concentration of the salt, the medium in which it is used, and the specific micro-organism grown upon it. If the ammonia produced in unit time in untreated soil is taken as 100 per cent, we obtain the values given in table VII for the various salts.

All except six of the salts stimulate bacterial activity. There is a wide variation, however, depending upon the specific salt. The cations arranged in a descending order would be Mn > Fe > Na > Ca > K > Mg. Although there is a wide variation, depending upon the cation and anion, it is interesting to note that it is not these elements which are to be recognized as plant foods, but the catalizers which head the list. This also appears in the case of the anion where the series is $SO_4 > Cl > CO_3 > NO_3$. The cations would appear to play a greater part as bacterial stimulants than do the

anions. This indicates a very marked acceleration of the speed with which the protein is transferred into ammonia, which would result in a greater available supply of this compound for the action of the nitrifiers. The speed with which the ammonia is oxidized to nitric acid also increases with the addition of various salts, as is brought out in table VIII.

TABLE VIII

PERCENTAGES OF NITRIC NITROGEN PRODUCED IN SOIL TO WHICH VARIOUS SALTS WERE ADDED, THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate
Sodium	142.0	101.0	100.0	100.0
Potassium	106.5	106.4	100.0	100.0
Calcium	100.0	102.1	196.7	100.0
Magnesium	123.2	106.5	101.2	140.7
Manganese	112.9	125.4	113.2	108.4
Iron	128.4	100.0	102.0	117.4

TABLE IX

PERCENTAGES OF NITROGEN FIXED IN SOIL TO WHICH VARIOUS SALTS WERE ADDED, THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate	Average
Sodium	102.6	102.7	104.4	107.3	104.2
Potassium	100.0	101.3	105.0	100.0	101.6
Calcium	167.6	109.9	102.6	103.3	104.0
Magnesium	102.2	101.9	101.7	101.9	101.9
Manganese	102.3	102.2	102.4	100.0	101.7
Iron	104.3	102.0	100.4	101.8	102.1
Average	101.9	103.3	102.7	102.3	.....

All but six of these salts increase the accumulation of nitrates in the soil. Two which did not stimulate the ammonifiers also failed to stimulate the nitrifiers. In four cases there was no agreement between ammonifiers and nitrifiers. The manganese and iron salts are both strong stimulants, as was the case with the nitrifiers; potassium, which is an essential element, is the least effective of the cations, whereas chlorine is one of the most active, being exceeded in activity only by the sulphate ion.

It is evident that the nitrogen available to higher plants would be very materially increased through the addition of salts to a soil,

and furthermore, it is usually those salts which are considered soil amendments that exert the greatest influence. What effect would these salts have on the total nitrogen of the soil? This is answered in table IX.

Again potassium is least efficient, while sodium is one of the most efficient. It is interesting to note that the nitrates, which are usually stated as retarding azofication, stimulate it to a greater degree than any of the other compounds. In this respect calcium nitrate is more efficient than any other salt. This probably indicates that it is best to add nitrates to the soil in this form. It could not be claimed that this compound was carrying available calcium to the soil, for this soil already contains some 40 per cent of calcium and magnesium carbonate.

It is evident that these salts would increase both total and available nitrogen in the soil. They would also increase the crop-producing power in another way, namely, by increasing both directly and indirectly the available potassium and phosphorous of the soil (10).

SOLVENT ACTION OF BACTERIA.—BROWN (3) found that twelve out of twenty-three bacteria isolated from soil exerted a definite solvent action on difficultly soluble plant food. One organism which produced no gas, but a large amount of acid, exerted the greatest solvent action upon calcium carbonate; while other organisms which produced gas (largely carbon dioxide), but not as much acid as the former, gave an action more marked than of the stronger acid producer upon the dicalcium and tricalcium phosphates. *Bacillus subtilis*, *B. mycoides*, *B. proteus vulgaris*, and *B. coli communis*, as well as several agar cultures from garden soil, were found (26) to be capable of dissolving the phosphates of bone and to a less extent those of mineral phosphates. The greatest solvent action was exerted in media containing sodium chloride, potassium sulphate, and ferrous sulphate. Even yeast (17) may play an important part in dissolving phosphates. KROBER, however, considers that the life activity of the bacteria, that is, assimilation of phosphorus by the living organism, plays little or no direct part in solution of the phosphates, but that the latter is due to the action of the organic acid and of the carbon dioxide produced.

The acids produced by bacteria act upon various phosphates, changing them to the soluble monophosphate, but the rate of solution varies widely with the different compounds. Tricalcium phosphate, in precipitated form, dicalcium phosphate, and tetracalcium phosphate of Thomas slag are much more rapidly dissolved than the crystalline or the so-called amorphous phosphates. The general reaction is as follows: $2RCOOH+Ca_3(PO_4)_2=Ca_2H_2(PO_4)_2+(RCOO)_2Ca$. The reaction takes place most rapidly in soils containing large quantities of organic matter due to the active fermentation taking place in such soils.

GRAZIA (6) considers enzyme action to play a part in the dissolving of phosphates in soil, for he found the addition of chloroform to a soil reduced bacterial activity and decreased the acid produced, but at the same time the solution of phosphates was increased. This is in keeping with the finding of BYCHIKHIN and SKALSKI (4).

The presence of ammonium chloride and sulphate in the cultural media is especially effective, according to PEROTTI (25), in increasing the solvent action of bacteria for phosphorus. PEROTTI considers the successive steps in the solution or decomposition of phosphorus in bacteria cultures to be as follows: (1) generation of acids, (2) secondary reactions in the solution, and (3) production of a soluble phosphorus containing organic substance. The first two of these are the result of the activity of the bacteria on the phosphorus, and the last is due to the metabolic assimilation of the microorganisms.

The oxidation of sulphur by soil bacteria at times may generate sufficient acid to play a very important rôle in the dissolving of soil phosphorus. HOPKINS and WHITING (15), however, consider that the nitrosomonus are of first importance in rendering phosphorus and calcium soluble, due to the nitrous acid produced from ammonia: $(NH_4)_2CO_3+6O=2\,HNO_2+H_2CO_3+2\,H_2O$. The resulting nitrous acid reacts with the raw rock phosphate rendering it soluble, thus: $Ca_3(PO_4)_2+4HNO_2=CaH_4(PO_4)_2+2Ca(NO_2)_2$.

Analyses showed that about one pound of phosphorus and about two pounds of calcium are made soluble for each pound of nitrogen oxidized, aside from the action of the acid radicles asso-

ciated with the ammonia. The carbonic acid would play an important part also in this reaction: $4H_2CO_3+Ca_3(PO_4)_2=2\,Ca(HCO_3)_2+CaH_4(PO_4)_2$. They found that neither ammonifying bacteria nor nitrobacter liberated appreciable quantities of soluble phosphorus from insoluble phosphates. Whereas this would readily occur in soil poor in calcium carbonate, yet in those rich in calcium carbonate there would be only small quantities of phosphorus liberated. This is the conclusion reached by KELLEY, but where the soluble phosphorus is rapidly being removed by the growing plant, there is little doubt but that the various soil organisms play an important part in rendering phosphorus soluble.

Moreover, it is quite evident that *Azotobacter* in their metabolism transform soluble inorganic soil constituents either into soluble or insoluble organic forms. This is especially true of phosphorus which is found in the ash of these organisms in such large quantities. The phosphorus, on the death of the organism, is returned to the soil in a readily available form, for STOKLASA has found that 50 per cent of the nitrogen of these organisms is nitrified within six weeks, and there is no reason for believing that the phosphorus would be liberated more slowly. There is also the possibility that many of the constituents of the bacterial cell may become available through the action of autolytic enzymes without the intervention of other bacteria (**19**).

It is further evident that an organism which possesses the power when growing under appropriate conditions of generating 1.3 times its own body weight of carbon dioxide during twenty-four hours (**34**) must greatly change the composition of the media in which it is growing. Water charged with carbon dioxide is a universal solvent, and will attack even ordinary quartz rock. Granite and rocks related to it are rather quickly attacked with the liberation of potassium and other elements. Likewise, it would act upon the tricalcium phosphate of the soil with the formation of more readily soluble phosphates, for this substance is four times as soluble in water charged with carbon dioxide as it is in pure water: $Ca_3(PO_4)_2+2CO_2+2H_2O=Ca_2H_2(PO_4)_2+Ca(HCO_3)_2$. Moreover, the nitrogen-fixing organisms produce, among other products, formic, acetic, lactic, butyric, and other acids. The

kind and quantity of each depend upon the specific organisms and upon the substance on which they are acting. These substances are sure to come in contact with some insoluble plant food which may be rendered soluble, for they have a high solvent power for the insoluble phosphates (32). The resulting salts of calcium would be attacked further by bacteria with the formation of calcium carbonate (5).

Whether these processes will give rise to an increase in the water-soluble plant food of the soil will depend upon whether the products of the second, the analytical reactions, exceed the products of the first, the synthetic reactions. We must not lose sight of the fact that although many of the organic phosphorus constituents may not be soluble in pure water, they may be more available to the living plant than are the constituents from which they at first were derived through bacterial activity. This being the case, variations may be expected in the results reported from laboratory tests. STOKLASA (33) found that bacterial activity rendered the phosphorus of the soil more soluble, whereas SEVERIN (27) in his early work found the opposite to be true. Others have found that the solvent action of bacteria for insoluble phosphates is in direct proportion to the acid secreted by the organism (26).

In a later work SEVERIN (28) obtained different results. He used three soils, one sterile, a second sterilized and inoculated with pure cultures of *Azotobacter*, and a third sterilized and inoculated with cultures of *B. radicicola* and *Azotobacter*. The solubility of the phosphorus increased 8 to 14 per cent over that in the sterile soil. The acid-producing organisms, due to the acid secreted and their intimate contact with the soil particles, possess the power of dissolving silicates. Moreover, arsenic greatly stimulates nitrogen fixation, and there is a relationship between this increased bacterial activity and the form and quantity of phosphorus found in such a soil (7).

Although the metabolic activity of *Azotobacter* gives rise to large quantities of phosphate solvents, these organisms transform phosphorus into organic phosphorus compounds less rapidly than do the ammonifiers (35). There are cases, however, in which bacterial activity has decreased the water-soluble phosphorus of

the soil and of raw rock phosphate (**36, 37**). This, however, does not indicate that it is less available, for, as pointed out by TRUOG (**38**), the mixing of floats with manure caused an immediate decrease in the solubility of the phosphorus in 0.2 per cent citric acid solution, yet when thoroughly mixed with the feeding area of the soil its availability was increased to such an extent that some species of plants apparently were able to secure almost an adequate supply of phosphorus from this material. The addition of manure to the soil greatly increased the carbon dioxide production, and for a short time measurably increased the solvent action on floats. Where there is a decrease for a time of water-soluble phosphorus in fer-

TABLE X

TOTAL WATER-SOLUBLE PHOSPHORUS PLUS ORGANIC PHOSPHORUS IN SOIL TO WHICH VARIOUS SALTS HAD BEEN APPLIED AND LEFT FOR THREE WEEKS, THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate
Sodium	124.0	99.8	.....	105.3
Potassium	106.6	105.7	.....	102.1
Calcium	101.6	105.9	88.4	99.1
Magnesium	103.1	105.3	108.4	85.2
Manganese	99.3	86.1	101.1	135.1
Iron	100.2	103.4	118.3	92.5

menting media, it is probably due to the formation of phosphoproteids within the bodies of the bacteria (**31**), and these would later be rendered soluble, due either to further bacterial activity or autolytic enzymes. This increased bacterial activity should and actually does result in an increased water-soluble and organic phosphorus of the soil, as may be seen from table X, in which the water-soluble phosphorus plus the organic phosphorus in the untreated soil has been taken as 100 per cent.

In all except seven cases where the salts had increased bacterial activities there also resulted an increase in the available phosphorus. Moreover, associated with this increase of available phosphorus go increased crops as found in field and pot experiments, and the significant feature of these facts is that there is also an increase in the phosphorus of these plants (**7, 10**).

Summary

Many salts when applied to a soil in small quantities increase the bacterial activities of that soil. This is manifest by an increased production of ammonia, nitrates, and soluble and organic phosphorus, together with an increased nitrogen fixation. Usually, although not always, those salts which become toxic in the lowest concentration are the greatest bacterial stimulants.

There is a very close correlation between toxicity of the various salts and the osmotic pressure produced in the soil, thus showing that toxicity is due in part to osmotic disturbances. Another factor of equal importance is the change in chemical composition of the protoplasm resulting from the formation of salts of the protein other than those normally occurring in the living protoplasm, thus incapacitating them for their normal functions.

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VASCULAR ANATOMY OF ANGIOPTERIS EVECTA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 290

HUGO L. BLOMQUIST

(WITH PLATES V–VIII AND EIGHT FIGURES)

In the study of the vascular anatomy of plants it has been realized that it is necessary to study the successive stages which the plant passes through in the progress of its development. Since it is the aim, first of all, to determine the position, interrelationship, and structure of the different vascular components of the mature plant, this procedure not only facilitates the work, but in most cases perhaps is absolutely necessary. Likewise, in the search for facts which are to show indications of phylogenetic relationship, it is unnecessary to point out that it is in the developmental stages that these are most likely to be found. This method has proved to be especially necessary in the case of the Marattiaceae, for the vascular structures in the mature plant are not only very complex and difficult to interpret, but as the plant develops there are marked changes appearing in the anatomical structures from stage to stage. In some of the previous investigations on the anatomy of the Marattiaceae the failure to follow this method has led to much confusion and incorrect interpretation of the facts.

Historical

Considerable attention has been given to the Marattiaceae in the last fifty years, due chiefly to the evidence from geological records that this group of ferns, or a closely related group, was of great abundance and wide distribution in the past, and to the unique morphological and anatomical features which this group presents. Most of the work done on the anatomy of the Marattiaceae has been rather scattered and incomplete. The reason for this has chiefly been the lack of material, as these ferns are restricted to rather inaccessible parts of the world. Although plants have been transported to many conservatories, this has not

appreciably altered the situation, because of the tardiness in the germination of spores and the slowness of growth of the gametophyte.

The investigations on the anatomy of the Marattiaceae up to 1900 have been reviewed by Miss SHOVE (12). It is evident that the work up to that time was rather unsatisfactory, as only mature stems were dealt with, and the material in most cases was limited to one specimen. Miss SHOVE also worked with a mature stem. Since then, however, several investigations have been carried out in which the younger stages have been studied. In 1902 FARMER and HILL (8) published the results of an investigation on the development of the vascular anatomy in *Angiopteris evecta*, which was supplemented by a paper on the younger stages by CAMPBELL (2) in 1909. A thorough investigation on the development of the vascular anatomy of *Marattia alata* was carried out by Miss CHARLES (7) in 1911. This was the first investigation on the anatomy of this group based on an abundance of material. In 1917 WEST (13) added another contribution to the knowledge of the vascular anatomy of this group in a paper on the anatomical structures of *Danaea* spp. and several of the other genera. This investigation also was based on an abundance of material.

In comparing the conclusions drawn in this literature, it is evident that the vascular anatomy of the plants of the six genera of the Marattiaceae is very complex, but shows a striking similarity in facts both in the developmental stages and the more mature plants. Differences which are reported are mainly in details and in the interpretation of facts. There seems to be an agreement of opinion that the sporophyte of the Marattiaceae is traversed by a central strand of vascular tissue, called a dictyostele, from which roots and leaf traces arise. This stele is at first solid, or protostelic, then passes to a more or less tubular form, a solenostele, which is soon broken open by leaf gaps which are at first wholly repaired, but later overlap, so that the central strand becomes a banded structure which appears crescent-shaped in cross-section. This at first is simple, but later becomes complicated by the appearance of commissural and medullary strands. Disagreement is shown in regard to the meristematic regions, the

presence or absence of an endodermis, the origin of lateral roots, and whether the central strand is of cauline or foliar origin. The relationship between roots and leaves and the vascular arrangements in the stipules are also points still unsettled.

Material and methods

The material used in this investigation was collected on the island of Tutuila, Samoa, by Dr. W. J. G. LAND, of the Hull Botanical Laboratory, during October and November 1912. It consisted of an abundance of young sporophytes of *Angiopteris evecta*, ranging from the first to the tenth leaf stages and a few somewhat more advanced.

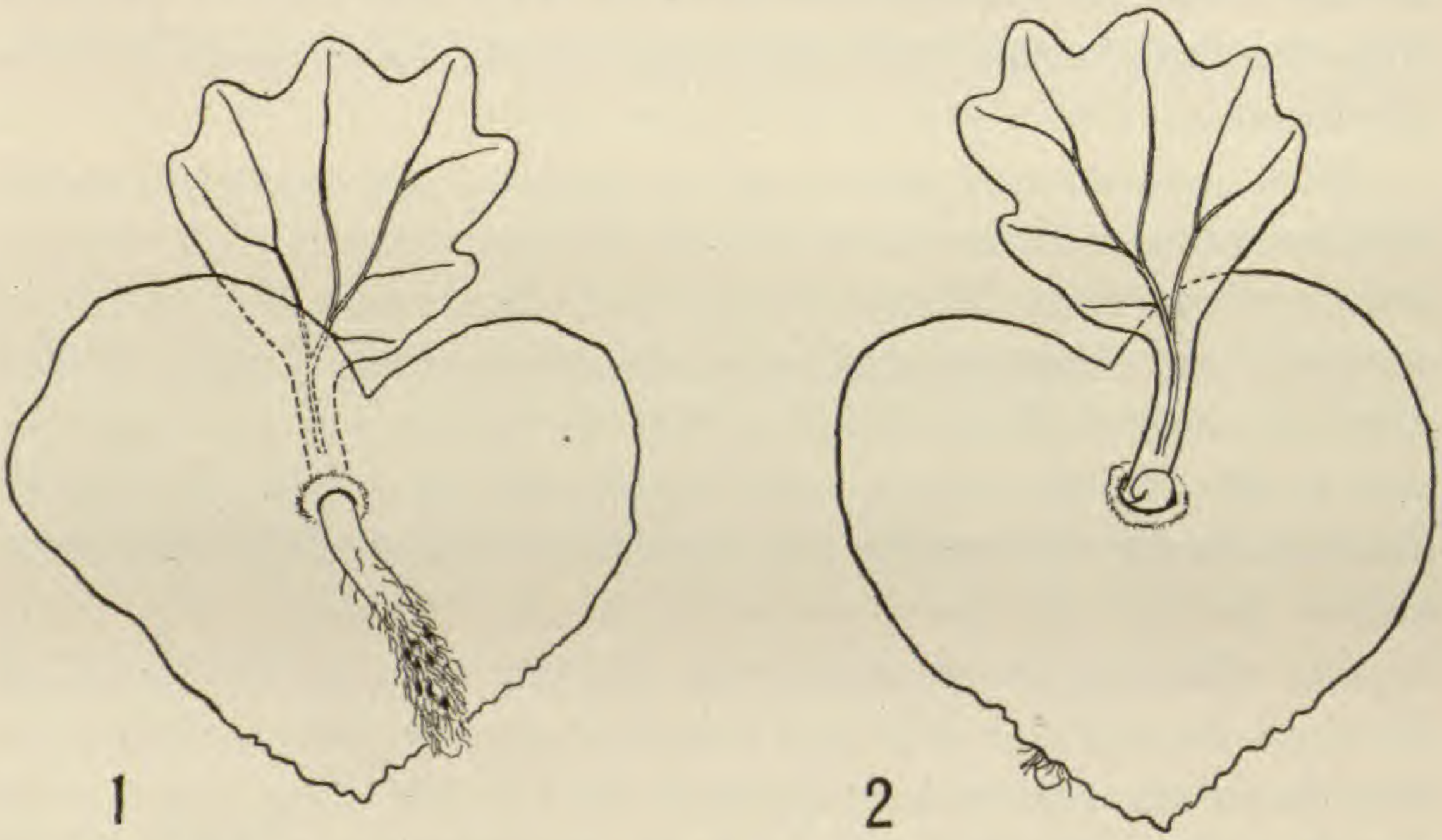
This material was imbedded in paraffin, and complete serial sections ranging from 10 to 20 μ in thickness were made with a rotary microtome. These sections were then studied from below upward, and diagrammatic reconstructions were drawn of the different stages. From these a final reconstruction was made in which the different stages were superposed one upon another to show as clearly as possible the successive stages which the plant passes through in the progress of its development. The most serious difficulty encountered was the fact that as the sporeling develops beyond the third and fourth stages, the lower part of the stem becomes more or less distorted, and in still older stages even decays. This difficulty was overcome by studying a close series of stages, which the material provided, selecting characteristic sections from the younger stages, and carefully matching these with sections representing the same morphological location in the older stages. The possibility of misinterpretation due to local variations, which are quite common in the anatomy of *Angiopteris*, was guarded against by studying several specimens of the same or approximately the same stage.

Investigation

External features

While the external features of *Angiopteris* have been described by several investigators, these will be reviewed in part to assist in relating the internal structures with the external features. The

first peculiarity of the young sporophyte of *Angiopteris*, as well as of all Marattiaceae, is the way in which the first leaf makes its exit from the gametophyte. As is well known, in most ferns the primary leaf grows first downward, and then emerges from under the gametophyte through the notch in the heart-shaped form of the latter. In *Angiopteris*, on the contrary, the first leaf pushes its way upward through the gametophyte and emerges on the dorsal side, as shown in figs. 1 and 2. CAMPBELL (3) attributes this behavior to the fact that the first division of the fertilized egg comes



FIGS. 1, 2.—Gametophyte and sporeling of *Angiopteris*, showing mode of exit of primary sporophyte organs from prothallus; ×4.

in transversely to the axis of the archegonium, or parallel with the axis of the gametophyte; while in most ferns it is usual to find the first wall perpendicular to the gametophyte, or parallel with the axis of the archegonium. In an unpublished paper by LAND (11) on the embryogeny of *Angiopteris*, the presence of a well defined suspensor is reported, and he suggests that this organ very probably is responsible for the initial upward growth of the primary leaf in the Marattiaceae. The suspensor has now been reported for two other genera, *Macroglossum* (5) and *Danaea* (1), and it is probable that its presence in the remaining genera awaits further investigations.

The next point to be observed is the appearance of the stem tip. About the time the first leaf has emerged from the gametophyte, a slight depression is seen on the dorsal side of the petiole where this passes into the root. At the bottom of this depression there is a well defined apical cell, which can easily be seen from longitudinal sections of the young sporophyte. The second leaf appears on the crest of this depression, usually about 130° from the first, while the corresponding root emerges directly below, next to the primary root. The succeeding leaves appear at approximately the same distance from the next older leaf that the second appeared from the first, thus forming a spiral arrangement which may be clockwise in one plant and counter-clockwise in another. This habit has an important bearing upon the development of the internal structure, as will be shown later. The succeeding roots grow through the cortex and appear from below the thickening stem, and later penetrate the sides. The relation between the roots and the corresponding leaves cannot be determined from external appearances after the third and fourth leaves have appeared. In none of the specimens studied had the roots branched, and no differences, except in size, could be discerned from the external appearances of the different roots.

A characteristic feature of the mature plants of the Marattiaceae is the presence of conspicuous fleshy stipules. In the first leaf of *Angiopteris* no stipules are visible, while in the second slight thickenings show their rudiments. No well developed stipules appear until the fourth leaf. At this stage the stipules appear as lateral swellings on the petiole as soon as the leaf is visible. As the leaf develops and the petiole elongates, the upper part of the stipule splits away from the petiole, and this gives rise to the more or less pointed lobes. In all sporelings examined there were no indications of dorsiventrality, either in external appearance or internal structure.

General internal structure

The general internal structure of a stem of *Angiopteris* which has developed beyond the first few stages may be divided into two main regions, the cortical and the central vascular. The cortex,

which is composed of rather large, thin-walled cells without intercellular spaces, is relatively thick, with an abundance of starch deposited in the cells surrounding the central region. In the younger stems cells filled with tannin are quite abundant, but in the older stages these are less common. This cortical area is traversed by the outgoing leaf traces, and numerous roots digest their way down through it. The leaf traces are single in the younger stages, but in the older levels these bifurcate before entering the stipules. Because of the spiral arrangement of the leaves, the leaf traces are arranged in zones which exhibit a spiral appearance in cross-section, in which the younger are closer to the center than the older. No fusing of leaf traces or a branch from one leaf trace with that of another takes place. The leaf traces pass outward directly.

The central region consists of the vascular tissue, imbedded in a ground tissue which may be called the central parenchyma. This parenchyma does not differ markedly from the cortical tissue except that the cells are somewhat smaller, have thinner walls, and less starch. In the first few stages there is a conspicuous endodermis, but in the older stages there is no such layer of cells which marks off the central region from the cortex, and the central parenchyma passes insensibly into the former. The central vascular tissue consists of a strand which appears more or less crescent-shaped in cross-section. The xylem is endarch and is completely surrounded by phloem. From one edge of this strand leaf traces are given off, and this contribution is always from the same edge in the same plant (figs. 3, 4). On the outer side of the other edge of this central strand a root stele is joined, usually at about the same level that the leaf trace is given off. The vascular tissue thus contributed to the leaf traces is made good on the other edge in two ways, by an increase of vascular tissue on the edge itself and by the addition of commissural strands. From the edge of each leaf trace abutting on the opening in the central strand a commissural strand is given off where the leaf trace is freeing itself from the central strand. This bends outward and passes diagonally upward and fuses with the opposite edge of the central strand just below where the next root above is attached (figs. 9–15).

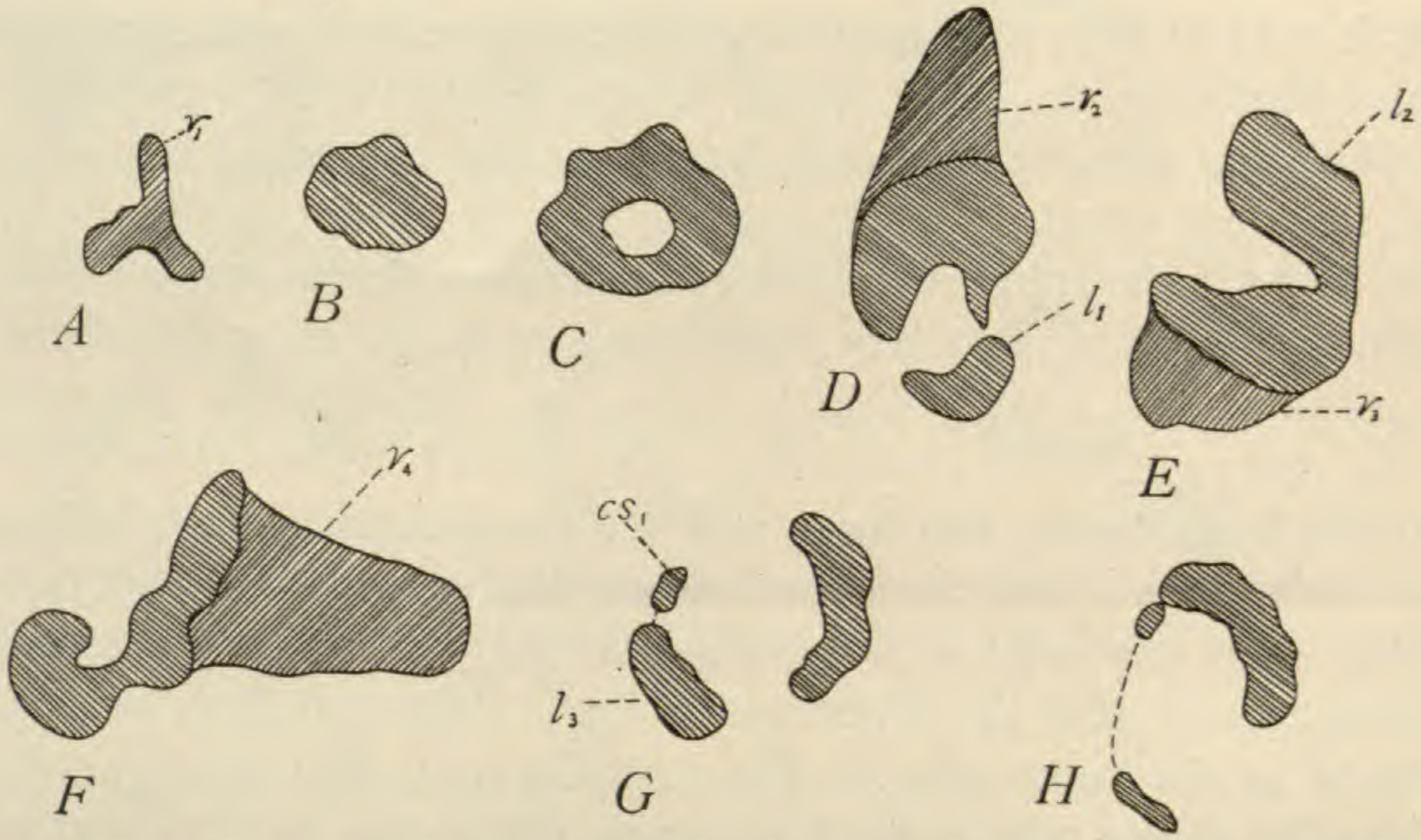


FIG. 3.—Diagrams of sections of stele, showing stele development up to third leaf (only xylem shown); sections not successive: r_1, r_2, r_3, r_4, first, second third, and fourth root traces; l_1, l_2, l_3, first, second, and third leaf traces; cs_1, first commissural strand; ×176.

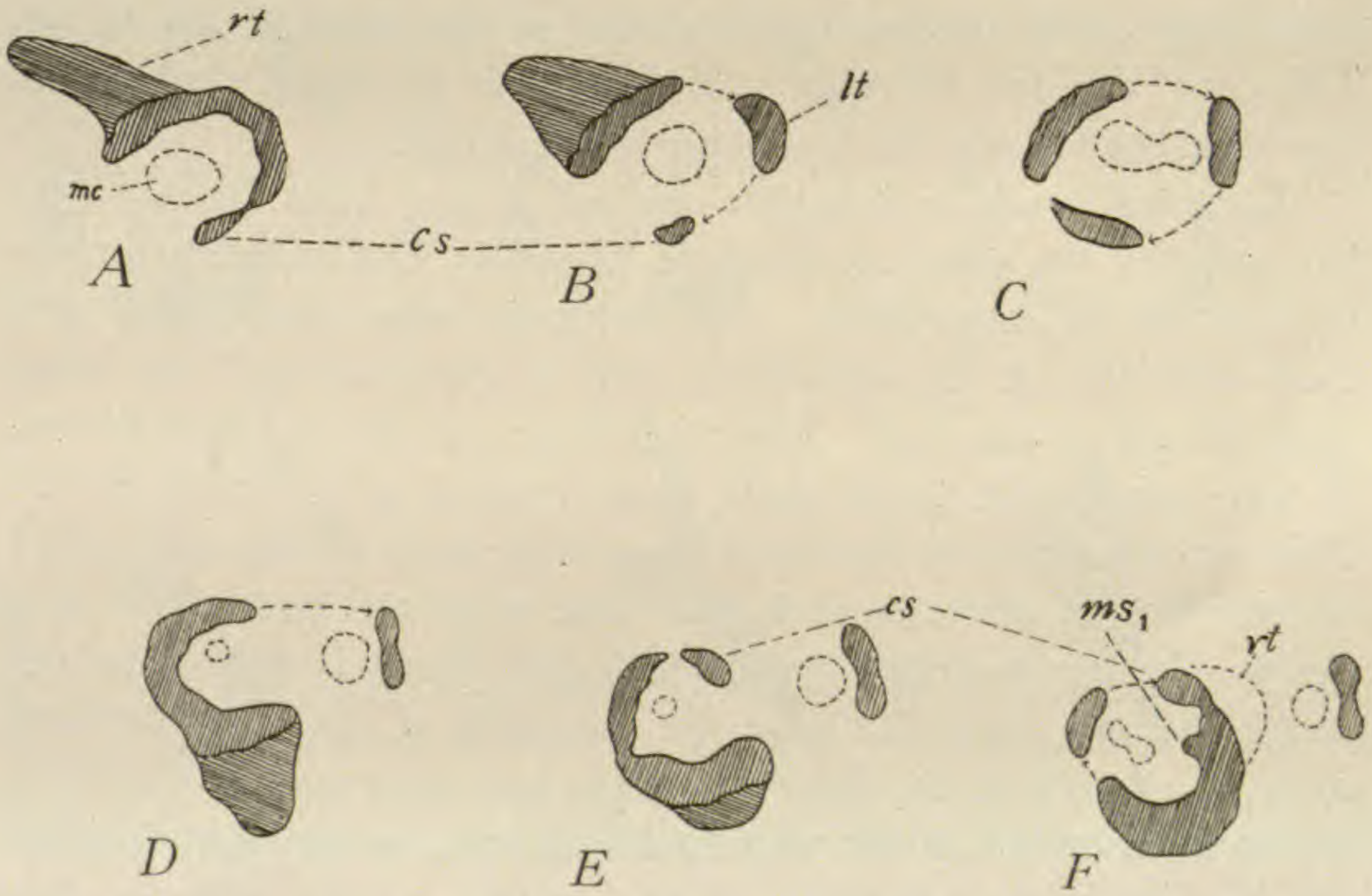


FIG. 4.—Diagrams of sections of stele, commencing slightly below where first medullary strand appears (only xylem shown); sections not successive: *rt*, root stele; *cs*, commissural strand; *lt*, leaf trace; *mc*, mucilage canal; ms_1, first medullary strand; ×80.

It is evident that by a continual loss of tissue from one edge and an addition on the other, in the successive sections, the crescents will appear to move in a circle, indicating that the strand is a more or less open spiral. The explanation for this spiral condition is to be found in the spiral succession of the leaves. In the stages above the eighth leaf, a medullary strand appears on the inner side of the central strand, usually slightly above and opposite the junction of a root stele. This passes diagonally upward, crosses the central parenchyma tissue, and fuses with the commissural strand before the latter has joined the central strand (fig. 5). In this condition they meet the edge of the central strand, and the commissural strand fuses with the latter and is lost. The medullary strand, fused to the inner side of the central strand, but retaining its identity, passes upward until the union of the root and the central strand has been cleared, when it frees itself and repeats its course across the central parenchyma tissue. This behavior of the medullary strand agrees in the main with the condition in *Marattia* as described by Miss CHARLES (7). In the lower levels of the stem a mucilage canal appears in the center of the parenchyma tissue. This canal divides wherever a leaf trace is given off, and one of the branches follows the inner side of the latter.

Each leaf trace is definitely related to one root. HOLLE (10) has reported the same condition in *Marattia*, but found two roots to each leaf in *Angiopteris*. The writer in some cases found two roots attached on the same level, and it appeared as if two roots were related to one leaf. After following out the series, however, it was found that these roots were related to different leaves. This variation will be discussed more fully later. The root which appears almost opposite each leaf trace is related to the next leaf above. In the younger stages each leaf trace is joined to the central strand directly above the insertion of its corresponding root (fig. 9); but in the older stages the leaf trace may be displaced to the right or left of the corresponding root, as the case may be, due to the spiral condition of the central strand (figs. 11–15). The distance between the root stele and the junction of the corresponding leaf trace above increases also in the successive stages.

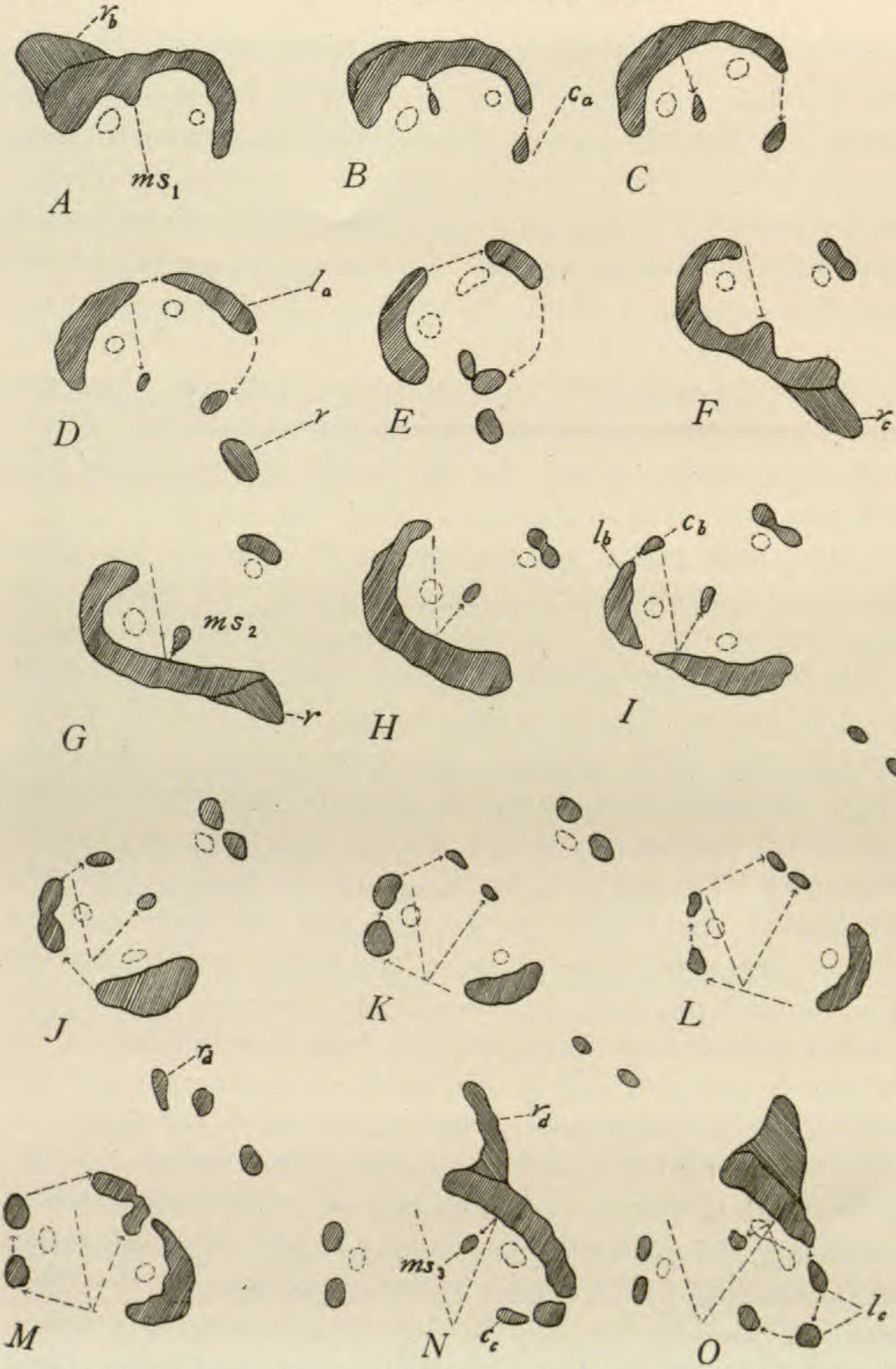


FIG. 5.—Diagrams of sections of stele, showing course of different vascular components after appearance of medullary strand (only xylem shown); sections not successive: *r*, root stele; *l*, leaf trace; *ms*, medullary strand; *c*, commissural strand; *mc*, mucilage canal; dotted lines with arrows indicate origin; and approximate courses of leaf traces, commissural strands, and medullary strand; ×16.

First leaf stage

The first few stages have been described by Farmer (8) and Campbell (2), and the present investigation adds little to their descriptions. The primary root is stated by them to be diarch. The writer found this to be true in some cases, but in as many cases the root was found to be triarch. Similar variations have been reported by Campbell in *Danaea*, *Botrychium*, and *Helminthostachys* (3). The endodermis is well organized and is separated from the protoxylem and protophloem by a uniseriate pericycle. In some places the protoxylem abuts directly on the endodermis, as has been reported previously (8). In the diarch stele there are two protophloem points and in the triarch three (figs. 16, 17).

The transition region in *Angiopteris*, as reported for other Marattiaceae, is a solid strand of vascular tissue with more or less irregular outl'ne (fig. 19). In passing from this region upward, some cells in the xylem remain unlignified, usually near the center of the stele, so that the latter may become more or less tubular in cross-section. This condition has been called a solenostele (fig. 25). In some cases, however, these parenchyma cells are more scattered, and the condition resembles what has been reported for *Danaea* by Brebner (1). In this genus the parenchyma cells appear without any relation to the center of the protostele. In either case the appearance of these unlignified cells marks the union of the next root stele and the corresponding leaf trace above. The writer agrees with Brebner that "the parenchyma . . . . called pith is probably simply due to the root-junction and the preparation of the departure of the leaf trace." No phloem is developed in the center of the tubular stele at this stage.

A longitudinal section of the sporeling at this stage shows a well defined apical cell at the stem tip (fig. 18). This apical cell has been reported by Campbell in *Macroglossum* (5), by Miss Charles in *Marattia* (7), and by West in *Danaea* (13). In a transverse section it was impossible to locate definitely this apical cell, but from the shape of the cells in the stem tip region it seems that this cell is four-sided. No procambial tissue is to be observed below the stem tip, a condition which has been reported by Brebner for *Danaea* and by Campbell for *Angiopteris*. The first differentia-

tion of vascular tissue is found to be related to the second leaf trace. The few tracheids which join up the first leaf stage with the second pass into the second root stele, while some pass directly upward and add to the structure of the second leaf trace (fig. 3 *D*, *E*).

Second leaf stage

The second root appears slightly above the transition region, about 130° from the first leaf trace. Fig. 20 shows this root in a very young stage. It is impossible to state from what tissue this originates, since it appears before any differentiation of tissues has taken place. The same is also true of *Marattia*. The second leaf trace appears directly above the second root. Some variation occurs at this stage; the second leaf trace may be joined to the third by both edges, in which a second tube is formed and the gap formed by the first leaf is repaired. In as many cases, however, only one edge is joined up with the third leaf trace, and in such cases the leaf gap is not repaired. In either case the phloem occurs on the inside as well as on the outside of the vascular tissue. The endodermis which was very evident in the first stage is more difficult to locate. Although endodermal thickenings occur, the characteristic organization is more or less broken up. In no cases was the vascular tissue observed to become a solid strand after the junction of the first and second leaf stages had been passed. In *Marattia*, on the contrary, the protostelic condition may appear more than once.

Third leaf stage

After the second leaf stage has been passed, the vascular tissue of the central region never appears as a tube. The third root stele originates on the level with the outgoing of the second leaf trace from the central region. At this stage the leaf trace comes off from one edge of the central strand, which is the vascular tissue leading up to the fourth leaf trace (fig. 3 *F*–*H*). The latter tissue now assumes a crescent-shaped form in cross-section. A few scattered tracheids may come off the free edge of the third leaf trace and wander back toward the central strand, or may end blindly before arriving there. These tracheids represent the rudimentary commissural strand, which in many cases is fairly

well developed at this stage (fig. 3 *G*, *H*). The leaf trace passes out to the petiole undivided, and the stipules, which are not conspicuous at this stage, have no vascular strands.

Fourth and fifth leaf stages

In the fourth leaf stage the commissural strand is well developed and separates from the leaf trace after the latter has passed a considerable distance from the central strand, then it approaches the opposite edge of the latter. This behavior is the same as shown in fig. 4 *A*–*C*. Meanwhile this edge of the central strand is approaching the commissural strand by the addition of vascular tissues, and when the two meet, fusion takes place (fig. 4 *C*–*F*). At this stage a mucilage canal appears in the center of the central parenchyma. This divides where the leaf trace goes outward, and one branch follows the inner side of the trace. The fifth stage is very much like the fourth except that the strands are larger and the commissural strand leaves the leaf trace closer to where the latter comes off the central strand. The central strand has increased in size so that the root stele reaches only half way around the other side. No well defined endodermis is discernible in this and the subsequent stages.

Sixth and seventh leaf stages

The sixth and seventh leaf stages differ from the fifth mainly in the stipular region, which will be discussed later.

Eighth and ninth leaf stages

In the eighth leaf stage a new feature presents itself in the appearance of a medullary strand. This strand is first visible coming off the inner side of the central strand opposite the junction of the ninth root (fig. 4 *F*). The medullary strand takes a diagonally upward course, crosses the central parenchyma, and fuses only partially with the commissural strand immediately before the latter has met the central strand. Both join the edge of the central strand just below the junction of the tenth root (fig. 5 *A*–*F*). The commissural strand becomes an integral part of the central strand, but the medullary strand remains visible, joined to the inner side of the central strand, and passes upward to the upper level of the

root junction (fig. 14). In these stages the leaf trace bifurcates while still within the cortex.

Tenth and subsequent stages

The tenth stage is very similar to the eighth and ninth, except that the different strands have increased in size. In cross-section the crescent shape of the central strand is less evident, especially

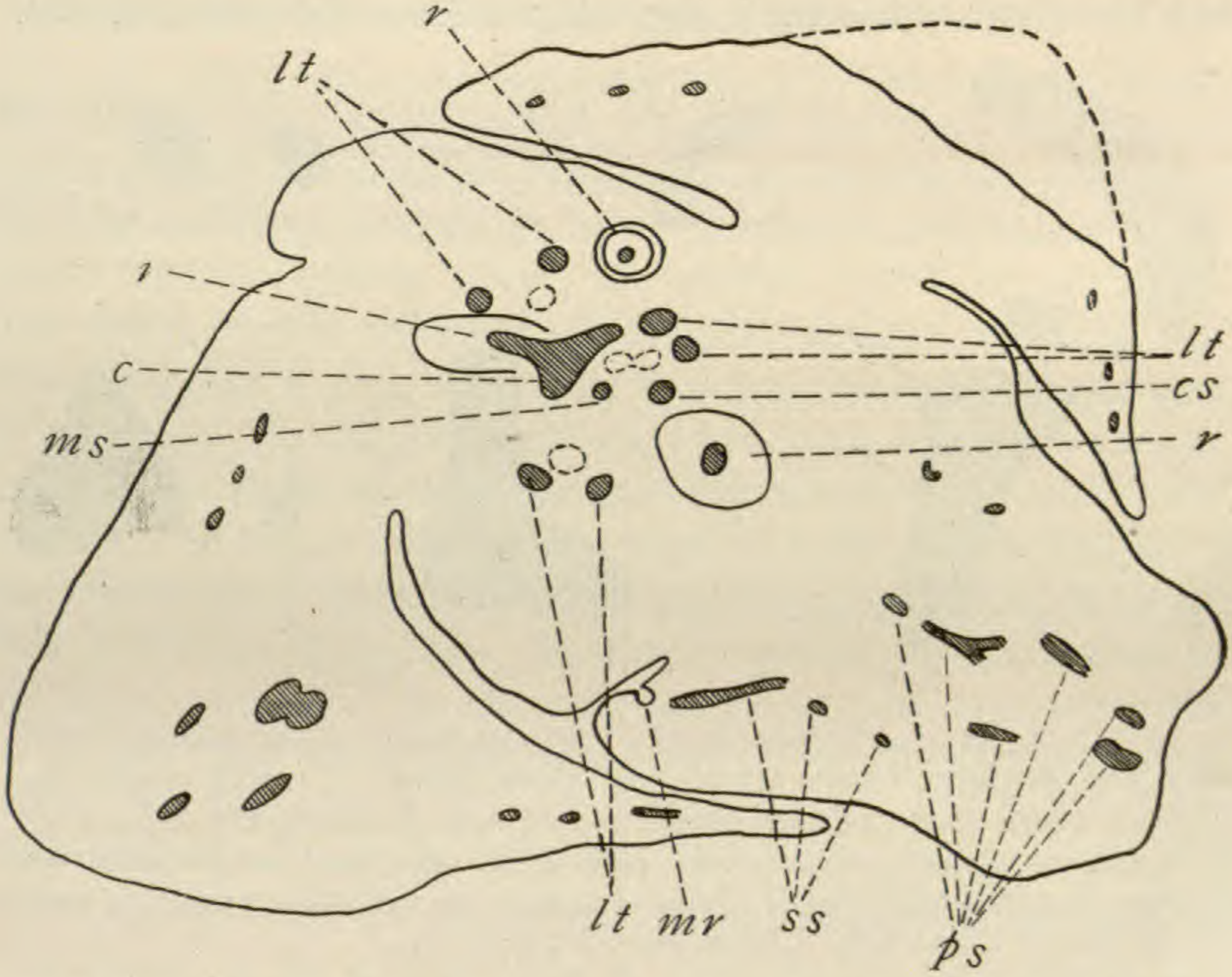


Fig. 6.—Diagram of section of stem above tenth leaf stage: *c*, central strand; *ms*, medullary strand; *cs*, commissural strand; *lt*, leaf trace; *ss*, stipular strands; *ps*, petiolar strands; *r*, root; *mr*, meristematic region of stipule; ×8.

in the region of a root junction. In some places the commissural strand and the central strand are very similar both in size and shape. The central and the commissural strands and the leaf traces are always endarch, while the medullary strand is exarch (figs. 21, 22). The commissural strand comes off close to the central strand, and the leaf trace bifucates close to the central region. At this and the subsequent stages five vascular strands may leave the central strand approximately on the same level; namely, the eighth leaf

trace (which usually comes off divided), a root stele, the commissural strand, and the medullary strand. A section of the sporophyte at this stage is shown in fig. 6.

In the more advanced specimens studied the exact stage could no longer be determined, owing to the distortion and decay of the lower regions. The difference between these and the previous stages was shown in the earlier bifurcation of the leaf traces, which may take place as the leaf is preparing to leave the central strand.

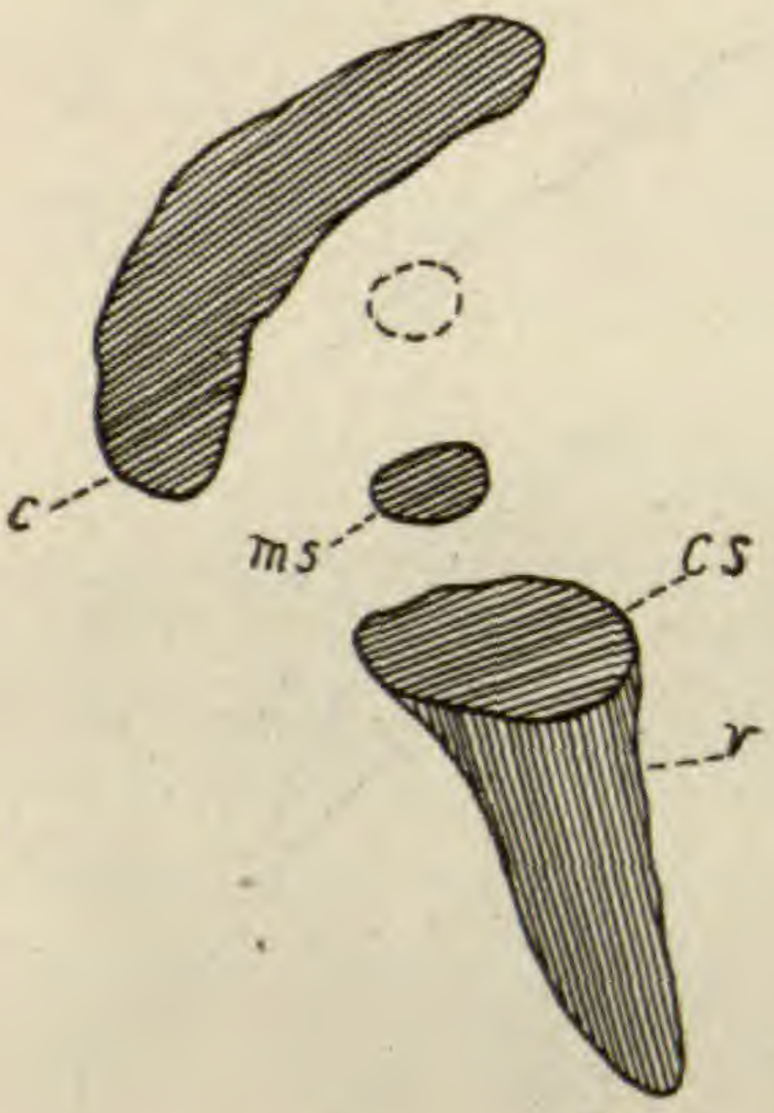


FIG. 7

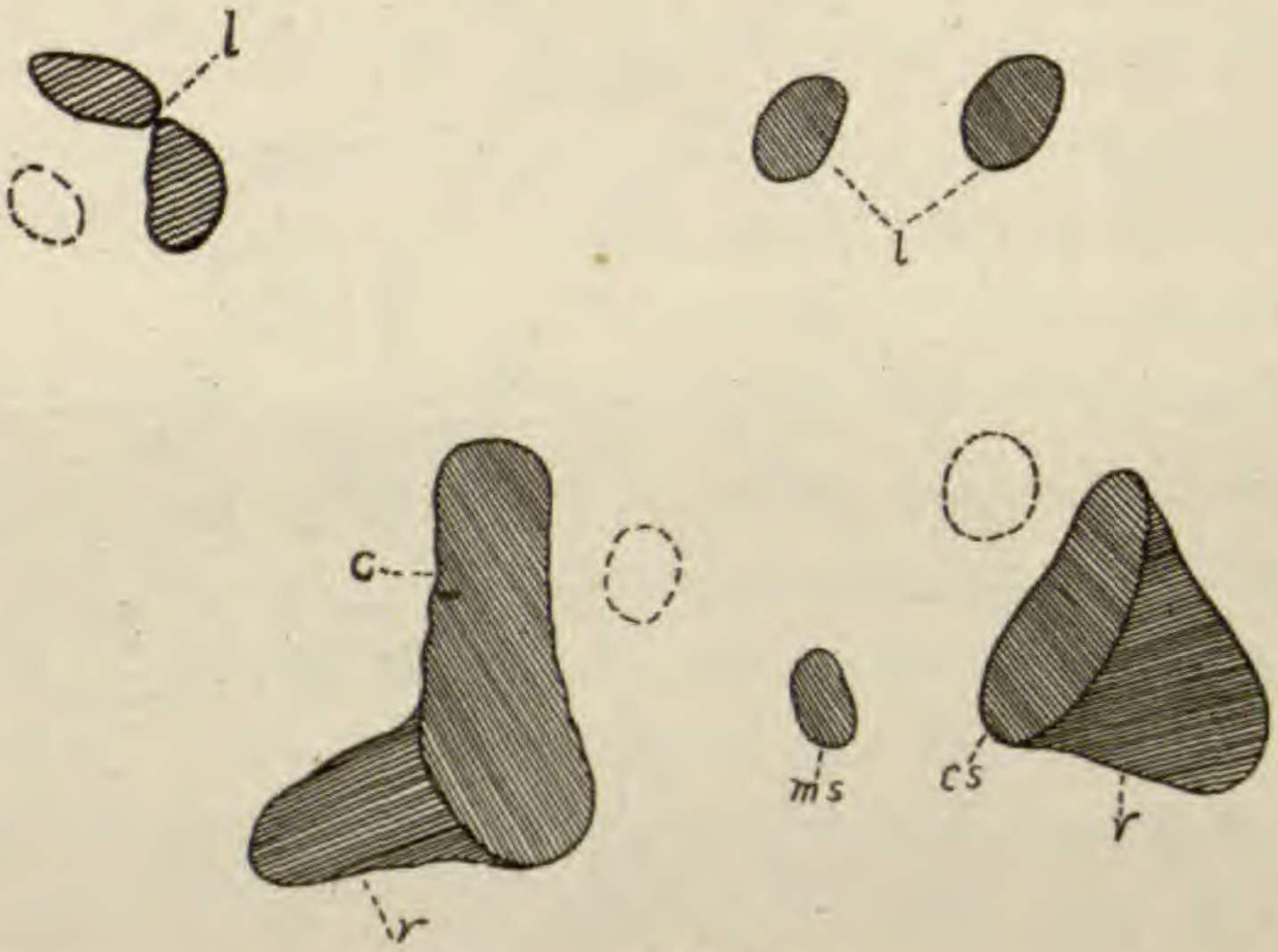


FIG. 8

FIGS. 7, 8.—Fig. 7, diagram showing root attachment to commissural strand before latter has joined central strand (only xylem shown); *c*, central strand; *ms*, medullary strand; *cs*, commissural strand; *r*, root stele; *l*, dividing leaf trace; ×38; fig. 8, diagram showing two roots on same level; one joined on central strand and other on commissural strand: *c*, central strand; *ms*, medullary strand; *cs*, commissural strand; *r*, root stele; *l*, bifurcated leaf trace; ×38.

The commissural strand also appears earlier, in some cases before the leaf trace is free. The two branches of the leaf trace bifurcate while within the cortex, so that here four strands from one leaf trace enter the stipular region. Considerable variation was observed in the attachment of the commissural strand. This may run up farther before merging with the central strand, and the root which is usually attached to the central strand may be inserted on the commissural strand (fig. 7). In some cases also a root may appear higher on the central strand, bringing two roots almost on the same level (fig. 8). In such cases apparently the two roots are related to one leaf trace. In following out the series, however, it was found that

the root appearing on the central strand was related to the next leaf above, while the one attached to the commissural strand corresponds to the leaf trace above that. The appearance of roots on the commissural strand seems to be a common occurrence in the older stems.

Vascular anatomy of stipules

As has already been mentioned, no conspicuous stipules appear until the fourth leaf. At this stage the leaf trace passes through the stipules as a single strand, to which two weakly developed xylem strands are attached, which pass to the stipular lobes. In the fifth leaf these strands are better developed, and the leaf trace shows signs of forking where the stipular strands are attached. The latter remain unbranched for some distance from the leaf trace, when each gives off usually two branches to the upper part of the lobes. In the sixth leaf the trace forks where the stipular strands are given off, but anastomosing takes place immediately beyond. In the seventh leaf the point of forking of the leaf trace has moved closer to the stem, and anastomosing is delayed longer than in the previous stage. Further branching has taken place in the stipular strands. In the subsequent leaves the point of forking of the leaf trace moves closer and closer to the central region, until finally this takes place as the leaf trace is preparing to free itself from the central strand. Likewise, the point of anastomosing of the branches is moved farther and farther away from the point of attachment of the stipular strands, until the leaf trace passes into the petiole still divided. Above the tenth leaf the two branches from the forked leaf trace bifurcate, so that four strands enter the stipular region. In this region further branching and anastomosing take place. The course of the vascular bundles above the tenth leaf is represented in the reconstruction in fig. 34.

The stipular strands which pass to the lobes of the stipule bend outward and pass to within a short distance of the epidermis, where they end blindly. The lowest (or the main strand), however, is terminated by a procambial strand which originates from a group of meristematic cells on the inner side of the edge of the stipule slightly above where this merges with the cortex. This meriste-

matic region was first visible in the seventh leaf (figs. 23, 31, 34). Such a region has been reported by GWYNNE-VAUGHAN (**9**) to occur in *Kaulfussia* and *Archangiopteris*, and WEST (**13**) reports the same for two species of *Danaea*. Although not reported for the other genera of the Marattiaceae, it is probable that this region is found in them also. GWYNNE-VAUGHAN suggests that this region might represent the rudiments of the adventitious buds, and WEST reports that this does actually occur in *Danaea nodosa*. While this region may give rise to adventitious shoots, it seems probable that its principal function is to build up the fleshy stipules. In none of the specimens of *Angiopteris* studied was there any evidence of the presence of adventitious buds in this region.

Conclusions

It is evident that *Angiopteris* presents an example of striking general variation in the vascular structures from stage to stage. How far this variation is continued remains to be determined from further studies of more advanced stages than have been dealt with in this investigation. From the facts observed in the stages studied, however, it seems probable that some of these variations are continued indefinitely as the plant increases in size and age. The most conspicuous of these variations are: (1) the elimination of the endodermis, (2) the appearance of commissural and medullary strands and the increase in their importance in the structure of the central region, (3) the repeated bifurcation of the leaf traces, (4) the placing closer to the central strand the point of attachment of the commissural strand and the point of forking of the leaf trace, and (5) the variation in the place of attachment of the root steles. Much of this variation tends to the breaking up of the central strand, a fact which points to a polystelic condition so characteristic of the phylogenetically advanced types of the different groups of vascular plants.

The total absence of a cauline procambium is interesting, as it suggests that the central vascular structure in the stem of the Marattiaceae, and perhaps other closely related ferns, is mostly if not wholly of foliar origin; that is, it consists of a sympodium of leaf traces. The definite relation between roots and leaves strongly supports this theory.

Summary

1. The general internal structure of the stem of *Angiopteris evecta* consists of two main regions, the cortical and central vascular. The relatively thick cortex is traversed by leaf traces and roots. The central region consists of a vascular strand which appears crescent-shaped in cross-section and is imbedded in a central parenchyma tissue. This strand gives off root steles and leaf traces. In addition, commissural and medullary strands appear in the central region.

2. The leaf traces are given off from one edge of the central strand, and this contribution is always from the same edge in the same plant. On about the same level that the leaf trace leaves the central strand a root is attached on the outer side of the other edge. The contribution thus made to the leaf traces is made good on the opposite edge in two ways, by an increase in the vascular tissue on the edge itself, and by the addition of commissural strands.

3. The commissural strand originates from the free edge of the leaf trace after the latter is freed from the central strand, and passes to the opposite edge of the central strand and fuses with it.

4. By a continual loss of tissue from one edge and an addition on the other the central strand assumes a spiral condition. This condition is due to the spiral succession of leaves.

5. In the older stages a medullary strand appears on the inner side of the central strand opposite the upper level of a root junction. This crosses the central parenchyma tissue and fuses with the commissural strand before the latter has fused with the central strand. After fusion has taken place, the medullary strand passes upward on the inner side of the central strand, fused to the latter but retaining its identity, until the root junction has been cleared, when it frees itself and repeats its course across the central parenchyma.

6. Each leaf trace is definitely related to one root. The root which appears almost opposite a leaf trace corresponds to the next leaf above. In the early stages each leaf trace appears directly above its corresponding root; but in the later stages the leaf trace is displaced to the right or left, as the case may be, of its corresponding root, due to the spiral condition of the central strand.

7. The leaf traces at first are single, but later bifurcate in the stipular region, but anastomose beyond. The point of forking moves closer and closer to the central strand. Further forking and anastomosing of the leaf trace take place in the more advanced stages.

8. One strand from each side of the leaf trace goes to the lobes of the stipule. Branches from this strand supply the stipular lobes with vascular tissue.

9. All the stipular strands end blindly except the lowest or main strand, which is terminated by a procambial strand originating from a group of meristematic cells on the inner side of the edge of the stipule. This group of cells probably helps to build up the fleshy stipule.

10. The absence of cauline procambium and the definite relation between roots and leaves suggest that the vascular tissue of the central region is a sympodium of leaf traces, and most if not all of the central strand is of foliar origin.

The writer wishes to express his gratitude to Dr. W. J. G. LAND for the valuable collection of material furnished for this investigation and for his advice, criticism, and encouragement during the progress of the work.

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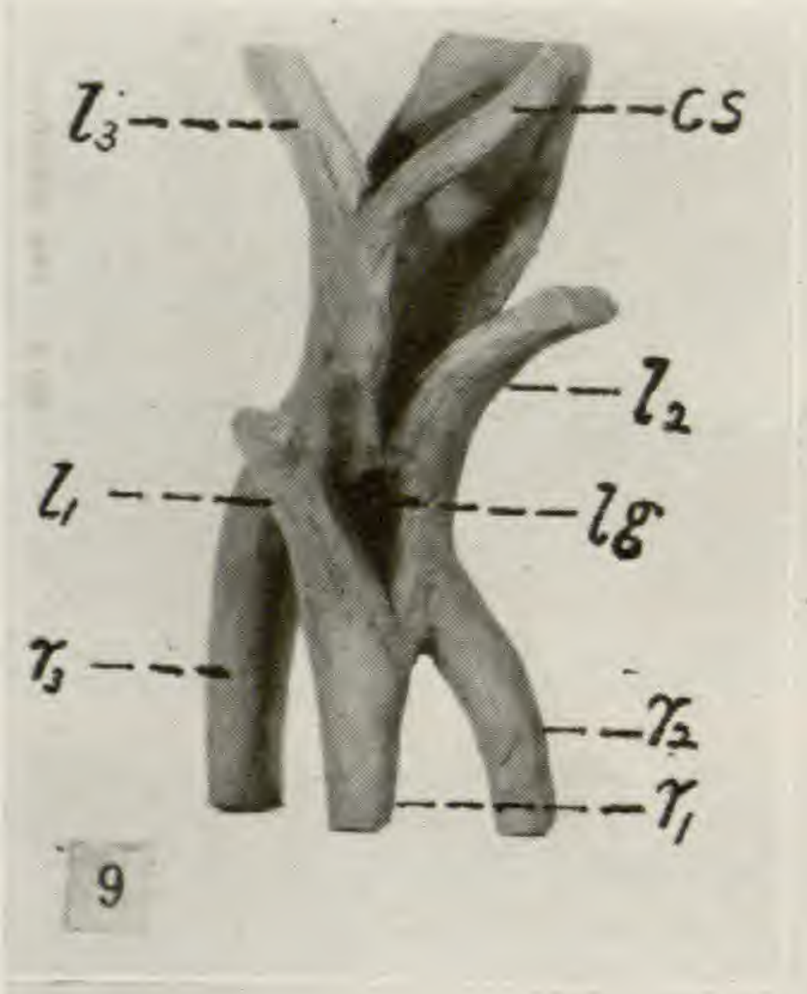

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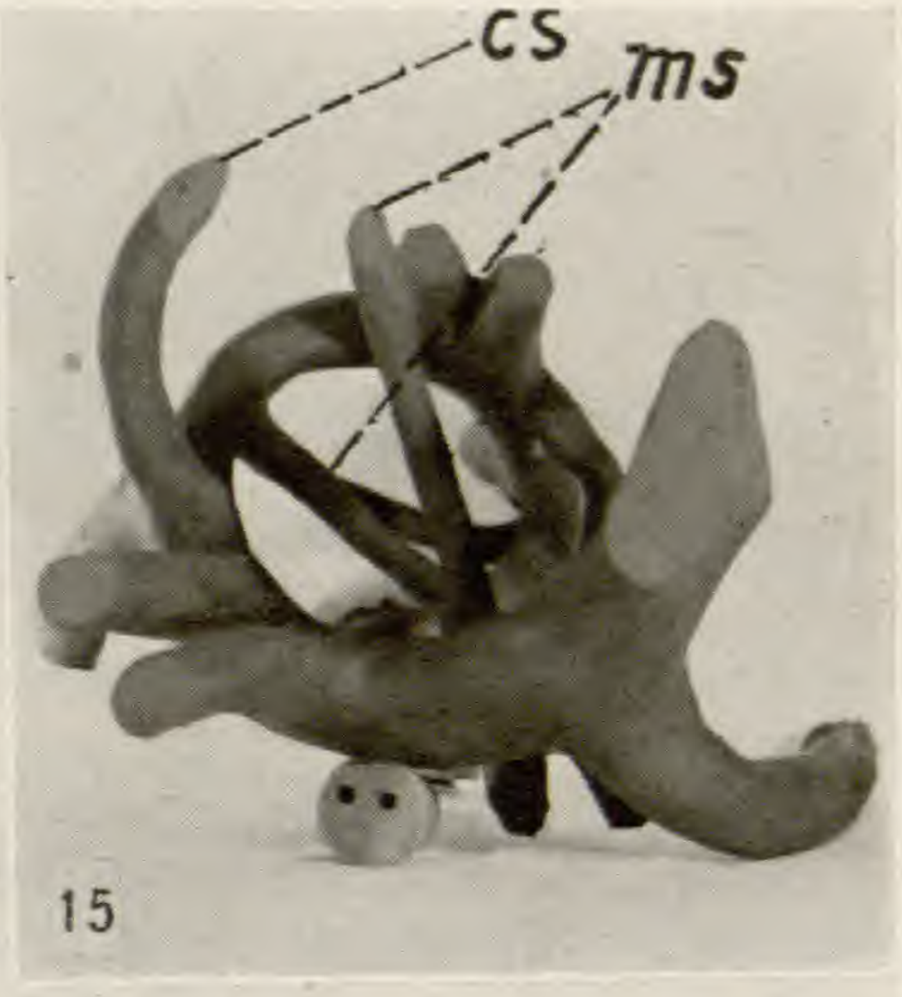

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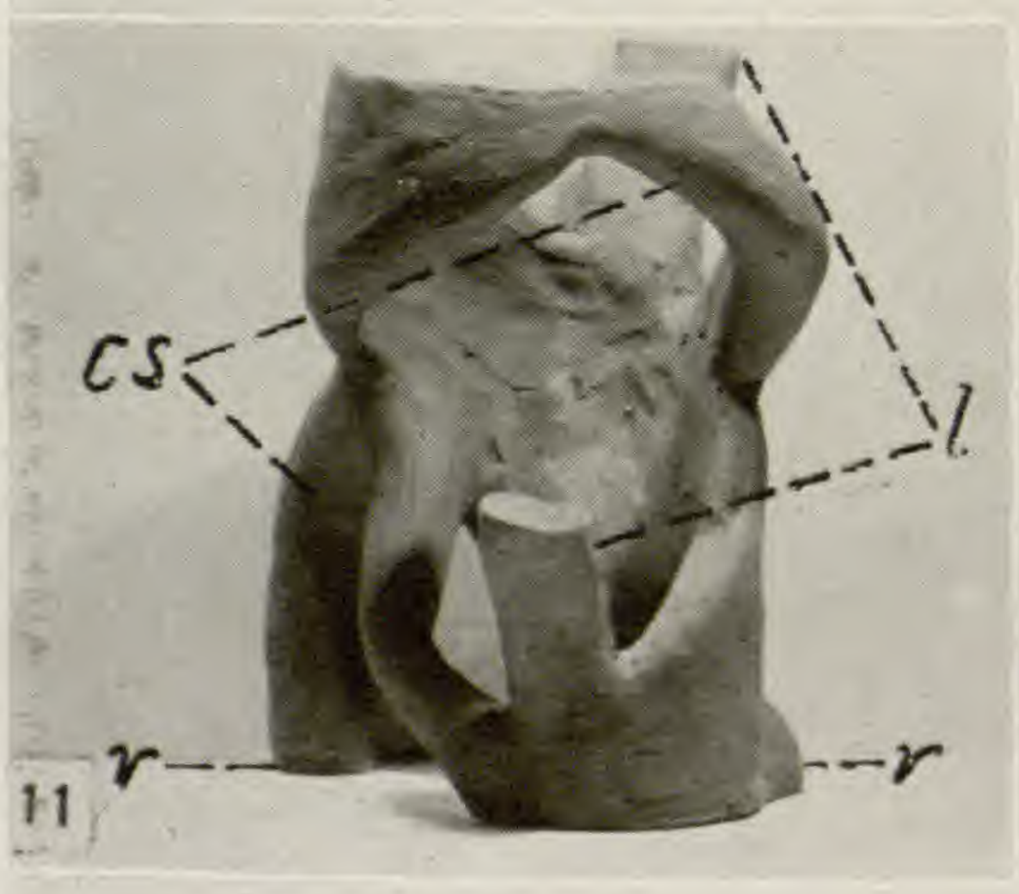

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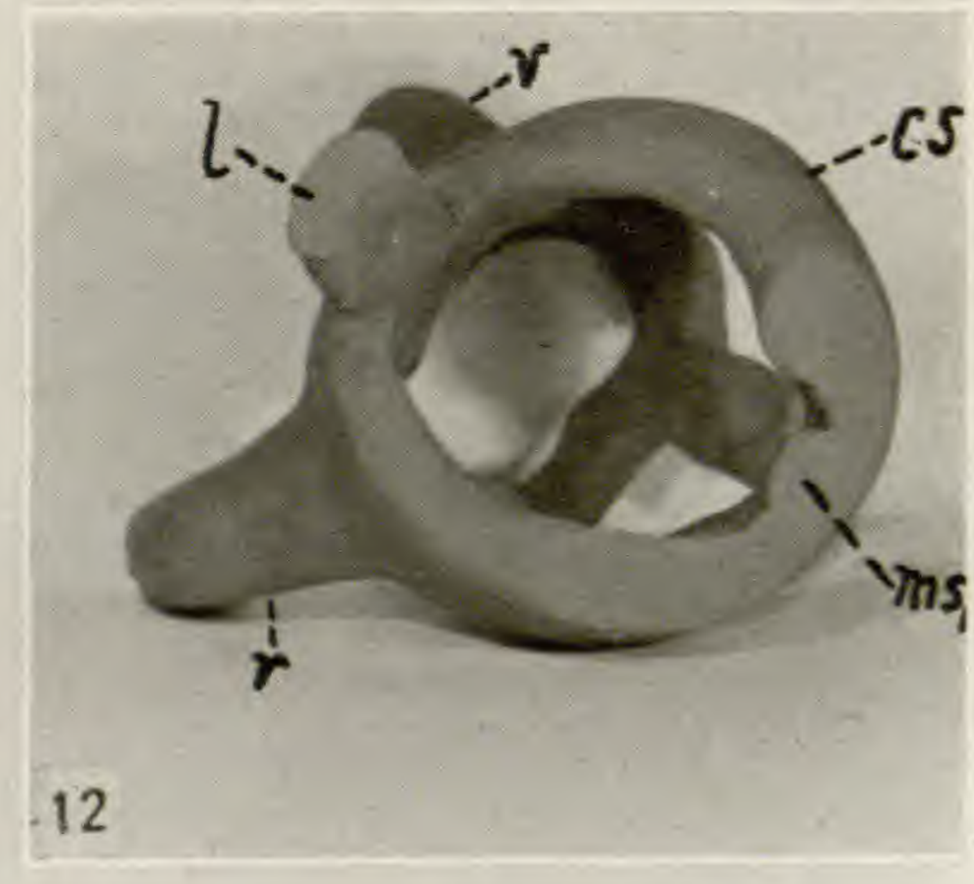

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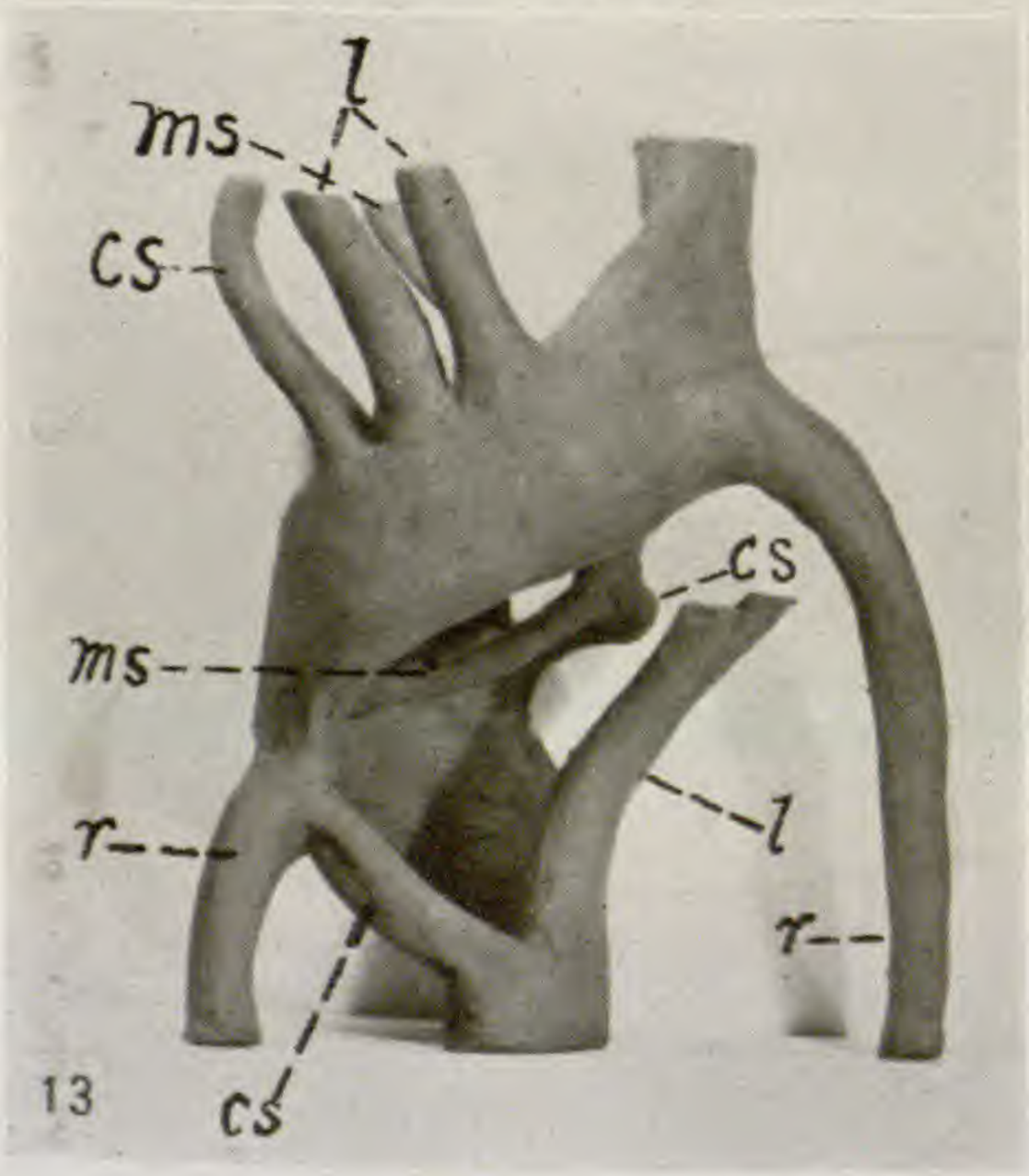

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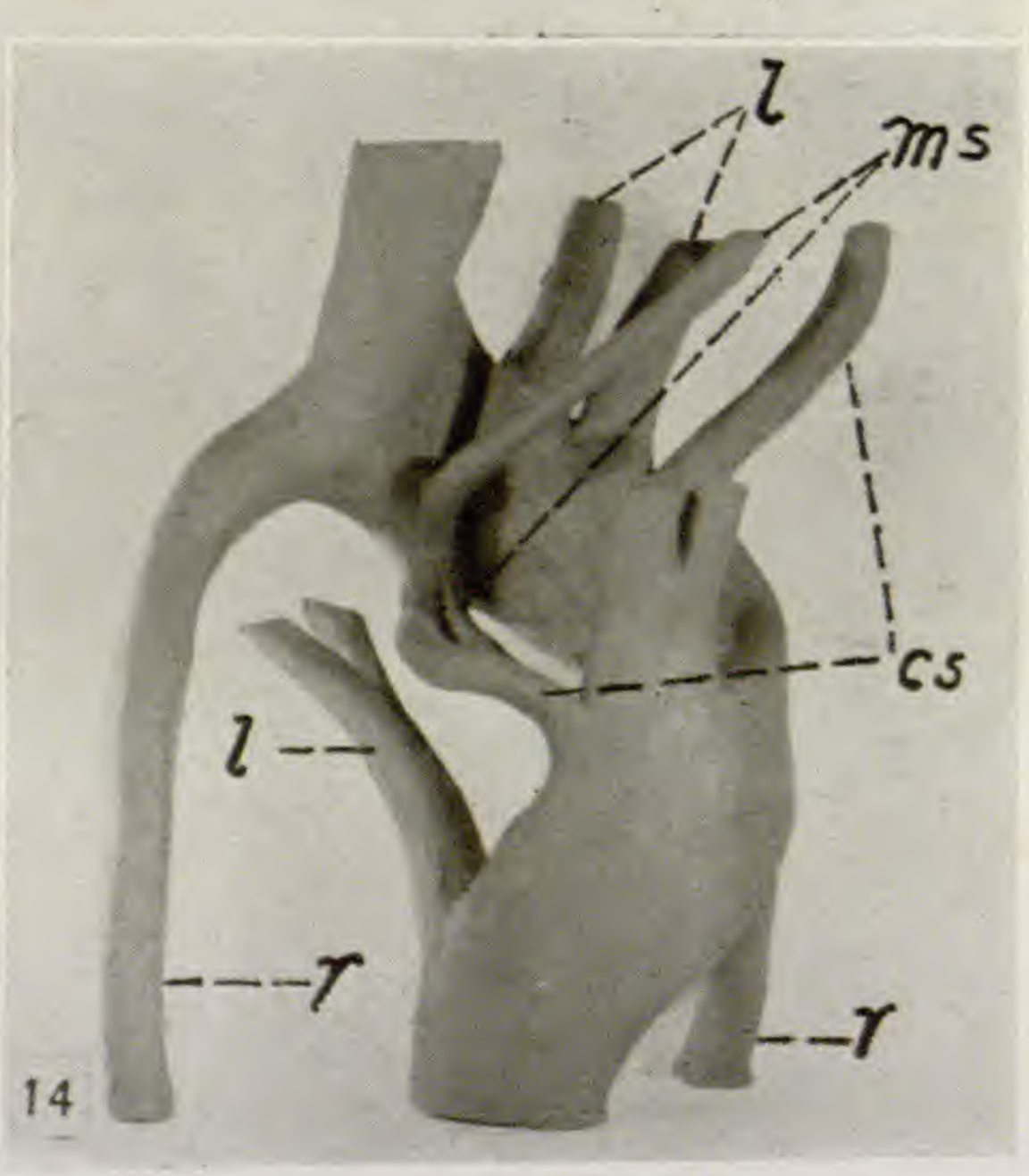

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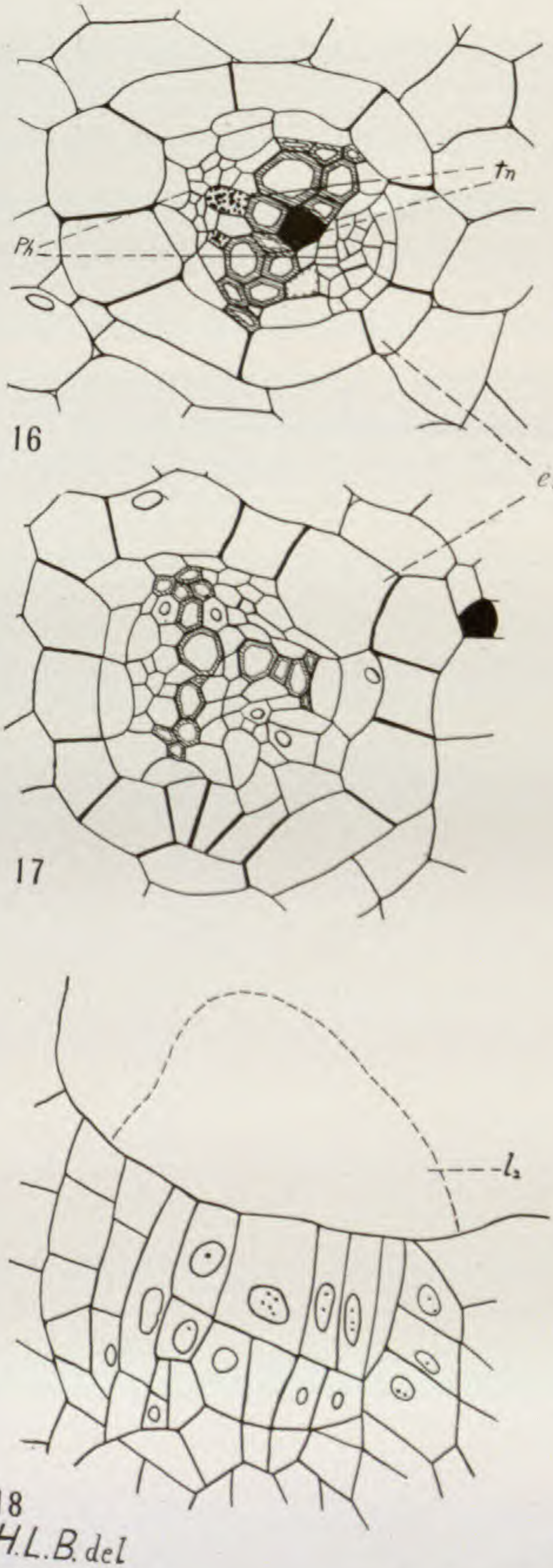

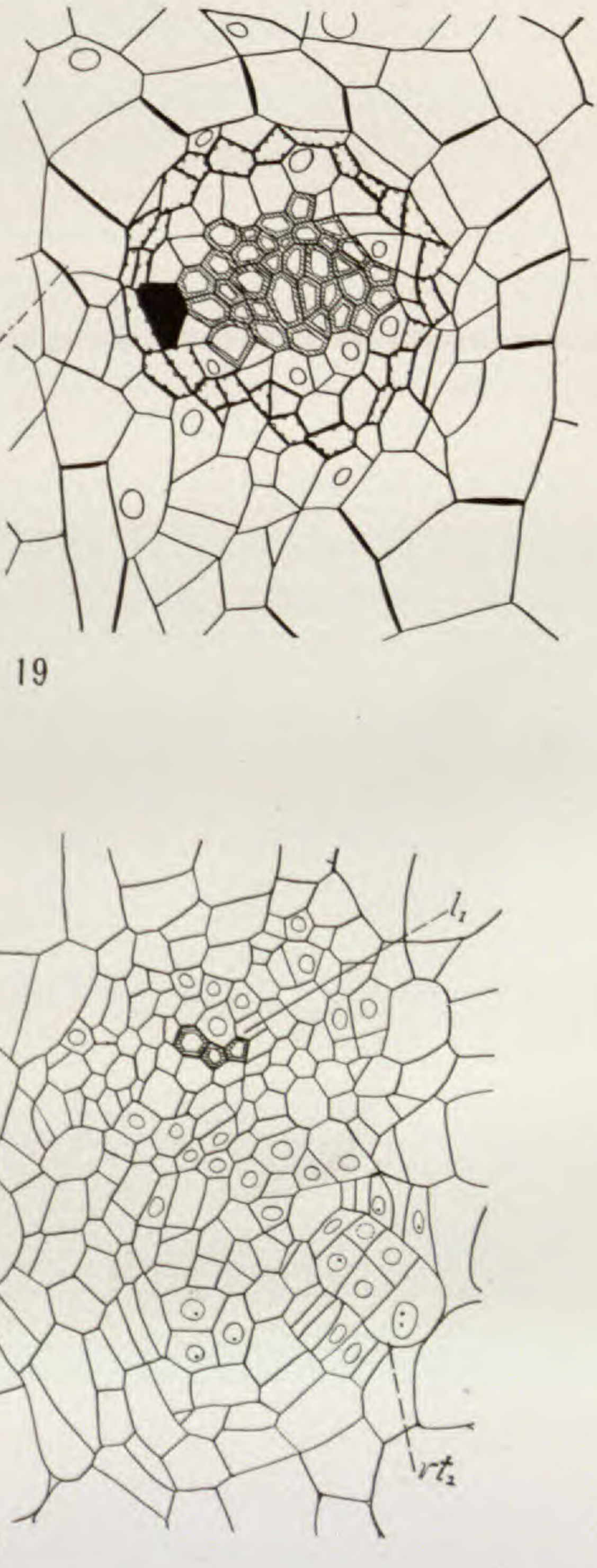

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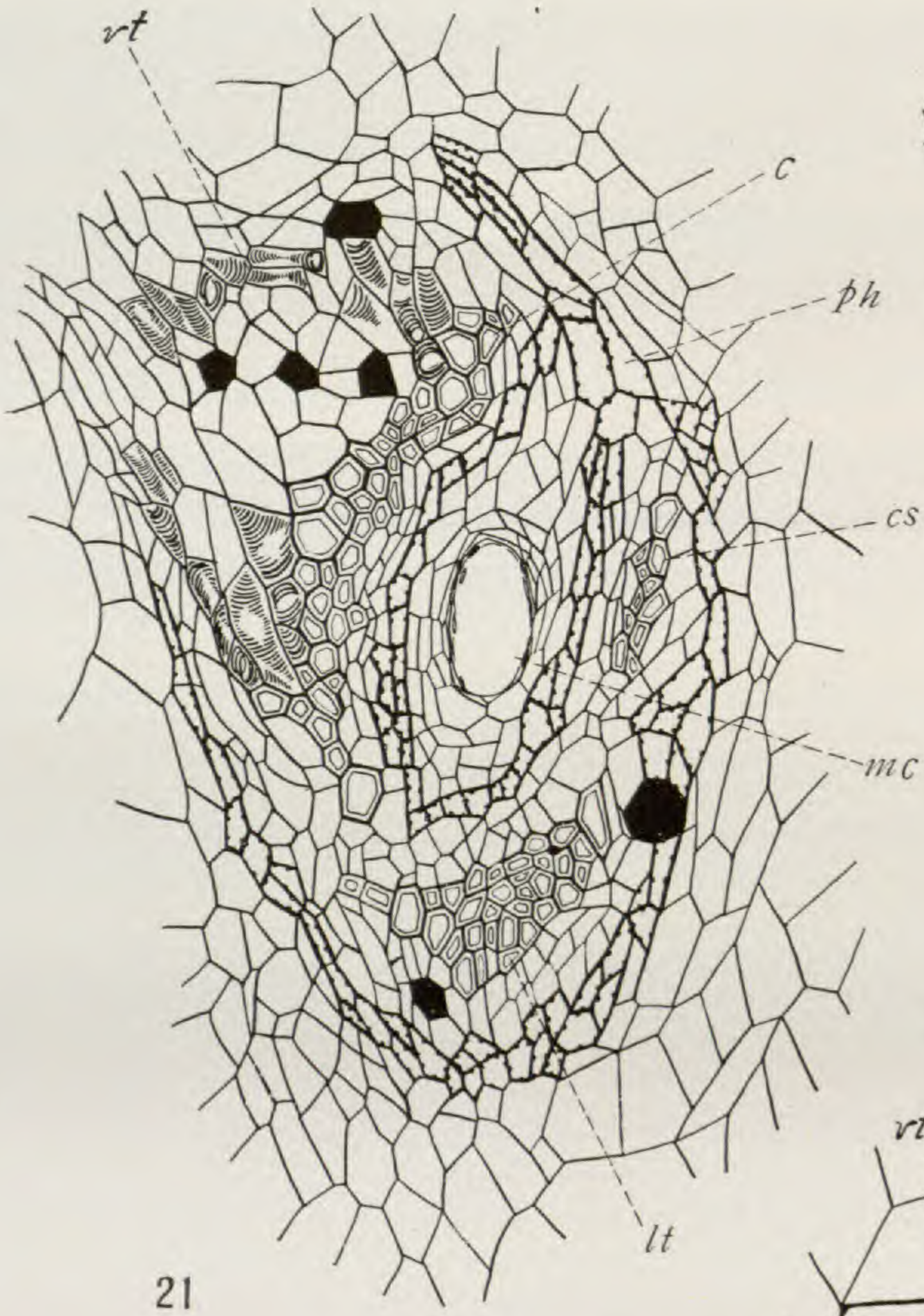

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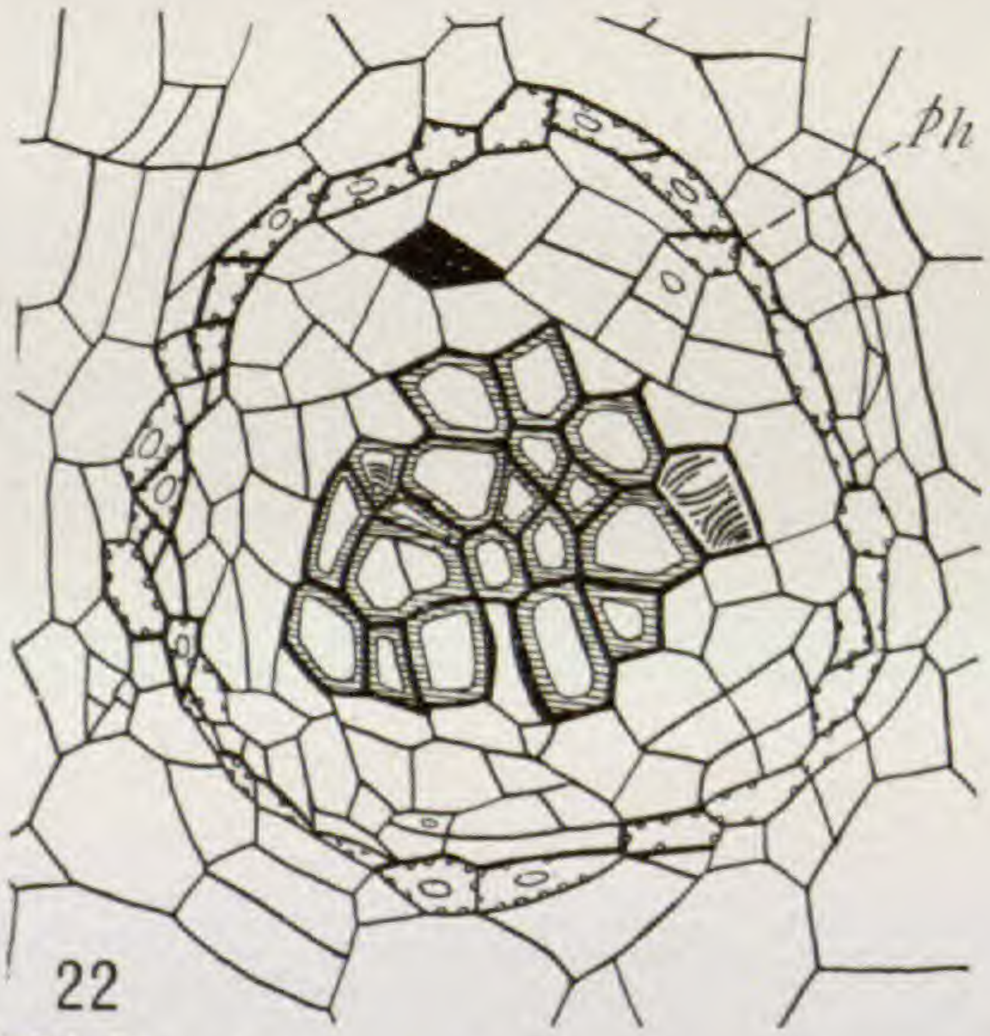

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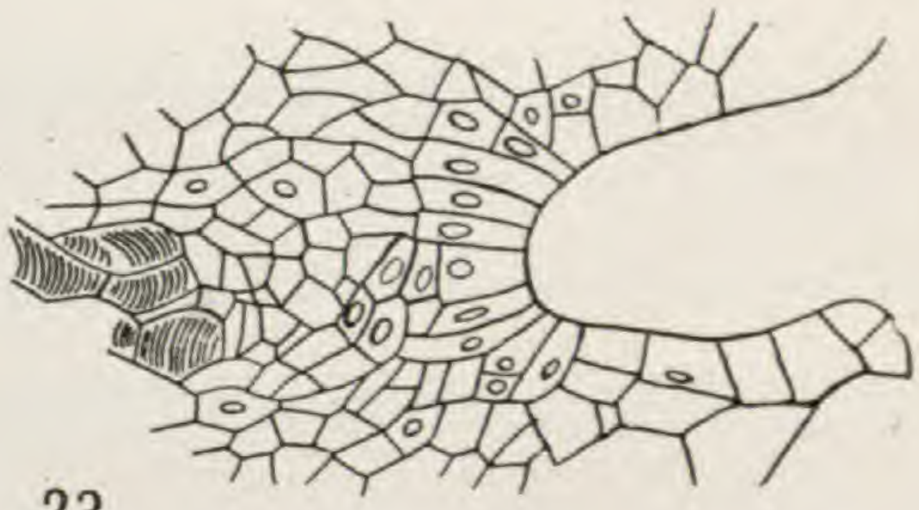

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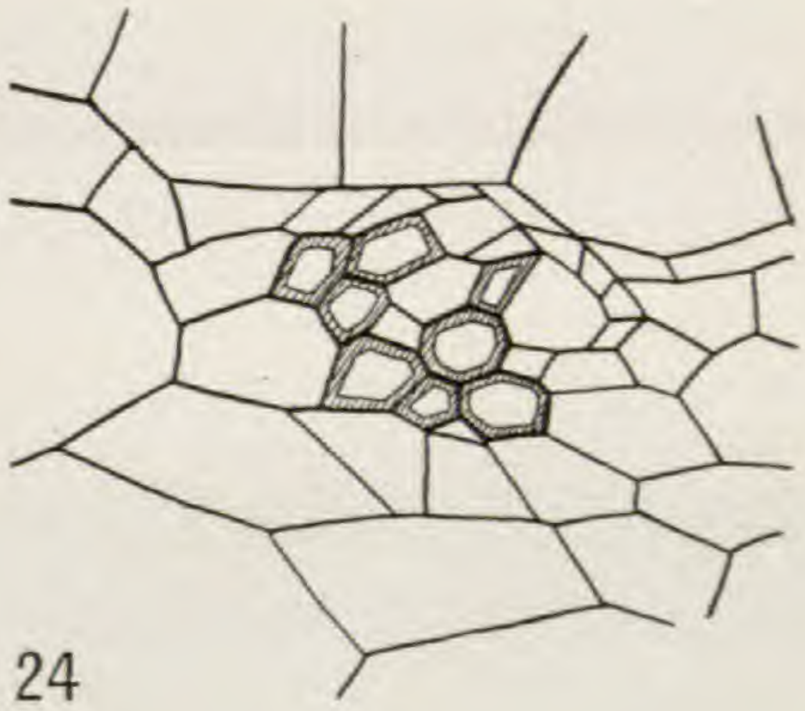

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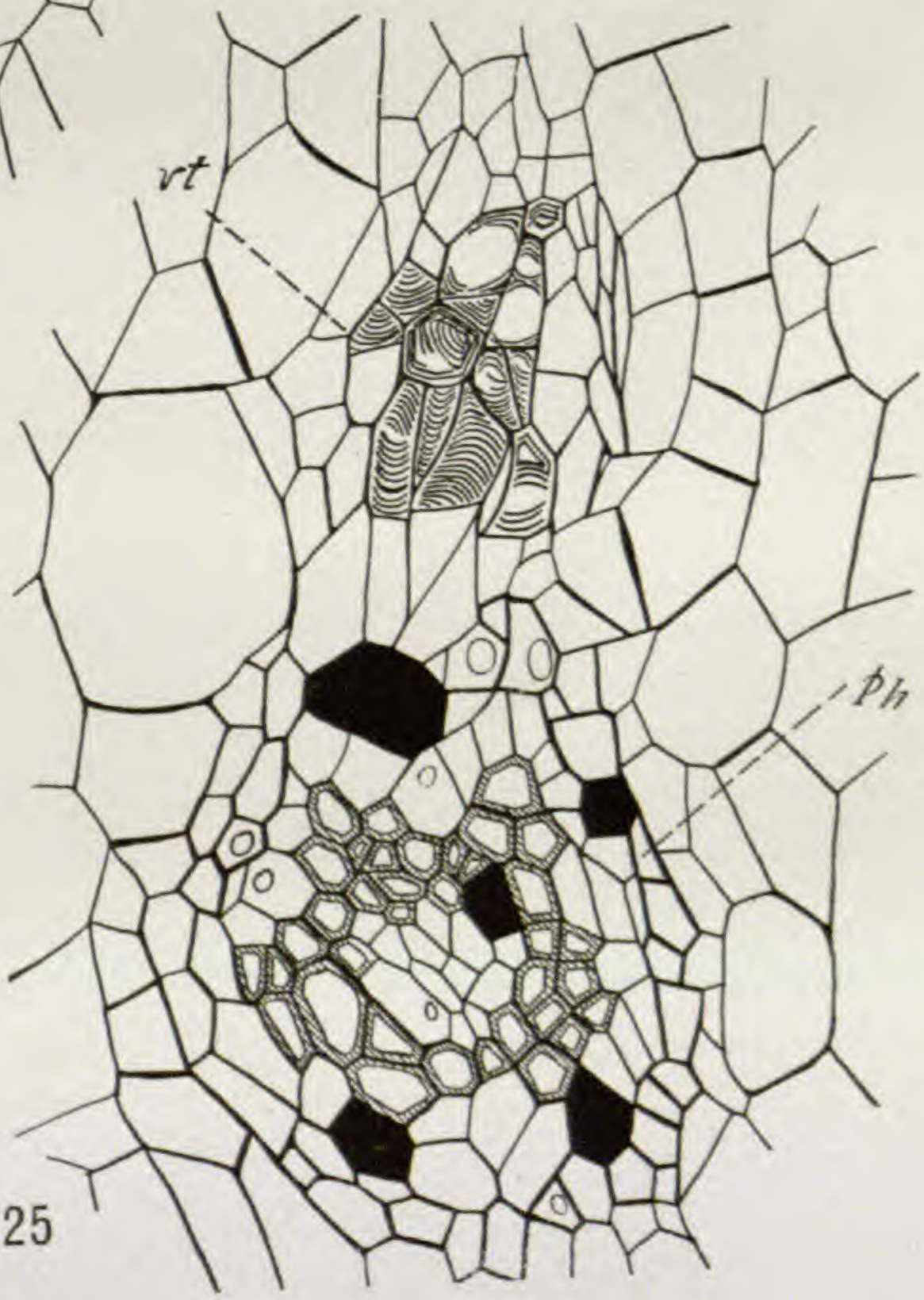

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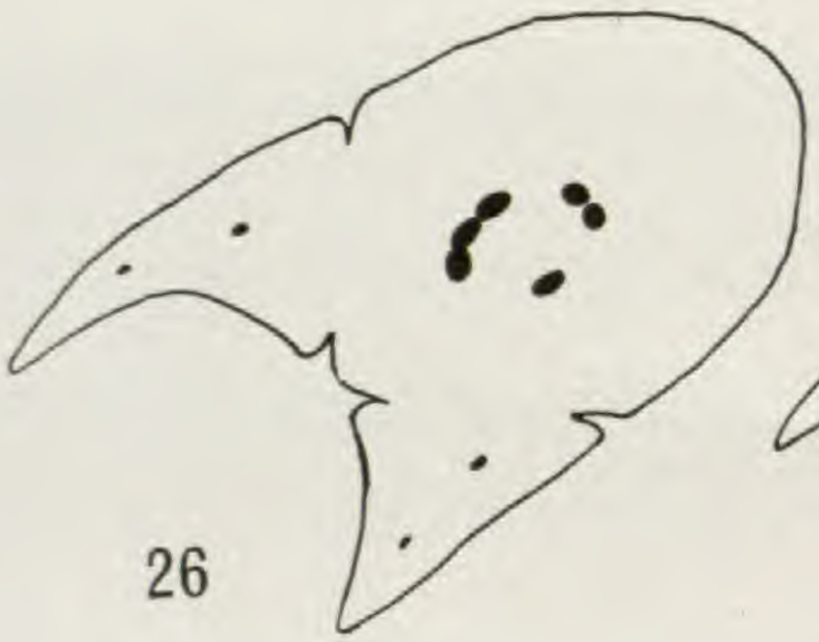

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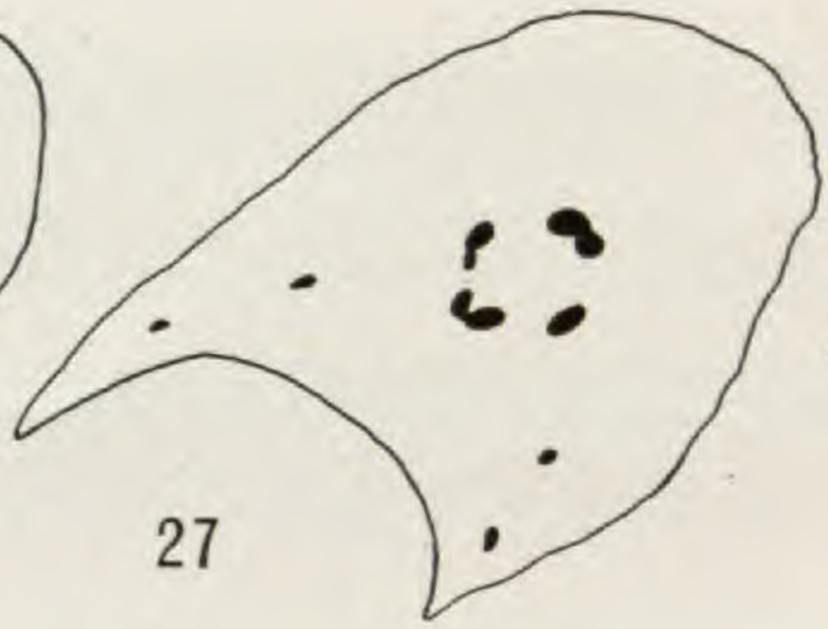

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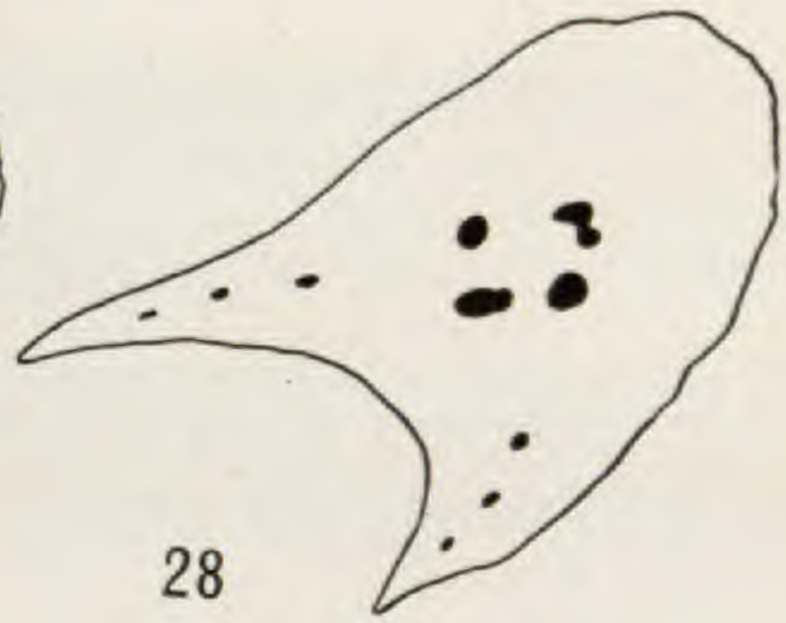

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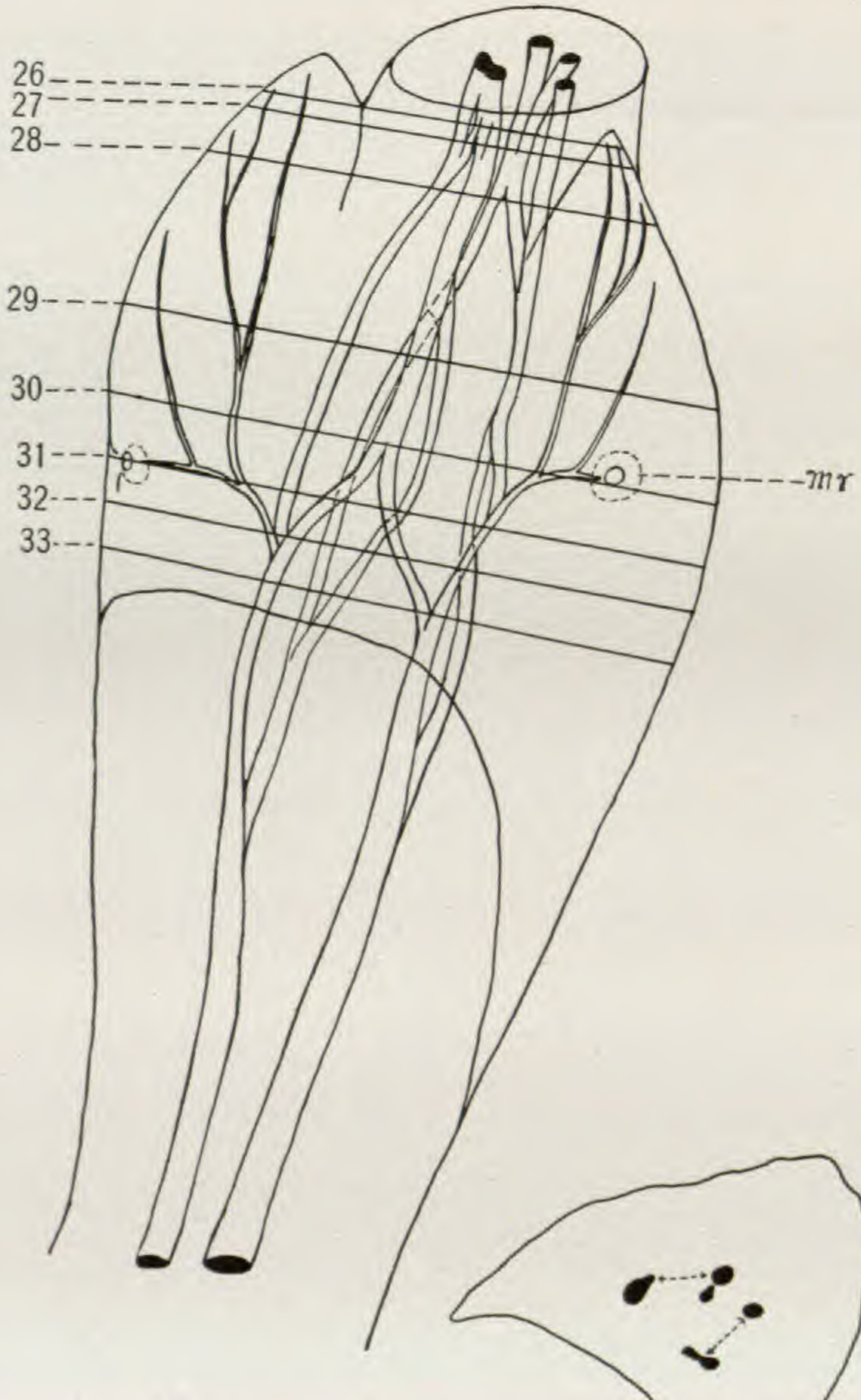

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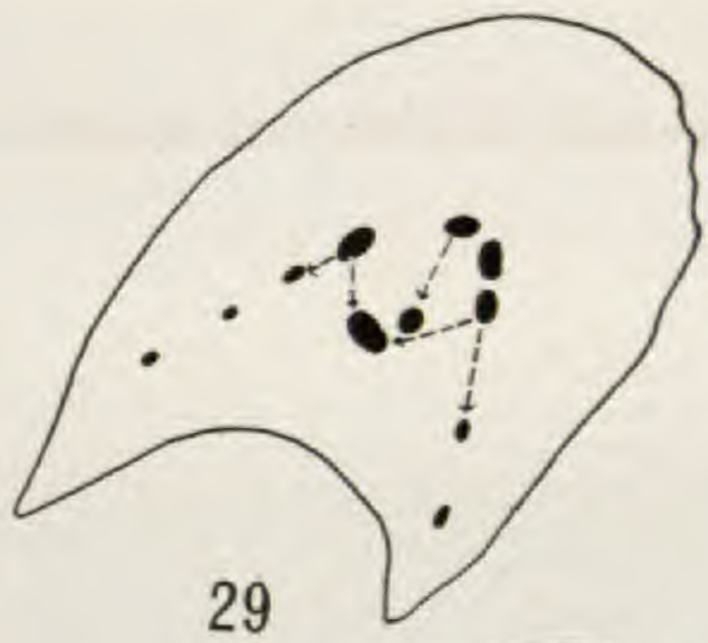

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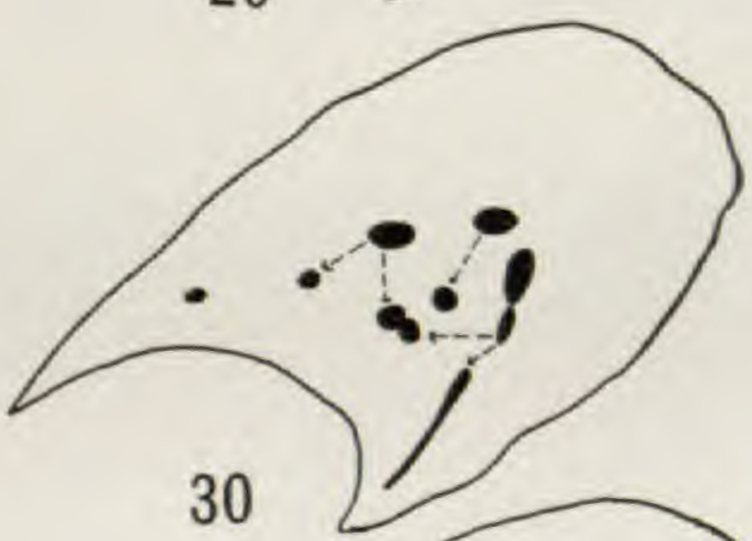

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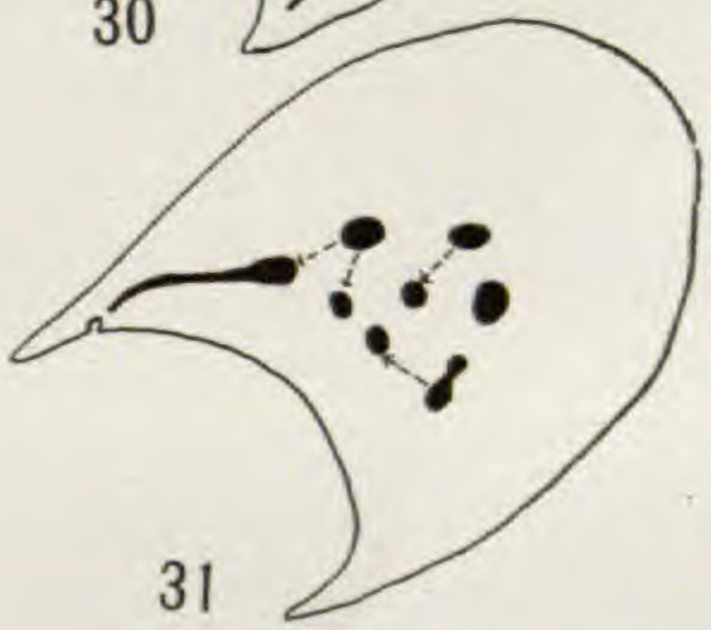

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EXPLANATION OF PLATES V–VIII

PLATE V

FIG. 9.—Model of stelar structure represented in fig. 3: r_1, r_2, r_3, first, second, and third root steles; l_1, l_2, l_3, first, second, and third leaf traces; *cs*, commissural strand; *lg*, leaf gap.

FIG. 10.—Same model viewed from above.

FIG. 11.—Model of stelar structure represented in diagram in fig. 4.

FIG. 12.—Same model viewed from above: ms_1, first medullary strand.

FIG. 13.—Model of stelar arrangement represented in fig. 5; abbreviations same as above.

FIG. 14.—Same model viewed from opposite side.

FIG. 15.—Same model viewed from above.

PLATE VI

FIG. 16.—Detail of a diarch primary root: *en*, endodermis; *ph*, phloem; *tn*, tannin cell; *pc*, pericycle; ×330.

FIG. 17.—Detail of triarch primary root; ×330.

FIG. 18.—Apical cell at stem tip: l_2, second leaf; ×330.

FIG. 19.—Detail of transition region; ×330.

FIG. 20.—Section of sporeling: rt_2, origin of second root; l_1, first leaf trace; ×330.

PLATE VII

FIG. 21.—Detail of central region on level of outgoing of fourth leaf trace: *c*, central strand; rt_5, fifth root stele; *cs*, commissural strand; *ph*, phloem; *mc*, mucilage canal; ×170.

FIG. 22.—Detail of medullary strand: *ph*, phloem; ×330.

FIG. 23.—Meristematic region terminating main stipular strand; ×180.

FIG. 24.—Detail of stipular strand; ×330.

FIG. 25.—Detail of stele above transition region showing medullated condition; rt^2, second root trace; ×330.

PLATE VIII

FIGS. 26–33.—Diagrams of sections of stipular region from advanced stage showing arrangement and inter-relation of vascular strands; ×8.

FIG. 34.—Reconstruction of sections: *mr*, meristematic region of stipule; reconstruction is slightly larger than sections to show better the courses of strands.

SYMBIOSIS IN A DECIDUOUS FOREST. I

W B. McDOUGALL

(WITH THREE FIGURES)

Introduction

In a previous paper[1] symbiosis is defined as the living together of dissimilar organisms, and the phenomena of symbiosis are classified as follows:

I. Disjunctive symbiosis
   1. Social
   2. Nutritive
      a. Antagonistic
      b. Reciprocal

II. Conjunctive symbiosis
   1. Social
   2. Nutritive
      a. Antagonistic
      b. Reciprocal

The writer has felt for some time that there is urgent need of work along the lines of these various types of symbiosis. Considerable work has been done on the relations of plants to their physical environment, but the work done on their relation to the biotic environment has not been in proportion to the importance of this phase of ecology.

Some five years ago the University of Illinois obtained possession of a sixty acre tract of forest. This land is located about five miles northeast of the University in Champaign County, Illinois, and is a remnant of a former much more extensive forest. It is known as the "University woods." Many of the primitive trees are still standing in this tract, although some cutting has been done in the past, and the forest was frequently pastured before it was acquired by the University. It may be said, therefore, to be only semi-primitive. It has been stated that there is very little if any natural forest vegetation in the state of Illinois at the present time, but the use of the word natural in such a statement is ill chosen and makes the statement inexact. It is true that there is very little if any such vegetation that has not been modified to a greater or less extent by man. Vegetation that has been influenced by man, however, is

[1] McDOUGALL, W. B., The classification of symbiotic phenomena. Plant World **21**:250–256. 1918.

not necessarily "unnatural." The plants in any forest in Illinois at the present time have life problems that are just as real, just as complex, and just as interesting from an ecological point of view as were the problems of plants in the primitive forests. We should make every effort, of course, to preserve as much of the primitive vegetation as possible, but no ecologist whose location is remote from primitive vegetation need feel that there is nothing he can do, for wherever plants grow there is ecological work to be done even though the land may all be under cultivation.

The work, a part of which is recorded in the present paper, was undertaken for the purpose of making a thorough study of symbiosis in all its phases in the University woods. The present paper

RAINFALL (IN INCHES) AT URBANA; AVERAGE FOR SIXTY YEARS

Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	TOTAL
2.16	2.42	2.92	3.47	4.04	4.11	3.89	3.04	3.60	2.21	2.49	2.24	36.89

TEMPERATURES AT URBANA; AVERAGE FOR FORTY YEARS

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Aver.
Maximum.....	57	60	72	83	89	94	98	96	93	84	72	58	80
Minimum.....	− 5	− 4	12	26	36	46	52	51	39	27	14	2	25
Mean.....	26.6	29.2	39.8	53.0	62.9	71.9	76.4	74.4	67.2	55.3	41.5	30.4	52.4

deals primarily with social disjunctive symbiosis, and it is the hope of the writer to follow it with others treating of the other types of symbiosis.

University woods

PHYSICAL ENVIRONMENT

CLIMATE.—Table I gives a summary of weather conditions at Urbana, Illinois, during 1919. The data for this table were kindly furnished and the table compiled by the Division of Soil Physics of the Agriculture Experiment Station of the University of Illinois. There were slightly more clear days in 1919 than usual, but on the whole the table is typical of the climate of this region. The following tabulations of average monthly and annual rainfall at Urbana for a period of sixty years, and of average maximum, mini-

mum, and mean monthly temperatures for a period of forty years, were compiled from data given by MOSIER.[2] It will be noted that the rainfall is adequate and is well distributed throughout the year. The average data of the last killing frost in spring is April 26, and that of the first killing frost in autumn is October 16, giving an average growing season of 173 days.

SOIL.—The soil of the forest is yellow-gray silt loam, an upland timber soil.[3] There are no streams within the forest, but the region is drained by a tributary of the Salt Fork of the Vermillion

TABLE I

WEATHER CONDITIONS AT URBANA DURING 1919

1919	TEMPERATURE			RAINFALL IN INCHES	MEAN RELATIVE HUMIDITY	WIND		NO. OF CLEAR DAYS	NO. OF PARTLY CLOUDY DAYS	NO. OF CLOUDY DAYS
	Maximum	Minimum	Mean			Average velocity	Direction			
January...	55	− 8	31.2	0.21	76.4	7.5	SW	16	6	9
February..	56	11	31.5	1.92	77.0	8.4	NW	10	3	15
March....	67	9	42.0	4.12	75.5	11.9	S	13	6	12
April.....	76	24	53.2	0.75	76.1	9.1	SW	7	6	17
May	92	38	60.8	3.29	78.6	7.4	NE	8	7	16
June.....	91	52	75.5	6.90	79.9	5.8	SE	10	13	7
July.....	96	56	81.3	2.66	59.3	6.1	SW	23	8	0
August....	94	50	73.9	3.85	63.4	6.4	SW	19	9	3
September.	92	42	70.5	2.47	68.1	6.5	SW	19	5	6
October...	88	33	58.4	5.59	79.8	7.0	SW	11	1	19
November.	63	16	39.1	3.37	78.3	8.9	SW	12	5	13
December.	51	− 4	23.9	0.12	77.6	7.4	SW	9	3	19
Total ...	.....	.....	.....	35.25	.....	.....	.....	157	72	136
Average.	76.8	26.6	53.4	2.94	74.2	7.7	SW	13.1	6.0	11.3

River. This ditch flows in an easterly direction some forty rods south of the present boundary of the forest. The forest itself is only gently rolling, but several acres in the middle eastern portion are several feet lower than the highest parts of the woods, and in the lowest part there is often standing water for a few weeks in spring.

As will be shown later, the vegetation in the lower part of the woods is quite different from that in the higher parts, and this difference seems to be almost entirely due to the difference in the

[2] MOSIER, J. G., Climate of Illinois. Univ. Ill. Agric. Exp. Sta. Bull. 208. 1918.

[3] HOPKINS, C. G., MOSIER, J. G., VAN ALSTINE, E., and GARRETT, F. W., Champaign County soils. Univ. Ill. Agric. Exp. Sta. Rep. 18. 1918.

amount of soil water. A number of measurements of soil temperature were made in both the higher and lower parts of the forest at depths of three inches and twelve inches, but temperatures taken in different parts of the forest on the same day and at the same depth were found to be almost exactly the same. Tests were made also for soil acidity in different parts of the forest, using the indicator method described by WHERRY.[4] The results of these tests showed that the soil of the forest is neutral throughout. Although no chemical analyses have been made, there is no reason for thinking that the mineral constituents of the soil are not practically the same throughout the forest.

THE PLANT COMMUNITY

METHODS.—Before the more special types of symbiosis can be studied adequately in any plant community, it is necessary to have a thorough understanding of the structure of the community and of the disjunctive social symbiosis, that is, of the gross interrelations of the component parts of the vegetation. In order to obtain this information, the entire forest was first staked out into one hundred foot quadrats. The only reason for choosing the one hundred foot quadrat instead of a unit of the metric system was that one hundred foot steel surveyor's tapes are readily obtained and are convenient to use. No attempt was made to have the quadrats exactly one hundred feet square. The tapes were fastened to the stakes by means of straps, and these were of such a length that on level land and with the tapes drawn tight the stakes would be about one hundred two feet apart. In the woods, however, with the tapes running among shrubs, between trees, over fallen logs, etc., and with the tape never very tightly drawn, the quadrats were approximately one hundred feet square. It was found that by making fractional quadrats across each end of the woods and part way along one side, to where there is a jog in the boundary line, there were then 216 full size quadrats (fig. 1).

Having subdivided the forest into quadrats, two methods were used for locating and mapping the components of the vegetation. The first method was used only for the trees and shrubs. Two

[4] WHERRY, E. T., Soil acidity and a field method for its measurement. Ecology 1:160–173. 1920.

FIG. 1.—Quadrat map of University woods showing distribution of the two dominant trees: o=*Acer saccharum*, —=*Ulmus americana*.

rows of quadrats were surveyed at the same time. A hundred foot tape was stretched between the first two quadrats of the first two rows. Then while walking through these quadrats in the directions indicated in fig. 2, counts were made of all species of trees and shrubs present. The frequency of the occurrence of each species of shrub was indicated by the numerals 1, 2, 3, and 4; no. 1 indicating only one to three specimens in the quadrat, no. 4 indicating great abundance, and nos. 2 and 3 representing intermediate degrees of frequency. In the case of trees the actual

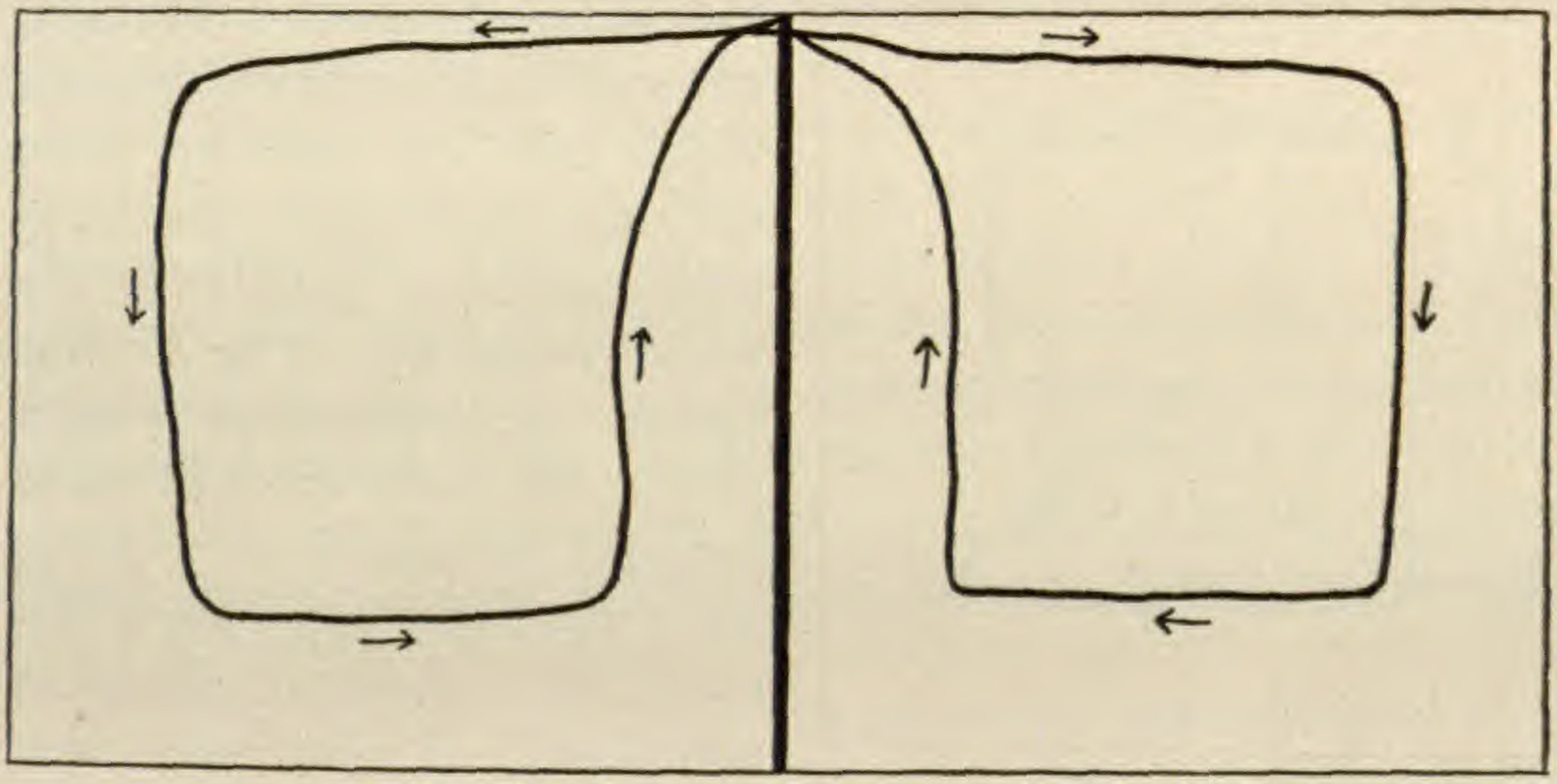

FIG. 2.—Two one-hundred-foot quadrats illustrating method used in making counts of trees and shrubs in University woods: heavy line between quadrats indicates position of steel tape; arrows show direction taken by surveyor in making counts.

number of individuals of each species was noted. After counts in these two quadrats had been completed, the tape was taken up and stretched between the next two quadrats and counts made there in a similar way, the procedure being repeated until the entire forest had been surveyed. Having secured these data, a distribution map was made for each species (fig. 1). No attempt was made to locate the individuals within the quadrat, but only to place them in the quadrats in which they occur. Each individual is shown on the maps, therefore, within one hundred feet or less of its actual position in the forest.

For the herbaceous vegetation a somewhat different method was used. Only one row of quadrats was surveyed at a time and no

tape was used. Instead, a pocket compass was employed for keeping on a line running through the middle of the row of quadrats, and each quadrat was surveyed by walking through it in the manner indicated in fig. 3. The middle of each quadrat after the first was found by pacing thirty-three paces from the middle of the preceding quadrat with the compass as a guide to the direction. The place was then marked by sticking into the ground a stick about four feet long which was sharpened to a point at the lower end and whittled off at the upper end so as to give a whitish surface which could be seen for some distance. This stick was carried from quadrat to quadrat as the work progressed. The various species of herbaceous plants present in each quadrat were recorded as nos. 1, 2, 3, or 4; no. 1 signifying rare, no. 2 frequent, no. 3 common, and no. 4 abundant. A map was then made for each species, showing its distribution and relative frequency of occurrence throughout the forest. In surveying for herbaceous plants, only those plants that were in bloom or were otherwise easily recognized were listed; and in order to make the lists complete the survey was repeated once each month during the growing season.

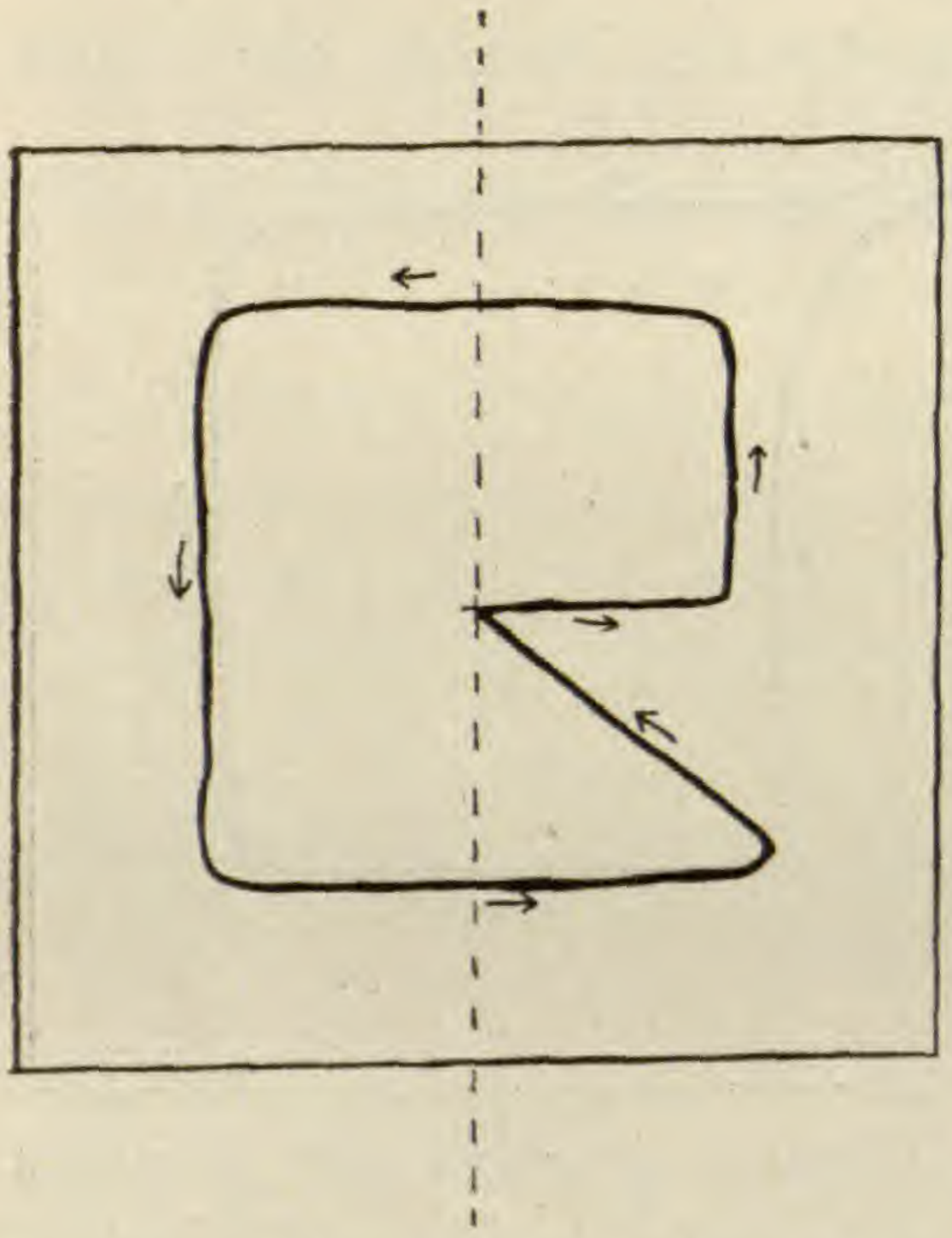

FIG. 3.—A one-hundred-foot quadrat illustrating method used in estimating frequency of herbaceous plant species in University woods: broken line with arrows indicates direction taken in locating center of quadrat; solid line with arrows shows directions taken in making survey.

RESULTS.—By this method a fairly complete list of the plants growing in the woods was obtained, together with the relative numbers of individuals and the locations of each species. No attempt has been made so far to include the bryophytes and the microfungi and algae in the survey, although it is hoped that this may be done later. The bryophytes are nowhere abundant, and

probably are of relatively little importance from the viewpoint of symbiosis or other ecological relations. This is obviously even more true of the algae in such a habitat as this. The microfungi, however, will have to be considered in some detail later in a discussion of their symbiotic relations with other plants. Aside from these three groups, the lists of species are believed to be fairly complete, except that there are probably a few late fall-blooming plants, mostly composites, that have been missed owing to the fact that the last survey of the season was interfered with by duties in connection with the opening of the University and the beginning of classwork for the year.

The lists of species which have been compiled include 31 trees, 12 shrubs, 6 lianas, 134 herbs, 5 ferns, and 83 higher fungi. These figures show that while there is by no means a paucity of species, yet for a sixty acre deciduous forest the flora cannot be said to be an especially rich one. It is not unlikely that the years preceding the acquirement of this property by the University, when it was frequently pastured and the public was allowed to dig up plants by the roots and carry them away at will, had their effect in reducing the number of species.

Acer saccharum and *Ulmus americana* are the dominant trees. These two are present in nearly equal numbers, the count showing 1987 maple and 2073 elm individuals. No other species are nearly so abundant as these two. The nearest competitor is *Fraxinus americana* with 537 individuals, then follow *F. quadrangulata* with 336, *Tilia americana* with 321, and *Carpinus caroliniana* with 303 representatives. All other species are represented by less than 300 individuals. These data show at once that the forest is typically hydrarch mesophytic and relatively mature, *Acer saccharum* being the most typical climax species of the region. While these species are all found more or less throughout the forest, the maple is much more dominant in the southern half and the elm in the northern half. In fact three-fourths of the hard maple and two-thirds of *Fraxinus quadrangulata* are in the southern half of the woods, while three-fifths of the elm, three-fifths of *F. americana*, and two-thirds of the blue beech (*Carpinus*) are in the northern half. It is the higher parts of the woods that are dominated by the maple,

while the lower parts are dominated by elm. In the area between quadrats 59 and 96 and vicinity (fig. 1), which is the lowest part of the woods, there are almost no trees except elms. I shall frequently refer to that part of the woods that is dominated by maple as the maple consociation, and that dominated by elm as the elm consociation.

The great majority of the shrubs are of two species, *Asimina triloba* and *Benzoin melissaefolium*. Both of these are very abundant, and are distributed throughout the forest except in the highest and lowest parts. No other shrubs are abundant except the species of *Crataegus*, which occur mostly along the border.

The subdominants of the herbaceous layer vary with the season as well as with the location within the forest. In the prevernal season *Claytonia virginica*, *Isopyrum biternatum*, and *Collinsia verna* often form extensive and conspicuous societies in the maple consociation. The two species of *Dicentra*, *D. cucullaria* and *D. canadense*, occur together as subdominants in a rather extensive prevernal society. *Phlox divaricata* and *Geranium maculatum* form less extensive but not less well marked societies. *Asarum canadense* is everywhere abundant, but because of its growth habit is not conspicuous. *Viola sororia* is abundant, but is scattered too much to be considered a subdominant. In the elm consociation *Floerkia proserpinacoides* forms an extensive and extremely dense society. Like the elm this plant is present throughout the forest, but is always thickest where the elms are dominant.

During the vernal season *Hydrophyllum appendiculatum* forms an extensive society in the maple consociation. Later *H. canadense* is a subdominant over less extensive but more closely occupied areas. *Cystopteris fragilis* is abundant over considerable areas, and locally is present in such numbers as to become a subdominant. *Podophyllum* is also common, but as usual forms local colonies rather than extensive societies. All of these societies extend into the elm consociation, but there is no subdominant that is characteristic of the elm consociation during this season.

The aestival season is characterized by the subdominance of *Laportea canadensis* over great areas. Like the dominant shrubs *Asimina* and *Benzoin*, the wood nettle is abundant throughout

the woods except in the highest and lowest parts. At the same time *Impatiens biflora* is subdominant in the lower parts of the elm consociation, while *I. palida* is only a little less prominent, and keeps largely to the somewhat higher parts of the same consociation.

During the serotinal season several composites are conspicuous, but perhaps the most characteristic plant is *Campanula americana*, which occurs nearly everywhere in the woods, but usually not abundantly enough to become subdominant. Finally, during the autumnal season species of *Aster* and *Eupatorium urticaefolium* are the principal subdominants.

Social disjunctive symbiosis

This is the type of symbiosis in which the organisms concerned are not in actual contact, at least not all of the time, and in which there is no direct food relation. It includes, therefore, all of the ordinary interrelations of dominant, subdominant, and secondary species in a plant community. These interrelations in deciduous forests have been studied and described by numerous authors. It will suffice here, therefore, merely to mention the salient features of the subject, and to point out their relative importance in the community under consideration. The dominant plants of a community, which in a forest are trees, are those which largely control the environment and so determine what other species may grow in the community. They have very important symbiotic relations, therefore, with all other members of the community through their direct or indirect control of light, space relations, water supply, and to a certain extent available food materials. From this point of view it is of interest to compare a plant community with a human community. In a human community man is the dominant species. As the dominant species he controls the environment to such an extent as to determine what other species may live in the community. Some of the other species usually found in a human community are the horse, dog, cat, mouse, fly, etc. Some of these are not present because man wants them to be, but because man is present and is controlling the environment in such a way as to make it possible for the other species to live in the community. These facts are just as true of the plant community. The presence of

some of the species is distinctly advantageous to the dominant plants, while that of others is just as distinctly disadvantageous, as for example the parasitic fungi, but they are all present because the dominant plants have made it possible by their control of the environment. In the human community we find a well marked division of labor among the individuals of the dominant species; some are engaged in supplying food, others in supplying clothes or fuel, others in administering the law, etc. In the plant community we find a somewhat comparable division of labor among the various species of the community, but not among the individuals of the dominant species. The function in the community of all members of the same species is the same, but some species have the function of manufacturing food, some for supplying a ground cover to check evaporation from the soil, some to act as scavengers in getting rid of dead bodies, etc. Another important difference between the human community and the plant community should be kept in mind. In the human community there are ordinarily more or less definitely organized activities carried on for the good of the community as a whole. On the other hand, in the plant community there is no altruism. It is a case of every plant for itself. The activities of certain species do result advantageously for the community as a whole, but this is due to chance circumstances, and the activities of course would be carried on just as vigorously if they were resulting in harm to the community. This fundamental difference between the two communities, however, is the natural result of the presence of consciousness in the human species and the lack of it in plants, and as soon as we leave that fact out of consideration the two types of communities become strikingly similar.

The individuals of any species, whether a dominant or a secondary species in a plant community such as the one we are considering, all make similar demands upon the environment. For this reason their relations seldom result in any benefits, but on the other hand there is constant competition between them for space, food, and often for other environmental factors such as light or shade. This is often just as true of individuals of different species which make such similar demands upon the environment as to merit being called ecological equivalents. Species which are ecologically

very different, on the other hand, often are incidentally very serviceable to one another. The trees, for example, furnish the shade necessary for some of the herbaceous plants and fungi, while the herbaceous plants furnish a living soil cover which prevents undue loss of the soil water which is needed in great quantities by the trees. The trees, likewise, as well as the shrubs, especially those near the border of the woods, serve as a windbrake which protects many smaller plants from the dangers of too high transpiration rates.

Of very great importance from the viewpoint of social disjunctive symbiosis is the phenomenon of leaf fall. The primary reason for leaf fall, of course, is the reduction of the transpiration surface during the season when absorption is difficult or impossible, and the primary cause is desiccation, but the effect of this habit on other members of the community is perhaps as important as its significance to the deciduous plants themselves. The fallen leaves form an efficient cover throughout the winter season, thus greatly reducing evaporation from herbaceous perennial plants as well as from the surface of the soil. The place of the fallen stems of herbaceous perennials in social disjunctive symbiosis is similar to that of the fallen leaves, as, likewise, is that of the dead bodies of annual plants.

Closely connected with leaf fall are the activities that are concerned with the decay of the fallen leaves. These are due mostly to bacteria and fungi. The bacteria and fungi are regular members of the community, and are living in social disjunctive symbiosis with the higher plants. They are able to live in the community only as a result of the presence of the higher plants, and they render a distinct service to the community by preventing the accumulation of dead bodies.

Although the phenomena just cited all result in benefit to certain members of the community, it must be understood that symbiosis does not necessarily imply any benefit to the symbionts, mutual or otherwise. In antagonistic symbiosis there is often more harm than benefit for at least some of the symbionts, while in social symbiosis there may be a mere tolerance of presence with neither harm nor benefit resulting to any appreciable extent. Thus many of the species in a community such as the one under

consideration are able to live together largely because the main parts of their absorbing systems are placed at different levels in the soil.[5] For example, *Circaea lutetiana* has its rhizomes only about one inch beneath the surface of the soil; the rhizomes of *Asarum canadense*, *Sanguinaria canadensis*, and *Thalictrum dioicum* are about two inches deep; those of *Podophyllum peltatum* and *Sanicula gregaria* average about two and one-half inches deep; the bulbs of *Allium canadense* are placed about three inches, and the corms of *Arisaema triphyllum* about five inches below the surface of the soil. The rhizomes of *Polygonatum commutatum* are produced about three inches down, and are later pulled down by root contractions to a depth of five or six inches. Along with these, of course, are the trees and shrubs which have absorbing organs at all depths to a distance of several feet. Still another factor which tends to make it possible for large numbers of species to live together in a forest community is the fact that different species carry on their more important activities at different times of the year, and so do not interfere with each other as much as they otherwise would. It is this that makes it possible to distinguish prevernal, vernal, aestival, serotinal, and autumnal seasons, each characterized by the prominence of different groups of species.

It is not necessary, however, to carry the discussion further along this line. It is recognized that there is nothing new in the preceding discussion except the point of view. In other words, the kinds of interrelations here pointed out are well known to most botanists, and for that reason it was considered unnecessary to go into much detail; but these interrelations have not usually been considered as cases of symbiosis. The reason for dwelling upon them here, therefore, has been to emphasize the fact that they are instances of the living together of dissimilar organisms, and so properly belong in a discussion of symbiosis. This proper point of view is preparation for further discussion of the other types of symbiosis, some of which are not so well known.

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EFFECT OF TEMPERATURE ON GERMINATION OF AMARANTHUS RETROFLEXUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 291

CLYTEE R. EVANS

(WITH FOUR FIGURES)

Introduction

The literature having a direct bearing on the effect of temperature on germination may be divided into two groups: one deals with growth in relation to temperature, and the other with delay in germination of seeds in general and of *Amaranthus retroflexus* in particular. In the first group the articles by SMITH (10), LEHENBAUER (7), LEITCH (8), BALLS (1), and KANITZ (6) on relation of growth to temperature are of interest. SMITH found that temperature, possibly internal temperature of the growing parts, may be a limiting factor to growth in *Furchraea* and *Agave*. LEHENBAUER, in his work on rate of growth of maize seedlings, found that the Van't Hoff law applies only at medium temperatures; at 31° C. the initial rate is not maintained, there being a falling off with time. He further found that the coefficients for 10° C. rise in temperature are greater at lower ranges of temperature (6.56 at 12°–22° C.), and less (0.06 at 33°–43° C.) at higher ones. He states that the optimum changes with length of exposure, and that there are not two optima, as stated by KOEPPEN. Miss LEITCH, in work with rate of growth of seedlings of *Pisum sativum*, found that the Van't Hoff law applies only from 10° to 28° or 30° C.; that there is the same type of gradation in the coefficients relating rate of growth to temperature that LEHENBAUER found; and that above 29° C. the relation of growth to temperature can no longer be expressed as a curve, so that a different curve must be constructed to express the rate of growth in successive time intervals. She defined the optimum temperature as the highest one at which the time factor does not enter. BALLS offers an explanation of the time factor, and says

that the Van't Hoff law applies approximately up to 30° C., then the growth rate acceleration decreases to a point which he proposes to call the "stopping point," a point below the lethal temperature.

KANITZ has written a monograph on the effect of temperature of life processes in which he cites over three hundred pieces of literature distributed among several fields. The following is based on DENNY'S review of this monograph. He derives formulae from those of BERTHELOT, ARRHENIUS, VON ESSEN, and VAN'T HOFF, by means of which the value of Q_{10}, the coefficient for 10° C. rise in temperature, can be calculated from experimental data at any two temperatures. These formulae are $Q_{10}=\left(\frac{k_2}{k_1}\right)^{\frac{10}{t_2-t_1}}$ and $Q_{10}=10^{\frac{10(\log k_2-\log k_1)}{t_2-t_1}}$, in which k_2 = rate of process at temperature t_2, and k_1 = rate of process at temperature t_1. He found that when he calculated results at short temperature intervals instead of long ones, Q_{10} is often not a constant at all intervals, but falls at high temperatures. He also states that many processes in plants and animals exhibit a temperature coefficient the same as the Van't Hoff one within certain temperature limits, and cites in this connection such plant processes as CO_2 assimilation between 0° and 37° C. (MATTHAEI); respiration of seedlings between 0° and 35° C. (KUIJPER); water intake of barley grains between 3.8° and 34.6° C. (BROWN and WORLEY); permeability of plant cells and tissues between 0° and 30° C. (RYSSELBERGHE); etc. Some of these processes show higher values of Q_{10} at lower temperatures, or temperatures near the minimum of the process.

BEAL (2) has found that seeds of *Amaranthus retroflexus* are long lived; that they are still viable after burial in the ground for thirty years. Delays in germination of seeds are due (putting aside the stimulus idea held by some workers) either to embryo characters such as immaturity of embryo, or need of fundamental chemical changes in the embryo preceding germination in a seemingly otherwise mature embryo (5), or to coat effects acting jointly with embryo characters. The embryo in the latter case is not dormant when naked and exposed to ordinary germinative conditions.

These coat effects may be of several kinds, namely, the almost complete exclusion of water from the embryo, as in some Leguminosae, the cutting down of oxygen supply below the minimum required for germination (9), or the high elasticity or breaking strength of the coats as compared with the force of the expanding embryo (3, 4).

CROCKER (3) and CROCKER and DAVIS (4) state that this last named coat effect is the chief cause of dormancy in seeds of *Amaranthus retroflexus*. This dormancy gradually disappears in dry storage, as is shown by a continual lowering of the minimum temperature for germination. On the other hand, wild oats, "rain barley," and a South American grass, *Chloris ciliata*, have a low maximum when not after-ripened, which rises as after-ripening progresses. Even in seeds of *Amaranthus retroflexus* which have been stored for a long time, incompleteness of after-ripening is indicated by the considerable lingering effects of the coats. Fully after-ripened seeds have their minimum for germination lowered by removal of coat restrictions. In fresh *A. retroflexus* seeds with coats treated, the minimum temperature for germination is the same as in dried seeds with coats treated. Vigor of the embryo of *A. retroflexus*, ability to respond in germinative conditions, and the rate of growth of the naked embryo under any given conditions is not affected noticeably by after-ripening. In this seed after-ripening seemingly is not a matter of after-ripening of the embryo, for embryos of fresh seed are of maximum vigor if coat effects are removed. That the breaking strength of these coats is lowered by a rise in temperature is shown by the fact that ripe seeds gathered from green plants will not germinate at temperatures lower than 40° C., but will germinate slightly at that temperature. *A. retroflexus* seeds are slightly inhibited by light at all temperatures, according to the unpublished experiments of CROCKER and DAVIS. This holds for seeds in which the coats are treated as well. Knowledge of these conditions was requisite to handling the material intelligently in finding the series of coefficients relating rate of germination of *A. retroflexus* to temperature changes.

Material and method

Three lots of seeds were used: one gathered in late summer or early fall, 1915, at Pullman, Washington; and the other two at Gary, Indiana, one lot in the late summer or early fall of 1914, and the other a year later. The experiments extended from March 15 to August 1, 1916. Thus after-ripening had proceeded for several months in two lots, and for one year and several months in the third lot. In some of the experiments, remaining coat effects were further eliminated by grinding some of the seeds with sand for four, six, or nine weeks, or treating them with concentrated H_2SO_4 for two or three minutes and then washing them with running tap water for two minutes. The optimum length of time for treatment with H_2SO_4 was determined by experiment to be two minutes for Indiana seeds, and three minutes for Washington seeds. Along with these treated seeds were run seeds with coats not treated. These seeds, treated and untreated, were then put in lots of one hundred in Petri dishes lined with moist absorbent cotton. The Petri dishes were placed in a cardboard box lined with opaque black paper, and the box then put in a refrigerator for forty-eight hours, where the temperature was below the minimum necessary for germination, to allow time for the seeds to soak before they were tested for temperature effects on rate of germination. At the end of this time the box containing the Petri dishes was kept at the desired constant temperature. The temperatures tested ranged from 9° to 42° C. Temperatures below room temperature were obtained in baths cooled with running water, and at room temperature and above, in a constant temperature incubator in which the variation was less than one degree. At one time only did the temperature vary more than this, and then it was in the water-cooled bath; this variation is indicated on the curves 1, 2, and 3, and in all the tables thus, (8°–10° C.).

The percentage germination at various intervals of time for each temperature tried was noted for all seeds in the box. The results were plotted as curves; one set of curves for each type of seeds, that is, one set of curves for Indiana seeds collected in 1915 and treated with H_2SO_4 for two minutes, another set for Indiana seeds collected in 1915 and ground with sand nine weeks, another

set for Indiana seeds collected in 1915 and untreated, another for Washington seeds untreated, etc. Eleven sets of curves were thus plotted, three of which are included in this paper as fairly typical ones. Not fewer than two hundred seeds of each type were germinated at each temperature, one hundred each in a separate Petri dish, so that all determinations were made at least in duplicate. In some instances four hundred seeds of each type were used, making quadruplicate determinations.

From these curves, three sets of which are given as typical, the length of time required for certain percentage germination at two different temperatures was read, and these readings and temperatures used in the KANITZ formula: $Q_{10}=\left(\frac{k_2}{k_1}\right)^{\frac{10}{t_2-t_1}}$, in which $k_2=$ rate of germination at temperature t_2, and $k_1=$ rate of germination at temperature t_1. The rate of germination in each case of course was the percentage germination divided by the number of hours required for this to take place. The results of these computations are given in tables I to XI.

Again similar experiments were run and attempts made to secure lots of seeds at the same percentage germination with hypocotyls the same length when the seeds were subjected to different temperatures. These readings were used exactly as were the ones from the curves previously mentioned, and as they gave the same type of coefficients, for the sake of brevity are omitted here.

TABLE I

INDIANA SEEDS, COLLECTED 1915, UNTREATED

Temperature interval (° C.)	Q_{10} at certain percentage germination*									
	12	14	20	28	30	40	50	60	70	80
11.9–16.3	11.69	12.14	11.91	17.6	.....	.....	.....	.....	.....	.....
16.3–25.3	.....	2.95	3.05	2.94	2.87	3.68	4.01	.....	.....	.....
25.3–36.4	.....	1.71	1.76	1.83	1.85	1.89	2.01	2.06	2.04	2.21
36.4–42	.....	1.14	1.13	Less than 1	Less than 1	Less than 1	1.14	1.28	1.49	1.37
16.3–36.4	.....	2.19	2.25	.....	2.25	2.55	2.74	.....	.....	.....
23.9–36.4	.....	1.68	1.72	2.11	2.02	2.29	2.41	2.96	2.94	3.53

* No germination at 8°–10° in 168 hours.

TABLE II

INDIANA SEEDS, COLLECTED 1915, GROUND WITH SAND NINE WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination*									
	12	14	20	30	36	40	50	60	70	80
(8 to 10)–16.3....	10.01	8.34	.....	.....	.....	.....	.....	.....	.....	.....
16.3–21.6.....	4.43	4.33	.....	.....	.....	.....	.....	.....	.....	.....
21.6–29.8.....	1.20	.....	1.31	1.30	.....	1.30	1.32	1.36	1.45	1.44
29.8–42.....	1.677	1.641	.....	.....	.....	.....	.....	.....	.....	.....
16.3–23.9.....	.....	.....	.....	.....	3.65	3.55	3.42	3.27	3.13	2.88
23.9–42.....	.....	.....	.....	.....	1.27	1.29	1.27	1.29	1.33	1.32

* 15 per cent germination at 8°–10° in 156 hours.

TABLE III

INDIANA SEEDS, COLLECTED 1915, TREATED WITH H_2SO_4 TWO MINUTES

Temperature interval (° C.)	Q_{10} at certain percentage germination							
	20	30	40	50	60	70	75	80
(8 to 10)–11.9...	1.778	2.118	5.91	.....	.....	.....	.....	.....
11.9 –14.45....	7.48	6.16	5.22	6.97	10.44	12.36	28.73	.....
14.45–23.4.....	3.69	3.81	3.82	3.83	3.66	3.70	3.74	4.27
23.4 –36.4.....	1.999	1.89	1.95	1.89	1.96	1.87	1.95	2.07
36.4 –42.....	1.93	1.74	1.45	1.32	1.23	1.27	1.25	1.161
14.45–28.9.....	2.81	2.92	2.73	2.39	2.18	1.76	.....	.....
21.6 –28.9.....	1.53	1.89	1.88	1.74	1.57	1.497	.....	1.903
14.45–36.4.....	2.63	2.53	2.57	2.53	2.53	2.49	.....	.....

TABLE IV

INDIANA SEEDS, COLLECTED 1915, GROUND WITH SAND FOUR WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination									
	4	8	10	20	30	40	50	64	70	80
(8 to 10)–23.9.....	4.82	4.74	.....	.....	.....	.....	.....	.....	.....	.....
16.3–23.9.....	.....	.....	.....	.....	.....	.....	.....	2.33	2.50	2.91
23.9–36.4.....	1.61	1.60	1.90	1.87	1.91	1.93	1.86	1.88	1.84	1.94
36.4–42.....	.....	.....	.....	1.28	1.24	1.25	1.13	1.02	1.10	1.09
(8 to 10)–21.6.....	5.75	6.05	.....	.....	.....	.....	.....	.....	.....	.....
23.9–29.8.....	.....	.....	2.79	1.93	1.91	1.83	1.75	.....	1.22	1.65

TABLE V

WASHINGTON SEEDS, COLLECTED 1915, UNTREATED

Temperature interval (° C.)	Q_{10} at certain percentage germination*				
	2	8	10	12	15
11.9 –14.45	8.173	10.32	11.75	.....	5.1
14.45–25.7	.....	2.40	2.37	.....	2.89
25.7 –36	.....	1.40	1.33	.....	1.38
36 –42	0.1928	.....	.....	.....	.....
23.9 –36	.....	1.15	1.11	.....	1.17
14.45–36	2.57	1.86	1.79	1.83	.....

* No germination at 8°–10° in 168 hours.

TABLE VI

WASHINGTON SEEDS, COLLECTED 1915, TREATED WITH H_2SO_4 THREE MINUTES

Temperature interval (° C.)	Q_{10} at certain percentage germination*					
	10	20	30	40	50	58
11.9 –14.45	5.77	5.77	5.66	5.15	1.86	1.0
14.45–23.4	3.82	3.40	3.30	4.05	4.52	5.17
23.4 –36.4	1.95	1.84	1.70	1.57	1.52	1.47
36.4 –42	2.36	1.37	1.21	1.21	1.13	1.08
21.6 –29.8	1.72	2.26	2.20	2.03	.....	.....
14.45–36.4	2.55	2.36	2.21	2.14	2.33	.....
14.45–29.8	2.56	2.32	1.98	1.60	.....	.....

* Only 2 per cent germination at 8°–10° in 84 hours.

TABLE VII

WASHINGTON SEEDS, COLLECTED 1915, GROUND WITH SAND FOUR WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination*						
	16	20	30	36	40	50	54
11.9 –14.45	9.07	8.84	8.09	6.74	.....	.....	.....
14.45–25.7	.....	.....	.....	3.05	.....	.....	.....
25.7 –37.6	.....	.....	.....	1.18	1.59	1.75	1.91
14.45–37.6	2.16	2.10	2.03	2.026	.....	.....	.....

* 8 per cent germination 8°–10° in 156 hours.

TABLE VIII

WASHINGTON SEEDS, COLLECTED 1915, GROUND WITH SAND SIX WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination*						
	10	20	30	40	50	60	70
16.3–23.4.....	2.28	2.10	2.05	.....	.....	.....	.....
23.4–42.....	1.45	1.36	1.24	.....	.....	.....	.....
16.3–21.6.....	2.62	2.80	2.58	2.67	2.34	2.29	.....
21.6–29.8.....	2.38	2.07	2.25	2.21	2.37	2.60	.....
16.3–29.8.....	2.48	2.32	2.37	2.38	2.35	2.47	2.65
29.8–42.....	.....	0.715	0.5799	.....	.....	.....	.....

* No germination at 8°–10° after 168 hours, and temperatures between this and 16.3° not tried.

TABLE IX

WASHINGTON SEEDS, COLLECTED 1915, GROUND WITH SAND NINE WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination*				
	10	15	20	22	30
16.3–21.6.....	2.29	.....	2.33	.....	2.79
16.3–23.9.....	1.99	1.74	1.48	1.44	.....
23.9–42.....	.....	1.65	1.79	1.85	.....
16.3–29.8.....	2.53	.....	2.54	.....	2.95
21.6–29.8.....	2.68	.....	2.64	.....	3.06

* No germination at 8°–10° after 168 hours, and temperatures between this and 16.3° not tried.

TABLE X

INDIANA SEEDS, COLLECTED 1914, UNTREATED

Temperature interval (° C.)	Q_{10} at certain percentage germination						
	12.5	15	20	30	40	50	60
11.9 –14.45....	12.82	9.60	13.15	16.85	.....	.....	.....
14.45–23.9.....	3.92	4.37	4.56	4.62	4.47	.....	.....
23.9 –42.....	.....	.....	.....	1.54	1.55	1.55	1.42
14.45–42.....	.....	.....	.....	2.42	2.23	.....	.....

TABLE XI

INDIANA SEEDS, COLLECTED 1914, GROUND WITH SAND TWO WEEKS

Temperature interval (° C.)	Q_{10} at certain percentage germination*						
	22	30	40	50	60	70	80
11.9 –14.45.....	8.75	8.88	8.30	8.001	6.65	11.42	.....
14.45–23.4.....	3.58	3.58	3.84	4.19	4.54	4.91	5.40
23.4 –36.4.....	1.92	1.91	1.89	1.89	1.92	1.88	1.68
36.4 –42.....	.....	1.24	1.22	1.18	1.10	1.06	1.35
14.45–29.8.....	2.91	3.03	3.24	3.41	3.72	3.94	4.35
23.4 –29.8.....	1.93	1.97	2.14	2.09	2.12	.....	.....
14.45–36.4.....	2.44	2.48	2.53	2.61	2.75	2.79	2.72

* 5 per cent germination at 8°–10° in 168 hours.

Discussion

In the effort to find a representative end point, many different ones were tried, with the result that almost any one proved satisfactory. These end points are Q_{10} at different percentage germination. A study of the tables shows that almost any percentage germination up to the total at the temperature might well serve for this representative end point, as in practically every series the coefficient for 10° C. rise in temperature is more than three when computed at temperature intervals near the minimum temperature for germination, and gradually decreases to about one or even less than one near the maximum. At some range between these, of course, Q_{10} falls between two and three. It is interesting that by computing through a long temperature interval Q_{10} may be found to be between two and three, when computations over shorter intervals of this longer one show Q_{10} not a constant but a variable. Take, for instance, from table III Q_{10} at the range of temperature 14.45° –36.4° = 2+, while Q_{10} at 14.45°–23.4° = 3.6 to 4+, 23.4°–36.4° = 1.8 to 2.0. Here Q_{10}, computed through a long interval where its values range from 4 to 1.8, gives an average value of 2+. This is the sort of thing to which KANITZ called attention. The series of coefficients computed through short temperature intervals agrees in general type with those given by LEITCH for effect of temperature on rate of growth of seedlings of *Pisum sativum*, with those given by LEHENBAUER for rate of growth of corn seedlings in

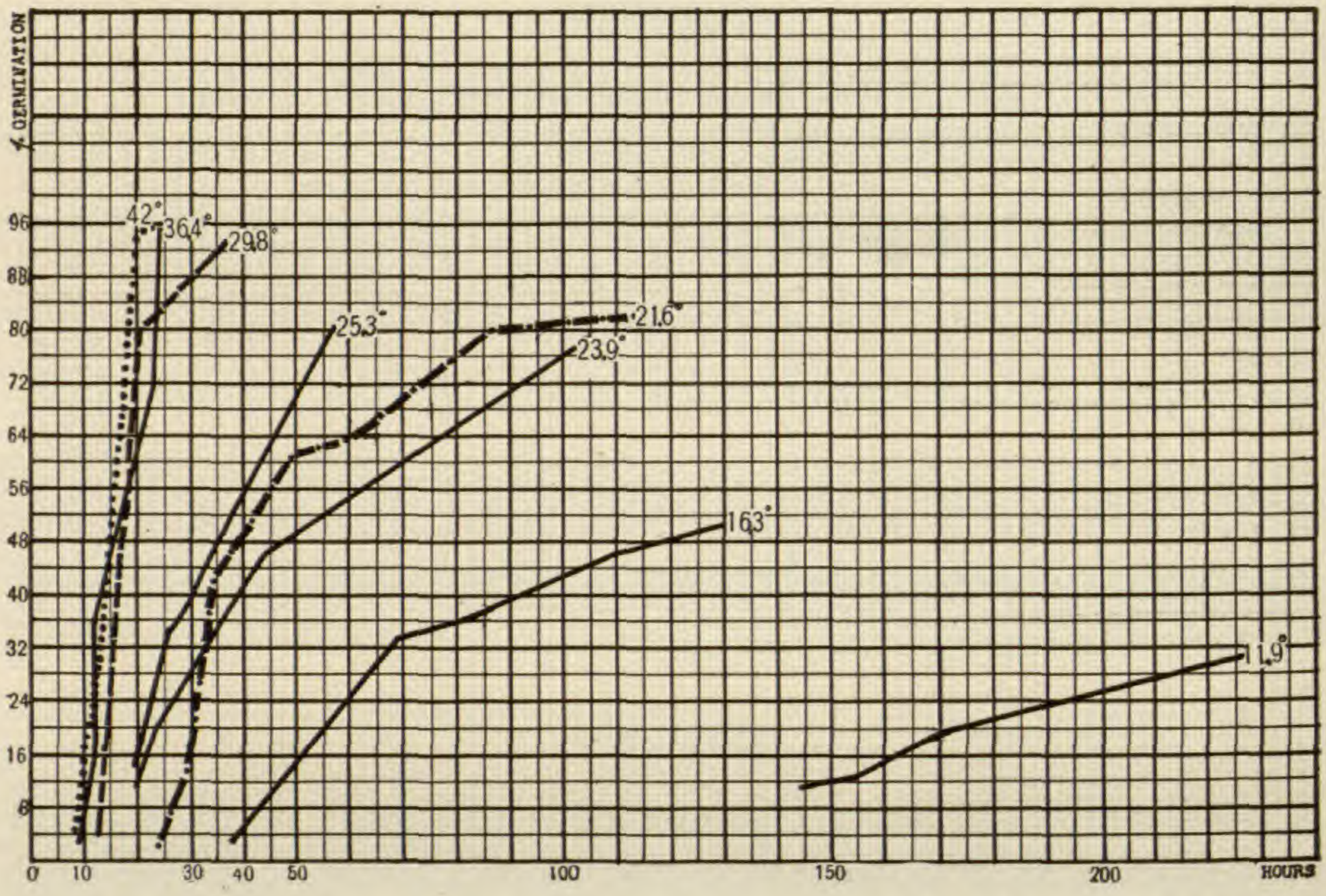

FIG. 1.—Rate of germination at certain temperatures (° C.) of seeds of *Amaranthus retroflexus*, collected at Gary, Indiana, in 1915, and untreated.

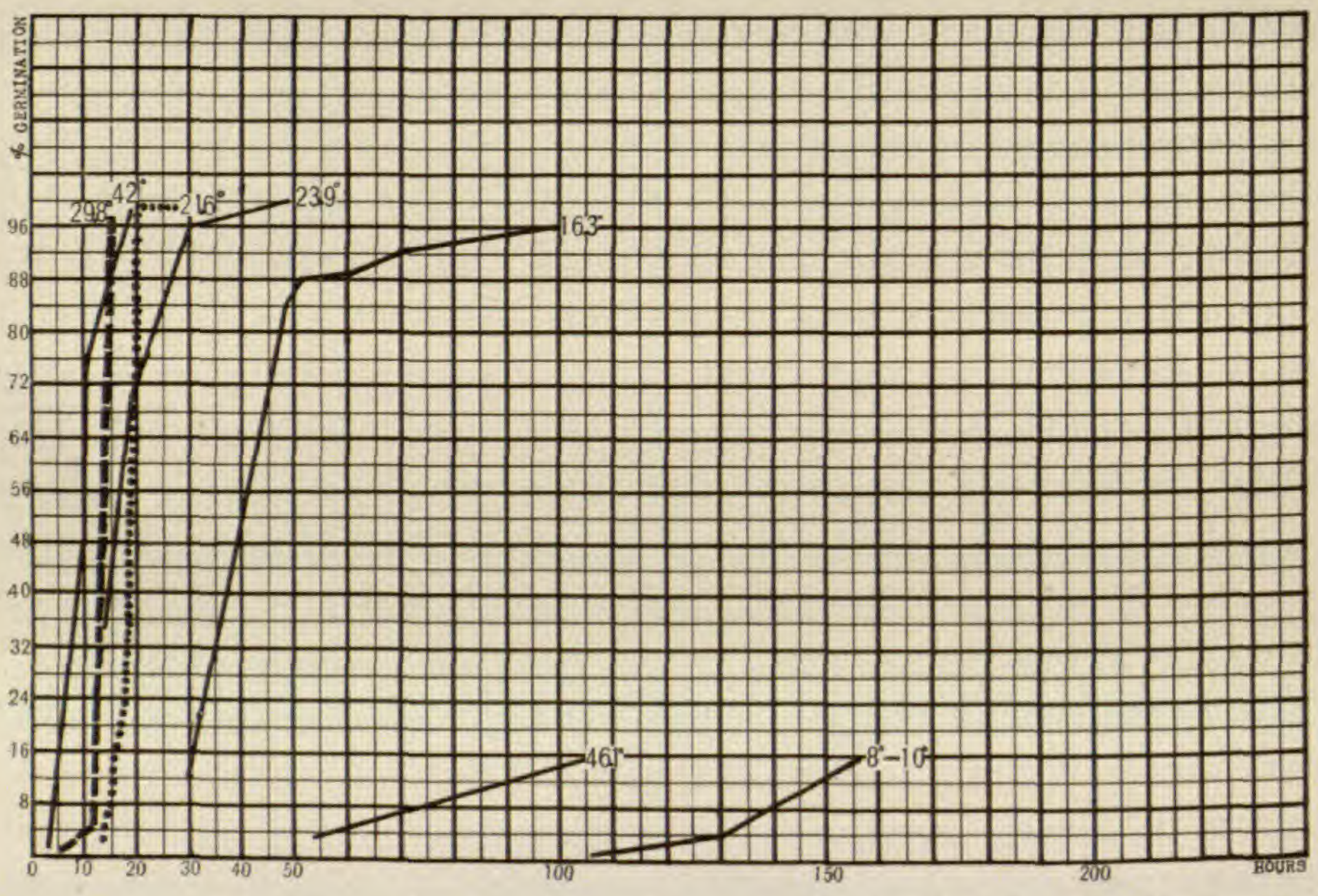

FIG. 2.—Rate of germination at certain temperatures (° C.) of seeds of *Amaranthus retroflexus* collected at Gary, Indiana, in 1915, and ground with sand nine weeks before being set to germinate.

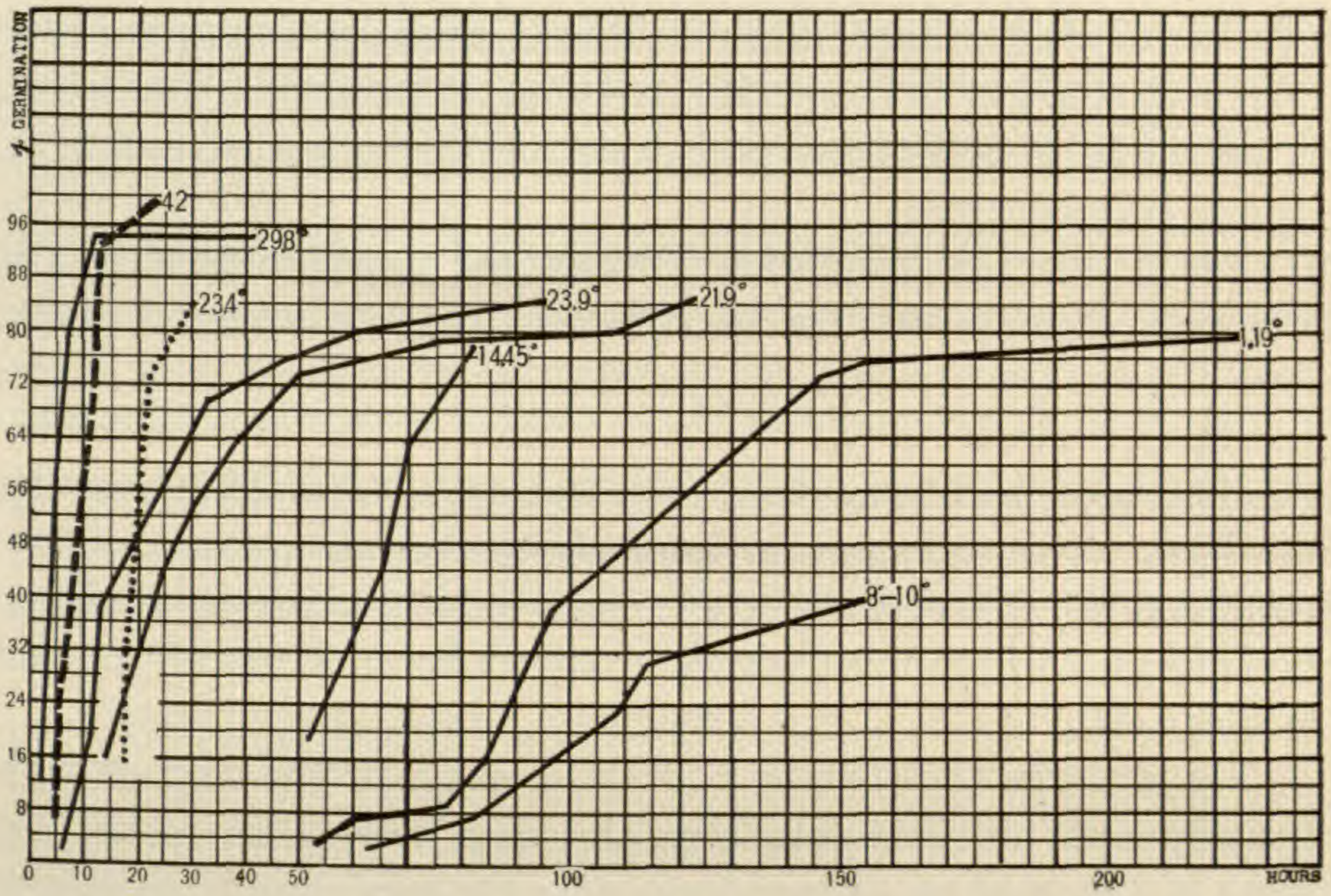

FIG. 3.—Rate of germination at certain temperatures (° C.) of seeds of *Amaranthus retroflexus* collected at Gary, Indiana, in 1915, and treated with concentrated H_2SO_4 two minutes before being set to germinate.

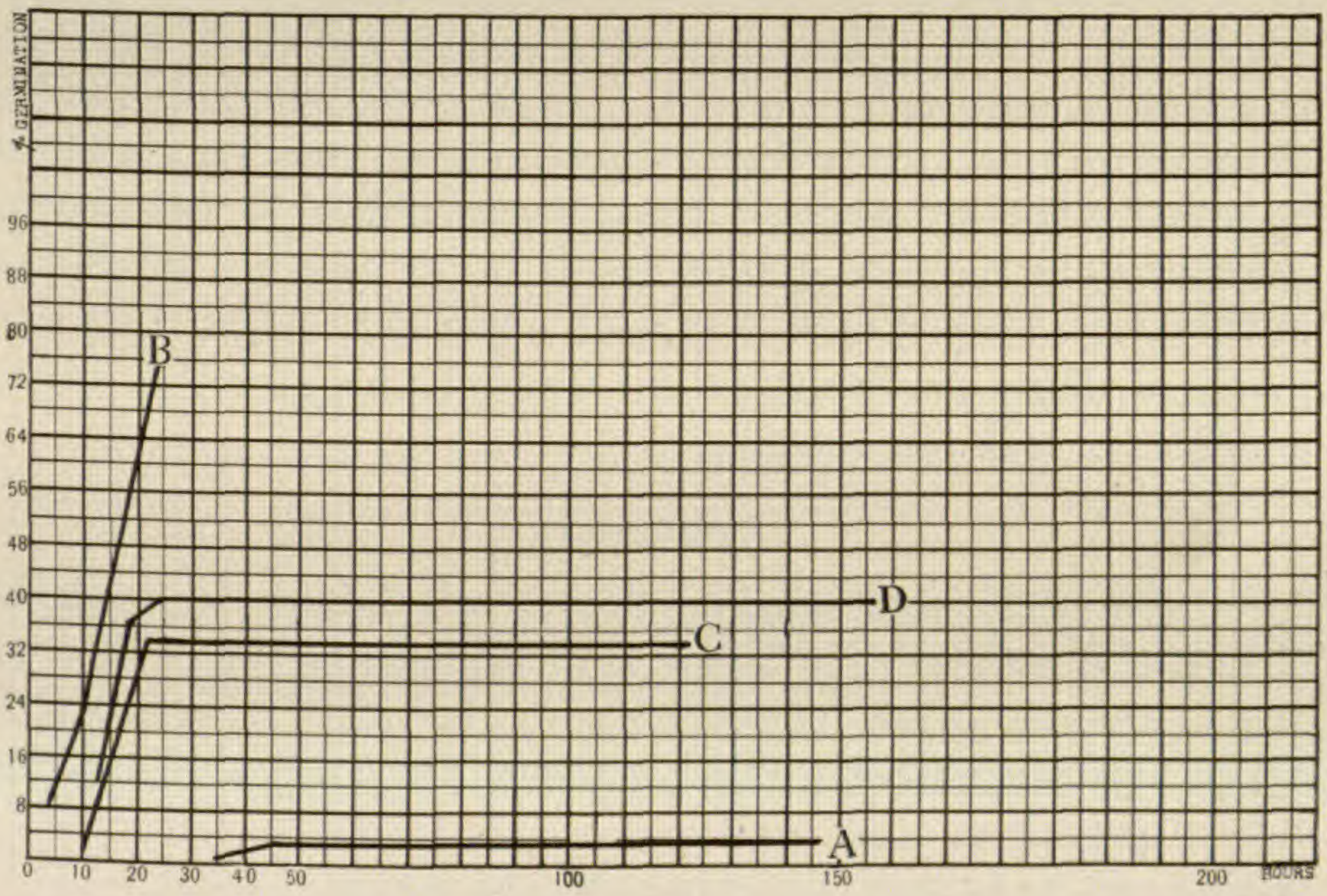

FIG. 4.—Relative rate and total percentage germination at 42° C. of *Amaranthus retroflexus* seeds collected at Pullman, Washington, in 1915: (*A*) untreated before being set to germinate; (*B*) treated with concentrated H_2SO_4; (*C*) ground with sand six weeks; (*D*) ground with sand nine weeks.

relation to temperature, and with those given by BALLS for rate of growth of sore-shin fungus in relation to temperature. Omissions in the tables of coefficients are due to lack of comparative data at certain temperatures.

Figs. 1, 2, and 3, included as representative graphs, show the difference in total percentage germination of seeds collected at the same time in one locality, and germinated at the same time at the same temperature but with seed coat intact in one lot, abraded by grinding with sand in another, and carbonized with H_2SO_4 in the third lot. This difference in total germination is greatest at low temperatures. Nevertheless, the coefficients for rate of germination computed from these curves in all lots run remarkably similar (tables I–III).

Fig. 4 shows the striking restricting effect of the coat at high temperatures, an effect paralleling the magnitude of the restricting effect of this coat at temperatures near the minimum temperature for germination. The removal of coat effects, either by grinding the seeds with sand or by treating with H_2SO_4, allows much more rapid germination at the same high temperature (42° C.) for the Washington seeds. Effects somewhat paralleling these were obtained for Indiana seeds at 46.1° C. In fig. 4 the effect of grinding the seeds with sand nine weeks is shown to be greater than the effect of grinding for six weeks. Concomitant with this slowness in germinating is the lack of anthocyanin in the seedlings developed at these high temperatures, and again apparently lessened amount in the seedlings developed at the lowest temperatures at which germination took place at all.

Conclusions

1. The coefficients relating rate of germination of seeds of *Amaranthus retroflexus* to temperature grade from high values as 10.01 (table II) at low temperatures, to low values as 0.001 (table II) at high temperatures, thus paralleling the coefficients relating rate of growth of seedlings and sore-shin fungus to temperature worked out by LEITCH, LEHENBAUER, and BALLS.

2. The general trend of these coefficients is the same for seeds only partially after-ripened, and for those with coat effects almost

completely removed by treatment with H_2SO_4 or abrasion with sand, as for instance 11.75 to 1.33 for Washington seeds untreated, and 5.77 to 1.37 for these seeds when treated with H_2SO_4 for three minutes.

3. In after-ripened seeds with coats untreated, the restricting effect of the coats shows particularly at low temperatures 8°–10° and 11.6°, and again at high temperatures, 42° for Washington seeds, and 46.1° for Indiana seeds. In both cases these effects can be lessened by treating the coats with H_2SO_4 or abrading them with sand.

Acknowledgment is due Dr. WILLIAM CROCKER for suggesting this problem, and guiding the work.

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NITROGEN FIXATION IN ERICACEAE

M. Cheveley Rayner

(with four figures)

Introductory

Since the middle of the nineteenth century it has been known that plants belonging to the Ericaceae form mycorhiza of a characteristic kind. Further knowledge of the relations between plant and endophyte in this group has only recently been forthcoming.

In 1915 Rayner[1] showed that the relationship in *Calluna vulgaris* is of a remarkable character, involving obligate symbiosis between the two organisms and a much more extensive distribution of the fungus throughout the green plant than had been suspected. As in Orchidaceae, root formation by seedlings is dependent upon early infection by the endophyte, failing which, development ceases and the plant perishes in the seedling stage. Unlike the condition in Orchidaceae, infection at the appropriate moment is provided for by the presence of mycelium on the seed coat, a condition ensured by the distribution of the endophyte throughout the vegetative tissues and eventually within the ovary chamber. These facts have been demonstrated with certainty in *Calluna*, and the evidence points to a similar condition throughout the family. Thus ovarial infection has been reported for many species in all the suborders of Ericaceae, and the inability of seedlings to complete their development without infection has already been proved for a number of these.

In such remarkable associations between flowering plants and fungi as are found in the orchids and in Ericaceae, it is of great interest to learn the exact nutritive relations between the symbionts. In orchids there is ocular evidence of digestion of mycelium by the cells of the root, and it is clear that by this means the plant can draw indirectly upon organic compounds of carbon and nitrogen in the soil. In the chlorophyllous orchids the endophyte can utilize

[1] Rayner, M. C., Obligate symbiosis in *Calluna vulgaris*. Ann. Botany **29**:97–153. 1915.

the products of photosynthesis, but in the nonchlorophyllous forms, such as *Neottia* and *Corallorhiza*, this is not so, and, on the observed facts, the mutual relationship appears to be one of parasitism on the part of the green plant. Indeed this condition has fully been demonstrated for *Gastrodia elata*, a remarkable non-chlorophyllous species found in Japan. It is certain, therefore, that one at least of the so-called "saprophytic" orchids is parasitic upon a fungus, *Armillaria mellea*, and that the establishment of this relation has become obligate for the full development of the plant. This is the more interesting in that the fungus concerned is parasitic in habit and invades the tuber of the orchid in the first instance in exactly the same manner as it attacks the tubers of potato, upon which it is commonly found as a parasite in Japan.[2] In orchids the fungi endophytic in the roots do not spread into the chlorophyllous tissues, nor is there any evidence that they can use atmospheric nitrogen.

In *Calluna* the evidence as to exchange of food materials between the two partners may be summarized as follows. There is no indication of digestion of mycelium by the root, nor are there any obvious symptoms of attack or defense beyond the fact that hyphae effect an entry in the first instance and spread from cell to cell. That this vegetative activity depends upon a supply of food drawn from the plant cells rather than from organic compounds in the soil, is suggested by the normal behavior of the symbionts when grown in solutions of inorganic salts in pure culture. In the shoot, active mycelium is not readily demonstrated although widely distributed in a reduced condition; active hyphae occur in the extensive air spaces of the leaves, and grow into the air from the surface of the shoot. Moreover, there is evidence that mycelium undergoes digestion by the mesophyll cells of the leaf, and also that the fungus can hydrolyze arbutin outside the plant (RAYNER, *loc. cit.*). With respect to nitrogen assimilation, there is cumulative evidence that the endophyte of Ericaceae can utilize atmospheric nitrogen in greater or less degree, and it is the purpose of this paper to present this as briefly as possible.

[2] KUSANO, S., *Gastrodia elata* and its symbiotic association with *Armillaria mellea*. Jour. Coll. Agric. Tokyo 4:1–66. 1911.

The experimental evidence in question is derived from three sources. (1) The work of TERNETZ,[3] who showed that certain fungi isolated from the roots of ericaceous species could utilize atmospheric nitrogen. (2) The work of the writer (*loc. cit.*), which supplied the necessary link connecting these fungi directly with Ericaceae, and also provided additional evidence of the ability of certain ericaceous species to utilize atmospheric nitrogen. (3) The work of DUGGAR and DAVIS,[4] who undertook a critical experimental review of the difficult problem of nitrogen fixation by fungi. The evidence provided by these workers will now be considered in historical sequence.

(1) The researches of TERNETZ were undertaken in connection with an attempt to isolate the root endophytes of Ericaceae, concerning which no information was at that time available. As a result of prolonged experiments, eight pycnidia-forming fungi were isolated, five of which were investigated for evidence of fixation of gaseous nitrogen. All forms isolated were referred by LINDAU and HEMMINGS to *Phoma*, and differed in the small size of the pycnidiospores (4–5 μ in length) from the species previously found associated with Ericaceae. The five forms experimented with were isolated from the roots of *Oxycoccus palustris*, *Andromeda polifolia*, *Vaccinium Vitis-Idaea*, *Erica Tetralix*, and *E. carnea*, and were named *Phoma radiciis Oxycocci*, *P. radiciis Andromedae*, *P. radiciis Vaccini*, *P. radiciis Tetralicis*, and *P. radiciis Ericae*, respectively. TERNETZ has put on record the interesting observation that these fungi, although isolated from plant species growing in close proximity, are specific strains, distinguishable by definite morphological and physiological characters.

The isolation of fungal species endophytic in the roots of plants is a matter of notorious difficulty, and their identity can only be proved by formation of mycorhiza typical for the species following upon inoculation from pure culture into the roots of seedlings free from fungal infection. Those isolated by TERNETZ were

[3] TERNETZ, C., Über die Assimilation des atmosphärischen Stickstoffes durch Pilze. Jahrb. Wiss. Bot. **44**:353–408. 1907.

[4] DUGGAR, B. M., and DAVIS, A. R., Studies in the physiology of the fungi. I. Nitrogen fixation. Ann. Mo. Bot. Gard. **3**:413–437. 1916.

believed to be the endophytes associated with the different species, but the necessary proof was lacking, inasmuch as seedlings of the latter were never obtained free from mycorhiza. All attempts to sterilize seeds failed, since sooner or later the roots of seedlings raised from such seeds showed the mycorhizal condition typical of the species in nature. The five strains of *Phoma*, as well as *Aspergillus niger* and *Penicillium glaucum*, were subsequently cultivated on media free from combined nitrogen. The cultures were carried on over a period of several years, were frequently repeated, and due precautions were observed with regard to purity of materials, adequacy of controls, and methods of estimation. It is recorded that none of the fungi investigated required a supply of combined nitrogen for healthy development or growth. They all fixed atmospheric nitrogen, but in very different degrees. The values

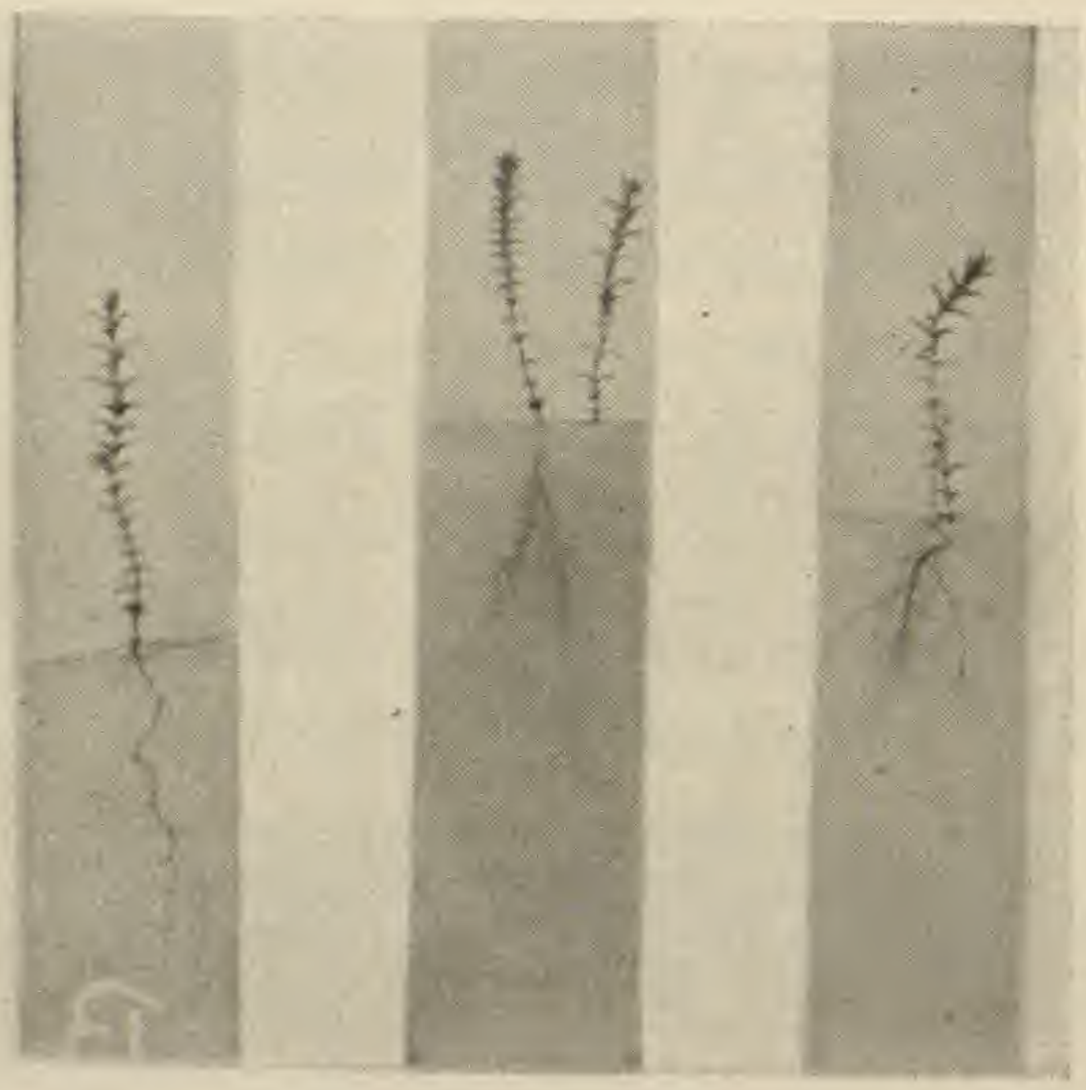


FIG. 1

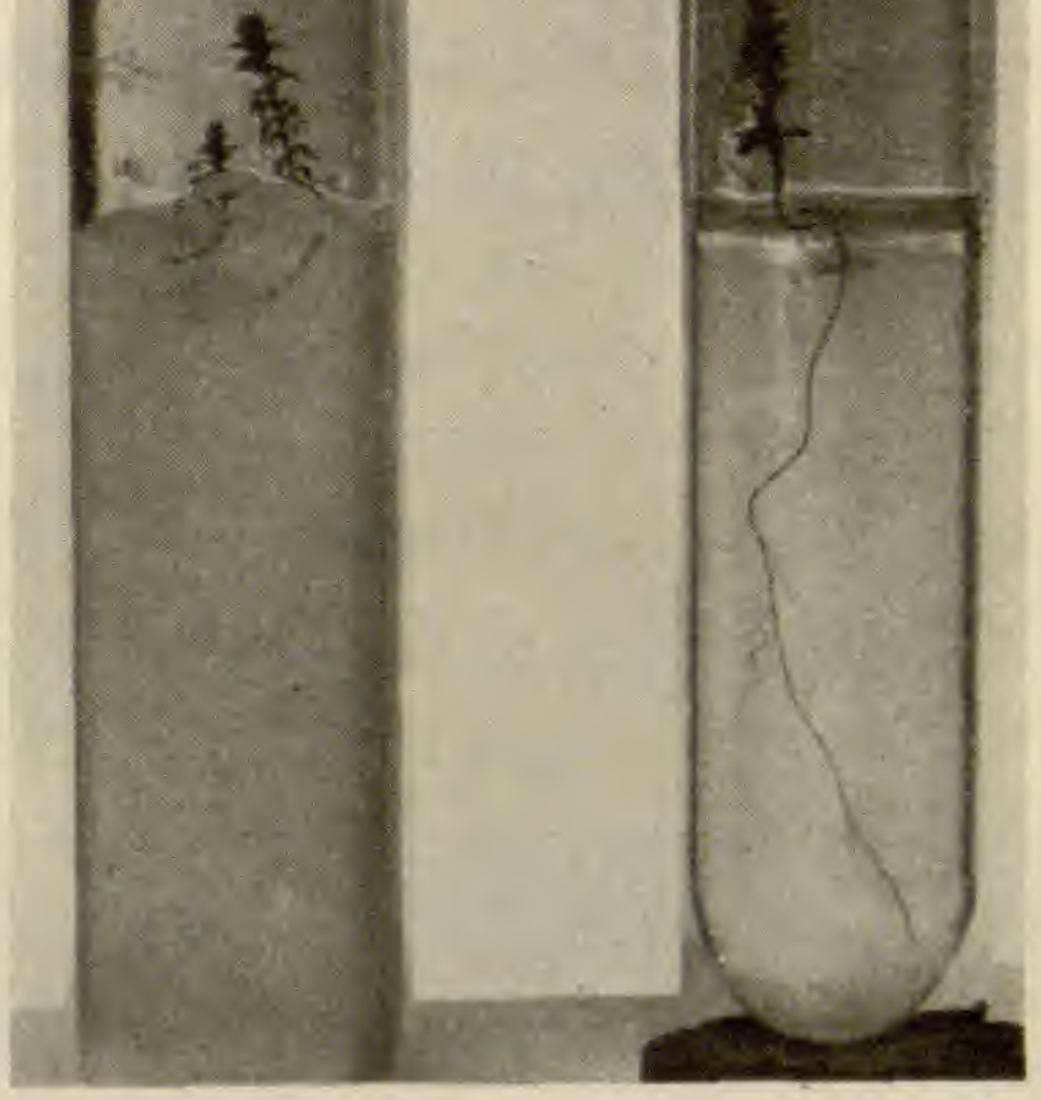


FIG. 2

FIG. 1, 2.—Fig. 1, *Calluna vulgaris:* representative seedlings (four months old) from large number grown in agar nutrient lacking nitrates; growth continued until root system occupied whole of rooting medium; shoots averaged 3.5–5 cm. length; fig 2, *Calluna vulgaris:* representative seedlings (three months old) from silica jelly cultures lacking combined nitrogen; silica nutrient in right-hand tube chanced to be more liquid in consistency than other tube, or than tubes shown in fig. 3, hence more vigorous root development; finer roots in upper part of root system not visible in photograph.

obtained for *Aspergillus* and *Penicillium* are too small to have any critical value, and agree in this respect with those of previous investigators. The highest capacity for nitrogen fixation was found in the strains of *Phoma* isolated from *Oxycoccus*, *Vaccinium*, and *Andromeda*, in which the values cited appear to be well outside the range of any possible experimental error. Table I, reproduced from the original paper by TERNETZ, shows the values obtained for *Phoma radiciis* as compared with those on record for the nitrogen-fixing bacteria.

TABLE I

RECORDS OF NITROGEN FIXATION BY FUNGI EXTRACTED FROM FOUR ERICACEOUS SPECIES, AS COMPARED WITH THAT EXHIBITED BY *Clostridium*, *Azotobacter* *Aspergillus*, AND *Penicillum*, FROM TERNETZ (*loc. cit.*).

ORGANISM	DAYS	DEXTROSE SUPPLIED		DEXTROSE USED (GM.)	NITROGEN FIXATION		INVESTIGATOR
		gm.	Per-centage		mg.	per gm. dextrose (mg.)	
Clostridium Pasteurianum..	20	40	4	40	53.6	1.34	Winogradski
Clostridium Pasteurianum..	20	20	2	20	24.4	1.22	Winogradski
Clostridium americanum...	30	1.25	0.25	1.25	4.6	3.7	Pringsheim
Clostridium americanum...	30	5	1	3.01	8.2	3.01	Gerlach and Vogel
Azotobacter chroococcus...	35	5	0.5	5	42.7	8.56	Gerlach and Vogel
Azotobacter chroococcus...	35	12	1.2	12	127.9	10.66	Ternetz
Aspergillus niger.....	28	7	7	1.1	1.9	1.71	Ternetz
Penicillium glaucum.....	28	7	7	0.7	2.8	3.8	Ternetz
Phoma radiciis Oxycocci...	28	7	7	0.85	15.3	18.08	Ternetz
Phoma radiciis Andromedae	28	7	7	0.67	7.3	10.92	Ternetz
Phoma radiciis Vaccinii....	28	7	7	0.71	15.7	22.14	Ternetz
Phoma radiciis Tetralicis...	28	7	7	1	4	3.99	Ternetz
Phoma radiciis Ericae.....	28	7	7	1.1	2.3	2.17	Ternetz

The three fungal strains concerned work much less energetically but more economically than *Clostridium* or *Azotobacter*. For example, for each gram of dextrose used, 22 mg., 18 mg., and 11 mg. of nitrogen were combined, as compared with values ranging from 1.2 mg. to 10.6 mg. of nitrogen per gram of dextrose for the nitrogen-fixing bacteria. These are the highest relative figures on record for nitrogen-fixing organisms.

(2) The evidence contributed by the writer was obtained in the course of an intensive experimental study of *Calluna vulgaris*, and supplied the link needed to connect the fungi isolated by TERNETZ

with their ericaceous host plants. In ignorance of the work of TERNETZ, the conclusion was reached independently that seedling roots become infected from the testa subsequent to germination. This view proved to be correct, and a pycnidia-bearing fungus was eventually isolated with comparative ease from unopened fruits. Proof of the identity of this fungus was then provided by reinoculation into seedlings grown in pure culture and raised from sterilized seeds. A remarkable condition of obligate symbiosis was thus put on record for *Calluna*, and the observations made by TERNETZ as to the specificity of the fungal strains in the different ericaceous species were subsequently confirmed. The characters of the endophyte isolated from *Calluna* agree with those described by TERNETZ, and the necessary proof is thus provided that the forms experimented with by this worker were actually those associated with the five plant species concerned. In view of this fact, the suggestion previously put forward as to nomenclature (*loc. cit.*, p. 125) should be withdrawn and the name *Phoma radiciis Callunae* accepted.

In the paper recording these facts, attention was drawn to observations bearing on the possibility of nitrogen fixation by the endophyte. Of these may be mentioned: (1) the vigor and longevity of seedlings germinated on filter paper moistened with distilled water (RAYNER[5]); (2) the wide distribution of the endophyte throughout the plant tissues, its development in the intercellular spaces of the leaves and emergence to the air from the surface of the shoot; (3) the evidence of digestion of mycelium by mesophyll cells (see footnote 1). The association of *Calluna* and other ericaceous species with soils deficient in nitrates in itself provides *raison d'être* for the remarkable biological relations between plant and fungus, assuming fixation of atmospheric nitrogen on the part of the latter.

Experimental observations

CALLUNA SEEDLINGS IN MEDIA LACKING COMBINED NITROGEN.—In experimental cultures, seedlings of *Calluna* grow readily under aseptic conditions in a dilute normal solution made with 1.2 per cent agar-agar. In order to test the possibility of cultivation in a

[5] RAYNER, M. C., The ecology of *Calluna vulgaris*. New Phytol. **12**:59–77. 1913.

substrate free from combined nitrogen, pure culture seedlings were planted in a similar medium lacking nitrates, both sets of seedlings being infected from a pure culture of the endophyte at planting. These cultures were first grown in 1915, and no special precautions were observed beyond the use of pure chemicals and freshly distilled water. The seedlings not supplied with nitrate grew surprisingly well. They

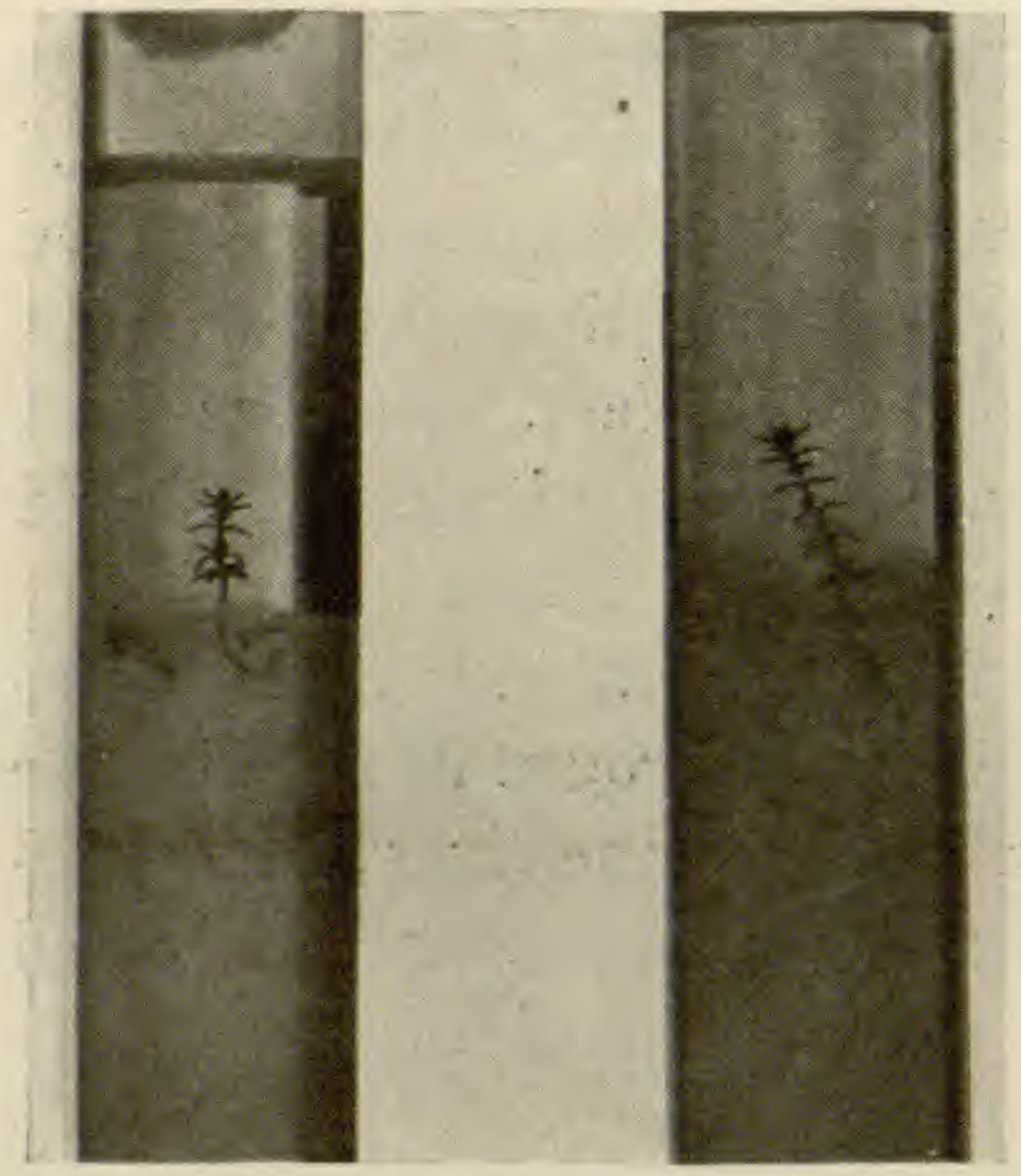


FIG. 3

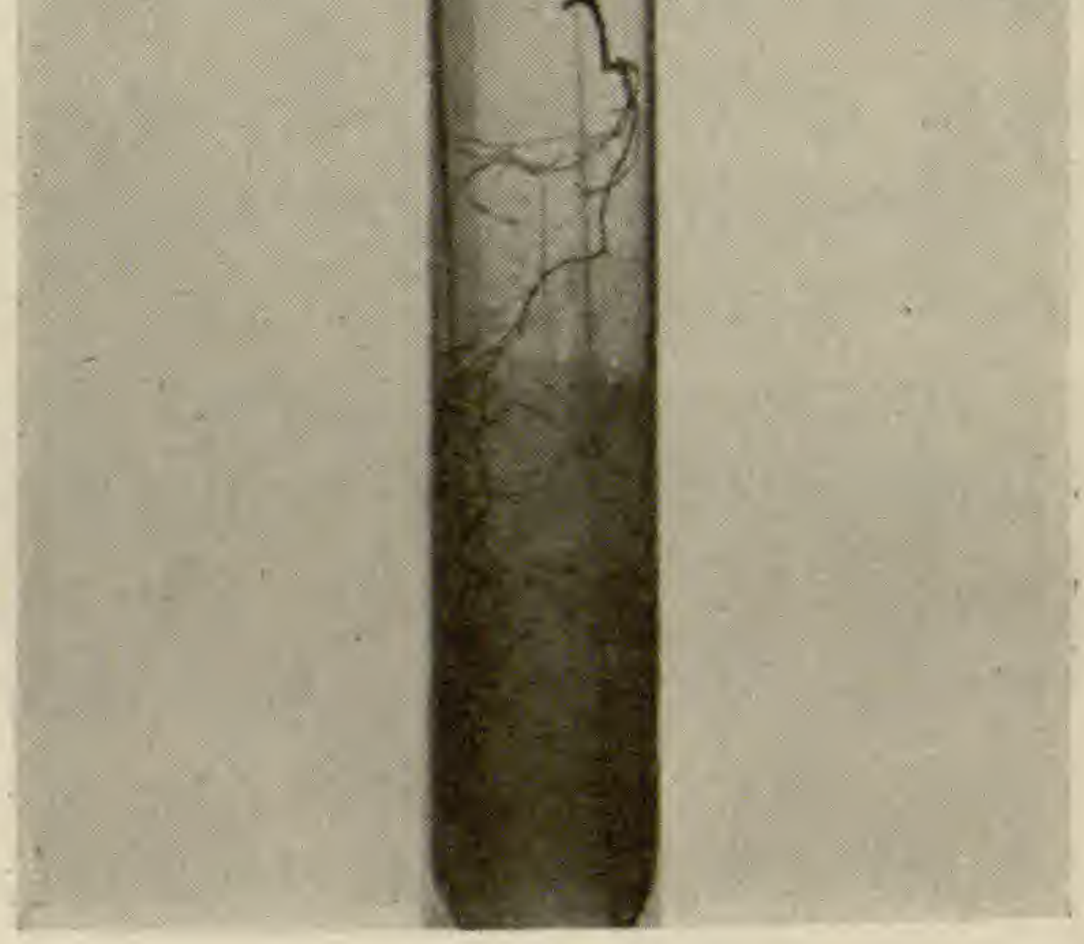


FIG. 4

FIGS. 3, 4.—FIG. 3, control seedlings planted simultaneously with those in fig. 2 in silica jelly nutrient containing combined nitrogen in form of KNO_3; fig. 4, same seedling as shown in right-hand tube of fig. 2, five and a half months old; shoot reached height of over 6 cm.

were, on the average, healthier than the controls, a brighter green, and of quite as vigorous growth. The controls were supplied with potassium nitrate in the proportion of 0.5 gm. per liter. They showed no differentiating features and unfortunately were not photographed. It should perhaps be mentioned that the cultures were grown in a small cold greenhouse away from the laboratory. Kjeldahl estimations of samples of the agar medium subsequently made yielded negative results.

It had been observed previously that *Calluna* seedlings thrive only in solutions of extremely low total concentrations of salts (0.05 per cent). The experiments just described show further that in cultures of "synthetic" seedlings, the use of a culture fluid of 0.05 per cent total concentration affords optimum conditions for growth.

The experiments have since been repeated, using every possible precaution to avoid contamination by traces of combined nitrogen. A similar solution of inorganic salts was made up in silica jelly prepared from specially purified materials and ammonia-free water. The cultures were planted in the autumn under unfavorable weather conditions, and seedlings did not root freely in the silica jelly, which seemed to offer mechanical difficulties. Otherwise, the results confirmed those already described. The seedlings deprived of combined nitrogen were green and healthy and grew at the same rate as the controls.

It may be objected that seedlings of *Calluna* could grow for several months on the seed reserves, and that this accounts for the vigor and longevity shown by seedlings supplied with distilled water only. Against this interpretation is the fact that seedlings germinated on moist filter paper from sterilized seeds not only form no roots, but make practically no shoot growth and quickly show symptoms of starvation such as yellowing and discoloration of the leaves. These symptoms are relieved by inoculation from a pure culture of the endophyte. Finally, there can be no doubt that the optimum conditions in artificial cultures for the establishment and maintenance of a properly balanced relation between plant and endophyte are supplied by a rooting medium of extremely low concentration of salts (for example, 0.05 per cent) lacking combined nitrogen. A fresh line of research is hereby suggested in order to ascertain whether the unfavorable symptoms shown by seedlings planted in culture solutions of higher concentration of salts can be specially correlated with the supply of nitrates. It is certain that a very small alteration in the character of the nutrient supplied to "synthetic" seedlings overthrows the normal balance and induces parasitism in the endophyte.

(3) ADDITIONAL EVIDENCE ON NITROGEN FIXATION IN PHOMA.—An indirect contribution to the subject has recently been made by

DUGGAR (*loc. cit.*). In the course of an experimental review of previous work on nitrogen fixation by fungi, DUGGAR has repeated and extended the observations of earlier workers on this subject, taking extraordinary precautions to avoid experimental methods open to criticism on the score of inaccuracy. Among the species thus investigated are *Penicillium* spp., *Aspergillus niger*, *Macrosporium commune*, *Glomerella Gossypii*, and *Phoma Betae*, as well as three forms of *Azotobacter* isolated from different soils. With regard to the four first named genera, DUGGAR'S work confirms that of previous observers, namely, that these fungi can utilize atmospheric nitrogen to a very slight extent. The amounts recorded are very small, and in DUGGAR'S opinion cannot be accepted as conclusive evidence of ability to fix atmospheric nitrogen. On the other hand, the values obtained by DUGGAR for *Phoma Betae* range from 3.022 mg. to 7.752 mg. per 50 cc. of culture fluid, a known amount of combined nitrogen being supplied. These values are of special interest for comparison with those recorded by TERNETZ for the forms of *Phoma radiciis* extracted from the roots of ericaceous species. Indeed, the evidence appears to be conclusive that ability to continue to fix atmospheric nitrogen exists in varying degree in *Phoma*.

The experimental results obtained by the writer indirectly support this view, and provide a basis for an intelligible explanation of one physiological aspect of the relation between green plant and fungal symbiont in Ericaceae; incidentally, they throw light on the proved ability of species such as *Calluna* and *Vaccinium* to thrive in soils deficient in nitrates. The degree of nitrogen fixation by the endophyte doubtless varies with the species concerned, and may operate as an important survival factor for the plant growing under competitive conditions.

Summary

1. In 1907 TERNETZ provided evidence that certain strains of *Phoma*, isolated from the roots of ericaceous plants, could utilize atmospheric nitrogen.

2. In 1915 the necessary proof that the fungi extracted by TERNETZ were actually the endophytes was provided by the

writer, who showed also that seedlings of *Calluna vulgaris* in pure culture thrive in a rooting medium lacking combined nitrogen.

3. In 1916 DUGGAR offered additional evidence for fixation of nitrogen by members of *Phoma*.

The experimental work of which this paper gives an account has been in part carried out at the Pilcher Research Laboratory, Bedford College, University of London, with the aid of a grant from the Dixon Fund of the University of London.

BEDFORD COLLEGE FOR WOMEN
UNIVERSITY OF LONDON

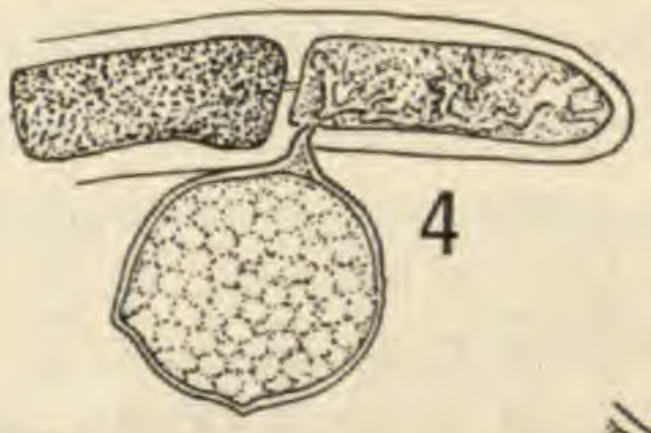
RHIZOPHIDIUM POLYSIPHONIAE IN THE UNITED STATES

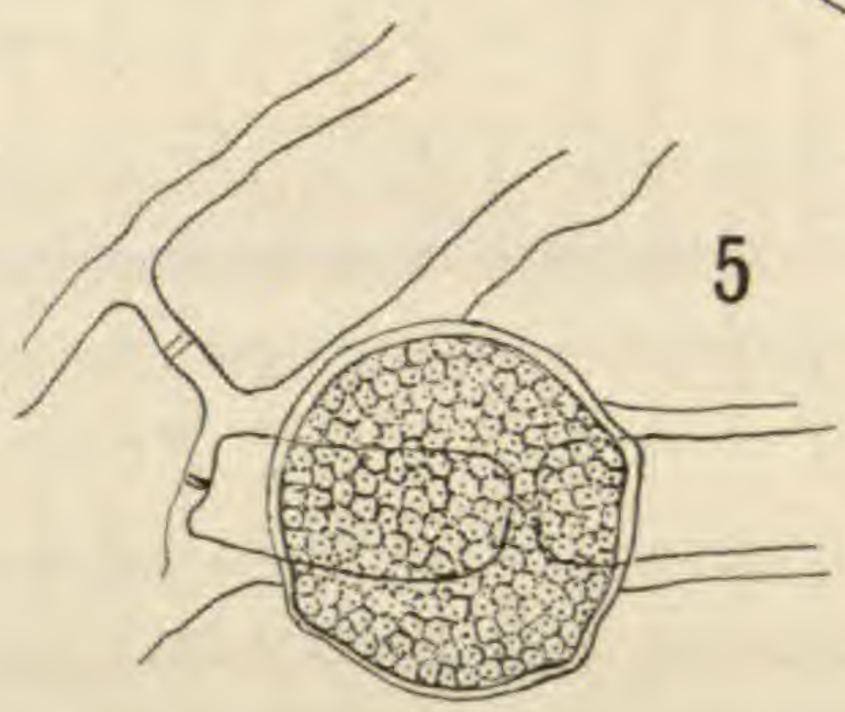
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **292**

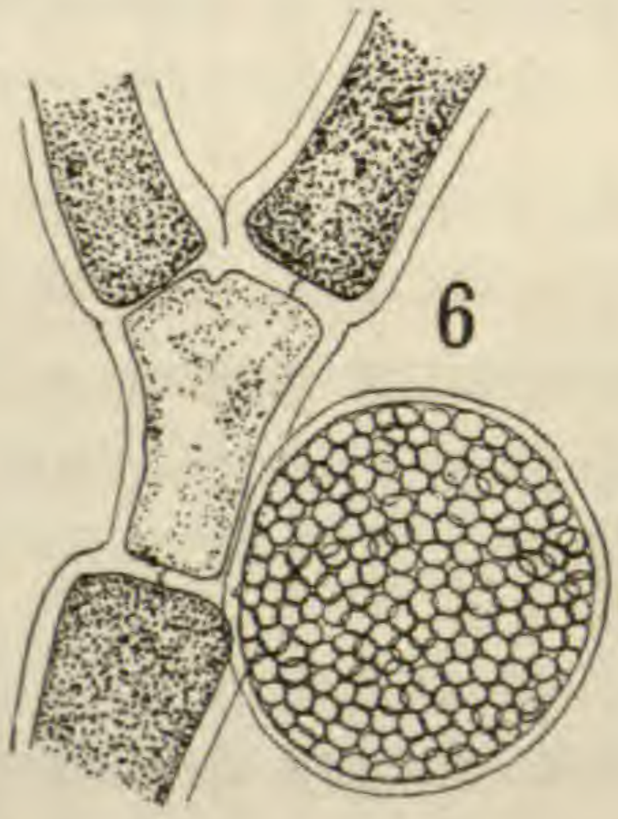
GEORGE W. MARTIN

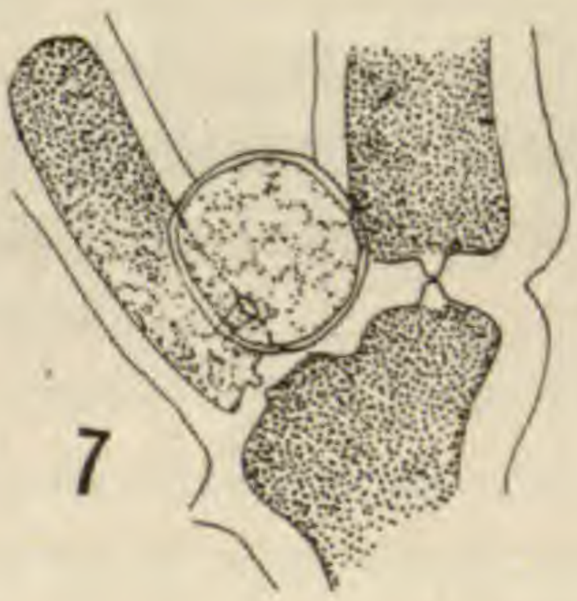
(WITH TEN FIGURES)

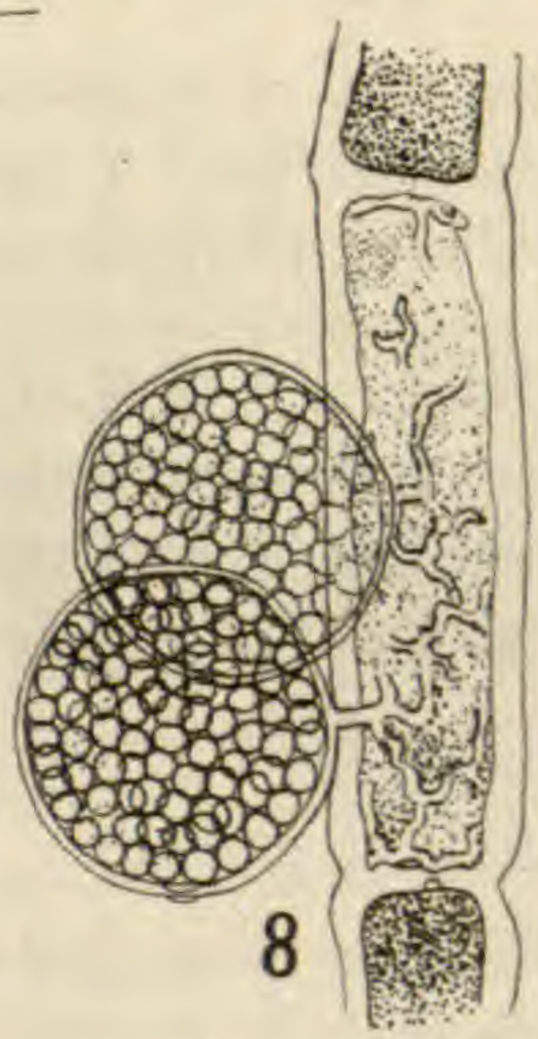
The morphology and distribution of the fungi parasitizing marine plants are still so imperfectly known that additional facts concerning any of them seem worth placing on record. Among some algal material collected by Dr. T. C. NELSON in Barnegat Bay, New Jersey, and sent by him to the writer preserved in formalin, occurred a small sterile plant of *Callithamnion* which was observed to bear numerous sporangia of a chytridiaceous fungus. The mature sporangia were globose or nearly so, from 22 to 39 μ in diameter, averaging 33 μ, and closely appressed to the attacked host cell, which could be sharply distinguished from the neighboring unattacked cells by the partial or nearly complete exhaustion and decoloration of its contents. In only one instance was more than one sporangium attached to a single host cell (fig. 8). The cell contents are destroyed first at the end at which the fungus is attached (fig. 3), and by the time the sporangium becomes mature the contents of the parasitized cell are, as a rule, almost exhausted (figs. 1, 2, 6, 8). In some cases a branched, rootlike mycelium, rather coarse for this genus, could be seen within the host cell and traced to the base of the sporangium (figs. 1, 2, 8, 9, 10). More frequently the mycelium could not be distinguished. The zoospores are from 2 to 3 μ in diameter, globose or somewhat irregular in shape, and are evidently liberated through an opening developed from a papilla, of which each sporangium bears from one to several (figs. 1, 4, 5). Each zoospore contains a nucleus or oil globule, and in addition a much smaller body, observed only in the spores remaining in a nearly

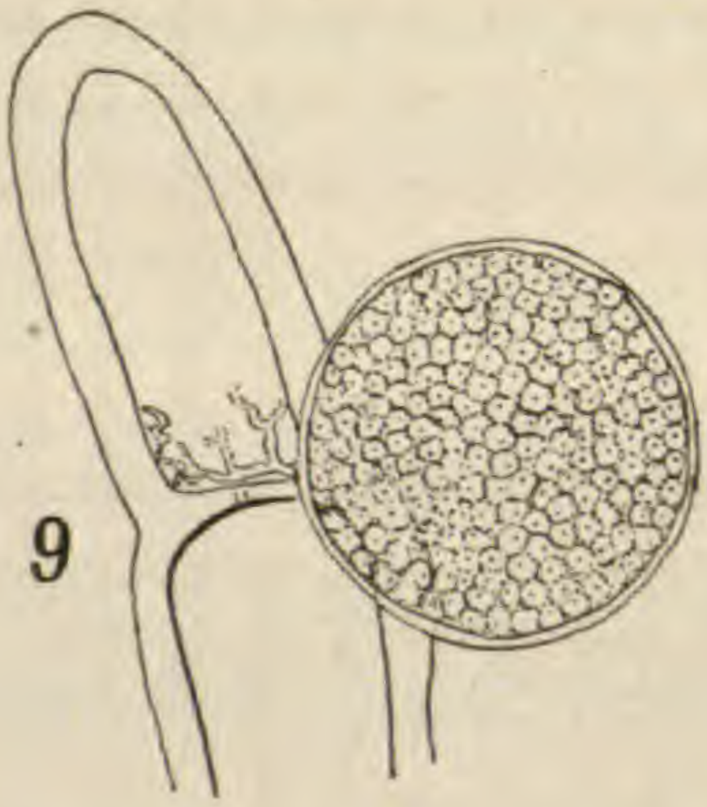


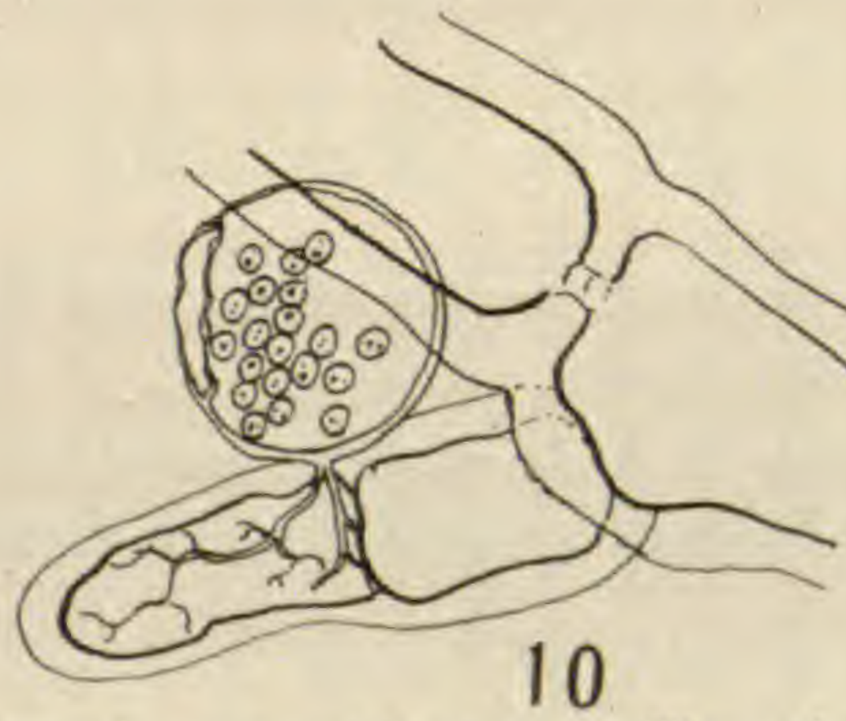












FIGS. 1–10.—Figs. 1, 2, mature sporangia, one with two papillae, showing mycelium in host cell; fig. 3, two immature sporangia showing exhaustion of host cells at end of which sporangia are attached; fig. 4, immature sporangium with two papillae; fig. 5, nearly mature sporangium from above, with three papillae (sporangium attached to basal cell of smaller branch); figs. 6, 9, mature sporangia, latter showing mycelium; fig. 7, immature sporangium, showing rootlike base; fig. 8, two mature sporangia on same host cell; fig. 10, nearly empty sporangium; spores with nuclei and blepharoplasts. Figs. 1–3, ×480; figs. 4–10, ×1000; all reduced to one-half in reproduction.

empty sporangium (fig. 10), presumably a blepharoplast. The wall of the sporangium is smooth and firm and about 1 μ in thickness.

According to the classification of SCHRÖTER[1] this fungus belongs to the genus *Rhizophidium*, of which several species have been reported as attacking hosts which occur in salt or brackish water. Of these, only *R. polysiphoniae* (Cohn) Petersen resembles the species under consideration. This fungus was originally described from Helgoland by COHN[2] under the name *Chytridium polysiphoniae*, as lacking a mycelium and with a dark colored membrane and a definite operculum, although the latter is not shown in COHN's later figure.[3] PETERSEN[4] redescribed the species as a *Rhizophidium*, stating that it possessed a definite mycelium, and, except in old specimens, a hyaline membrane, and reports it as occurring in several localities in Denmark on *Polysiphonia*, *Ceramium*, *Delessaria*, and *Callithamnion*. The correspondence between this species and the form from New Jersey is not complete, since in the latter no suggestion of a definite operculum could be seen, and some of the sporangia had at least three, possibly more, papillae. Nevertheless, the resemblance between the New Jersey species and the descriptions and figures of COHN and PETERSEN is so close that it seems inadvisable, without studying living material, to regard them as distinct.

UNIVERSITY OF CHICAGO

[1] SCHRÖTER, J., Chytridineae. In ENGLER and PRANTL, Die Natürlichen Pflanzenfamilien 1:[1] 1892.

[2] COHN, FERDINAND, Chytridii species novae marinae. Hedwigia 4:169–170. 1865.

[3] ———, Beiträge zur Physiologie der Phycochromaceen und Florideen. Archiv Mikr. Anatomie 3:1–60. 1867.

[4] PETERSEN, H. E., Contributions a la connaissance des Phycomycetes marins (Chytridinae Fischer). Overs. Danske Videnskabernes Selskabs. Forh. 1905:439–488.

CURRENT LITERATURE

BOOK REVIEWS

Transpiration of plants

BURGERSTEIN'S[1] second volume on the transpiration of plants constitutes a supplement to his well known work published in 1904. It presents a critical summary of the literature on transpiration down to and including the year 1920. The first volume was based on 394 publications, while in the second volume 505 have been added, many of which were published in English, and an unusually large number by women. The first volume contained 30 chapters, the second 32. They parallel each other very closely, but a comparison of the two brings out rather clearly the more recent trend of this line of investigation. Many of the subjects mentioned in the text in the first volume are here given separate chapter treatment. Thus the principal advances made in the study of the transpiration of plants during the last sixteen years are clearly indicated. A number of new terms and phrases appear in the second volume which were not used in the first, and most of which are concerned with an attempt on the part of the investigator to obtain a more satisfactory basis for the comparison of the amount of transpiration of different plants at different times and under different conditions.

Relative transpiration is the ratio of transpiration of any plant at any time to the water loss from a standardized water surface, or from any other water-evaporating surface exposed under the same conditions as is the plant, and for the same length of time. In most of the papers in which this term is used the assumption has been made that evaporation is a correct measure of the environmental conditions affecting transpiration, and that therefore any variation which occurs in the plotted graph showing ratio of transpiration to water loss is due to some adjustment on the part of the plant. This assumption is made without sufficient reason and is not well supported by experimental data.

Index of transpiring power differs from relative transpiration only in that a cobalt paper is introduced as an indicator of relative water loss; consequently any errors inherent to the relative transpiration method are not eliminated by this method, and there is also introduced the uncertainty connected with the use of the cobalt paper. Both of these methods have been used extensively and have stimulated an unusually large amount of investigation. While the accuracy of the results must be questioned, it is undoubtedly true that our knowledge of transpiration has been greatly advanced by their application.

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[1] BERGERSTEIN, ALFRED, Die Transpiration der Pflanzen. Zweiter Teil (Ergänzungsband). Jena. 1920.

Specific transpiration is really an expression of the rate of drying of plants. It is the percentage of the total water content of the plant lost during a definite time. The loss would naturally be very high in plants with low water content and very low in plants such as succulents, which have very high water content.

Correlative transpiration has been used in the sense of relative transpiration, and also to express the inter-relationship between transpiration of leaves in the shade and in the sun, bringing out the fact that leaves in the sun will often withdraw the water from the leaves in the shade, and although their transpiration is much more rapid than leaves exposed in the shade, will continue fresh while those in the shade wilt.

Water requirement is used in two different ways. In the broadest application of this word it is synonymous with the expression "water relation of plants," but by many it has been used in a narrow sense to signify the ratio of water consumption to dry matter produced during the growth of a plant.

Many new methods have been employed, both for determining the stomatal openings and measuring the water loss. The epidermis has been fixed either in alcohol or picric acid and the openings measured by microscopic examination. The rate of the flow of air through the leaf, or the porometer method, which has several modifications, has enabled the experimenter to estimate the relative difference in the openings of the stomata. To this method has also been applied automatic recording devices.

One of the simplest and most useful methods for determining whether or not the stomata are open is the infiltration method. Absolute alcohol, petroleum ether, or other fluids, when dropped on a leaf with open stomates penetrate into the mesophyll. This penetration is easily observed, and the technique is so simple that observations can be made rapidly.

Several modifications of the gas diffusion or gas infiltration method have been employed by different workers. A large variety of potometers and atmometers have been devised. Nothing especially new has appeared among the porometers, but a number of new types of atmometers have been employed. These consist chiefly of porcelain filters or of filter paper saturated with water which is allowed to evaporate and the loss determined by weighing.

In the measuring of transpiration loss no entirely new methods have been devised, although great improvements have been made on the older methods. Automatic weighing devices have been greatly improved, and there has been considerable improvement also in the methods of measuring transpiration by collecting the transpired water. There are now several types of automatic instruments, chiefly of the step-by-step type, which give satisfactory records of transpiration loss.

Efforts to find algebraic equations or formulae by which transpiration can be estimated from the observed environmental conditions, has resulted in a clearer understanding of the factors affecting transpiration, but no entirely satisfactory equations have been deduced. In fact, experimental data are hardly sufficient at the present time to enable one to evaluate properly such factors as wind, light, etc., and this, combined with the uncertainty of the

effect of stomatal movement, makes the problem an especially difficult one. It is true, however, that the effect of external conditions on transpiration, determined usually by comparing transpiration at different places at different portions of the day or year, is now much better understood than at the time of publication of the first volume. These results have been obtained usually with the help of either painstaking direct weighings, or by the use of automatic transpiration records, the evaporation, temperature, sunlight, wind, and wet bulb depression having been simultaneously determined.

Water requirement of plants used in its narrow sense is the amount of water consumed by a plant during its period of growth in the production of a unit weight of dry matter. It is evident, therefore, that any factor which affects transpiration and any factor which affects the growth of plants will modify this ratio. Where conditions are most favorable for growth the water requirement is likely to give the lowest value, while if conditions are not favorable for growth, even though the transpiration rate be relatively low, the water requirement will still be high. The practical value of this measurement in connection with the production of cultivated crops has led to a large number of determinations covering many of the more important crop plants. The relative consumption of water during growth and the effect of environmental conditions on the water requirement are less easily determined than the effect of environmental conditions on transpiration, since not only do the conditions control the rate of transpiration, but also affect the relative rate of growth for production of dry matter.

Although very little work has been done on the effect of insufficient soil moisture on the transpiration of plants, a great mass of data has been accumulated on the amount of moisture in the soil at the time plants wilt. Although results are somewhat conflicting, it has been found that there is a relatively definite percentage of soil moisture content, beyond which the movement of moisture in the soil is so slow as to make it practically impossible for a plant to supply its transpiration demand from the mass of soil through which its roots ordinarily extend. This moisture content has been referred to as the wilting coefficient.

Many papers have dealt with structure and morphological investigations for lessening transpiration. Although the value of transpiration in reducing the temperature of leaves has been brought out by a number of investigators, its value in relation to the nutrition of plants has not fully been admitted.

The difficulty of getting together so large a volume of English literature, especially at a period when war made access to literature from English speaking countries difficult or impossible, must have been very great. This work is a valuable summary, and no investigation of transpiration is feasible without first consulting it. It is impossible to bring into the work all of the material contained in the original papers, and these should always be consulted. A careful perusal of this work shows clearly that there is not a single line of investigation at the present time which does not afford a good starting point for further research.—H. L. SHANTZ.

Lake Maxinkuckee

The Department of Conservation of the state of Indiana has recently published a remarkable monograph on Lake Maxinkuckee, a physical and geological survey by EVERMANN and CLARK.[2] Lake Maxinkuckee is a small glacial lake similar to thousands of other lakes in the northern Mississippi Valley states, and because of this similarity any study of Lake Maxinkuckee or conclusions drawn therefrom would be typical of other lakes in this region. The work was begun and chiefly financed by a bureau of the United States government, now known as the Bureau of Fisheries. There is a general feeling of surprise and disappointment that the results were not published by the United States government. The Department of Conservation of Indiana, however, is to be congratulated, not only for the excellent manner in which the monograph is published, but also for having saved it from the shelves of discarded manuscripts. The monograph is more than a simple study of the lake, for it treats of the animal and plant life of the lake, as well as the physical surroundings and the life around it and in the air above it. It gives a vivid presentation of the physical, hydrographic, and meteorological features which belong to the lake, and a record of the animal and vegetable life in and about it.

The physical conditions discussed relate to the location, altitude, size, and form of the lake, and the character of the surrounding country. A list of the streams which feed the lake is given, as well as a most interesting account of the ice beach. Under hydrography is discussed the depth of the lake, the topography and character of its bottom, with special discussion of certain deep places, its level, the stage of water, and the volume of outflow. Under meteorology is discussed the sky, the air, the pressure and temperature, the winds, rain, frost, and even the snow, fog, and dew. Then follow exhaustive tables relating to the water temperature and the condition of the water and the formation of the ice. The most interesting paragraph in this chapter relates to the turning over of the lake each fall. Although this phenomenon was not actually observed, it was shown by an elaborate series of soundings just when the change must have taken place. Emphasis, however, is given to the biological features, to which two-thirds of the first volume and all of the second are devoted. The largest part of the biology of course is given to the fishes, since this was the primary object of the investigation. It is stated that 64 species were found in Lake Maxinkuckee. These are all described fully, with many interesting notes on their habits and food value, while there are many suggestions about angling. Many of the fishes are illustrated in color. Thirty species of mammals are recorded, and there are interesting notes about all of them, especially the two species of wolves which once inhabited this part of Indiana, and the disappearance of the beaver and porcupine is noted. Much attention is given to the birds and especially

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[2] EVERMANN, B. W., and CLARK, H. W., Lake Maxinkuckee, Vol. 1. pp. 660. *pls. 36. fig. 23.* Vol. 2. pp. 512. 1920.

to the water fowl, and 175 species are recorded. Considerable space is given to insects and the lower forms of animal life, and especially to those which are of value to the fish fauna of the lake.

The larger part of the second volume is devoted to the flora of Lake Maxinkuckee and its vicinity. This includes a special chapter on the aquatic flora and its uses, and a chapter on the algae, of which 76 species are mentioned. The volume closes with an annotated list of the ferns, fern allies, and seed-bearing plants found in the lake and the surrounding basins, of which 838 species are listed. The arrangement and nomenclature is that of the second edition of BRITTON and BROWN'S *Illustrated Flora*. The remarks about the various plants are very readable, while some of the observations are quite unique and have heretofore been unrecorded.

Besides the scientific value of the monograph, its important educational value should not be overlooked. Dr. EVERMANN, the senior author, although now a well known scientist, was originally a teacher in our elementary schools, and the educational importance of scientific research has always been emphasized by him. This book should be made a most helpful guide to the science teachers in our high schools and colleges who wish to do field work. It forms a model for the study of the lake or river valley or even the pond or creek in one's own locality. If the science teachers in the ninety-two counties of Indiana alone should use it with their classes in the study of local problems, a mass of information about the state would be accumulated, and a wonderful interest in nature study would be developed.—J. N. ROSE.

NOTES FOR STUDENTS

Taxonomic notes.—The vascular plants collected by the Canadian Arctic Expedition of 1913–18 on the Arctic coast west of the 100th meridian have been published by MACOUN and HOLM,[3] the latter completing the determinations after the death of MACOUN. There have also been included three other collections from the same region. The enumeration includes 230 species, Compositae including 23, Gramineae 22, Ranunculaceae 19, Cruciferae and Saxifragaceae each 18, etc. The largest genus represented is *Saxifraga* with 15 species, followed by *Carex*, *Salix*, and *Ranunculus* each with 12 species. Some interesting comparisons are made with the flora of Greenland and of the west coast of Alaska.

EVANS[4] has published a detailed study of the liverwort genus *Riccardia*, "often known as *Aneura*," as it is represented in Chile. He recognizes 25 species, of which 3 are new and 17 new combinations. The descriptions are very full, so that the presentation is morphological as well as taxonomic.

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[3] MACOUN, JAMES M., and HOLM, THEO., Report of the Canadian Arctic Expedition 1913–18. **5**: Part A. 1–51. *pls. 13.* 1921.

[4] EVANS, A. W., The genus *Riccardia* in Chile. Trans. Conn. Acad. Sci. **25**: 93–209. *figs. 13.* 1921.

SCHLECHTER,[5] in monographing the tribe Thismieae of Burmanniaceae, recognizes ten genera, the following two being new: *Scaphiophora* and *Triurocodon*.

PERKINS,[6] in monographing the African species of *Pycnostachys* (Labiatae), recognizes 33 species, 8 of which are new. The same author has also monographed the African species of *Achyrospermum* (Labiatae), recognizing 12 species, 3 of which are new.

BROWN,[7] in naming a collection of plants from southeastern Congo, Rhodesia, and South Africa, has described 30 new species, and also a new genus (*Alistilus*) of Leguminosae.

DIELS,[8] in continuation of his investigation of the flora of Micronesia, has published the following families: Myrtaceae, Myrsinaceae, Elaeocarpaceae, Asclepiadaceae, Scrophulariaceae, and Gesneraceae.—J. M. C.

Citrus diseases in the Orient.—The study of citrus diseases in the Orient is of particular interest and importance since most of our cultivated citrus fruits undoubtedly had their origin in this region. REINKING'S[9] recent paper therefore, is timely and interesting. A description of the diseases, a discussion of the causal organism, and suggestions regarding the control measures proper for each is given. A summary showing the citrus varieties found in each country, with the diseases to which they are subject, is given, also a list of scale insects and fungi parasitic on scales. Fourteen good plates, devoted chiefly to illustrating citrus canker (*Pseudomonas citri*), bark rot (*Diplodia*), and pink disease (*Corticium salmonicolor*) complete the article. The two latter diseases, occurring in the Philippines and unknown in the United States, are apparently of major importance, warranting every precaution against their spread or introduction into new territory. A "black spot" disease occurring in South China, of unknown cause, is also regarded as serious.

Particular attention is given to the degree of susceptibility to citrus canker shown by different species, hybrids, and relatives of citrus planted out at Los Baños, Philippine Islands. Observations of this character have an important bearing on the selection of material for culture in regions exposed to canker

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[5] SCHLECTER, R., Die Thismieae. Notizblatt Bot. Gart. u. Mus. Berlin-Dahlem. **8**:no. 71. 31–45. 1921.

[6] PERKINS, JANET, Die afrikanischen *Pycnostachys*-Arten. Notizblatt Bot. Gart. u. Mus. Berlin-Dahlem. **8**:no. 71. 63–77. 1921; Die afrikanischen *Achyrospermum*-Arten. *Ibid.* 78–82.

[7] BROWN, N. E., New plants from tropical and South Africa collected by Archdeacon F. A. ROGERS. Kew Bull. 1921:no. 8. 289–301.

[8] DIELS, L., Beiträge zur Flora von Mikronesien und Polynesien. II. Engler's Bot. Jahrb. **56**:529–577. 1921.

[9] REINKING, OTTO A., Citrus diseases of the Philippines, South China, Indo-China, and Siam. Philippine Agriculturist **9**:121–179. 1921.

infection. Hybrids having one or more resistant parents show in many instances promising resistance. The discussion, representing as it does some forty diseases and pests in the Philippines and nearly an equal number in the Asiatic countries visited, emphasizes the need for intensive studies of plant diseases in the regions where they have been long established.—W. T. SWINGLE.

Stelar morphology.—In his presidential address to the Royal Society of Edinburgh, BOWER[10] emphasizes the importance of the principle of similitude (GALILEO) in the investigation of the stelar morphology of the higher plants. He argues that, inasmuch as the surface of an organ or tissue varies only as the square of its linear dimensions, but the bulk as the cube, the larger a plant is the more dependent it will be upon its form and detailed structure, not only for its stability, but also for the performance of its functions of absorption and transit of liquids and gases. This will apply not only to the external surface, but also to those internal surfaces which limit one tissue tract from another. Upon the basis of this premise, he concludes that in the ontogeny and phylogeny of ferns the form of the vascular tissues is largely dependent upon the size of the plant and of its various organs. Thus, as the fern plant and its foliar appendages become larger, the simple and presumably primitive protostele tends to become involuted, medullated (solenostely), or dissected into separate strands (polycycly, perforation, dictyostely).

BOWER's correlations between size, form, and function are very suggestive, and deserve careful consideration, particularly by students of the phylogeny of the vascular cryptogams. It must be admitted, however, that there is a considerable element of uncertainty in interpreting such correlations. The fact that complex structures tend to occur in large plants does not prove necessarily that size is the primary factor in their evolution, although such a conclusion appears to be extremely plausible.—I. W. BAILEY.

Deccan vegetation.—The ecological problems of many portions of India are complicated by the density of the population and the intensity of the grazing. The rainfall of 27 inches in the Deccan coming during the months from June to October, preceded by a very hot and dry period, causes the erosion of fields denuded of vegetation by drought and grazing. In such a region the study of natural vegetation in areas protected from cattle has been begun by BURNS and CHAKRADEV[11] as a preliminary to work on the improvement of grazing lands. Permanent quadrats were established within barbed wire inclosures. Native grasses such as *Andropogon monticola* and *Iseilema laxum* appear to be able to establish themselves completely, and it seems

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[10] BOWER, F. O., Size, a neglected factor in stelar morphology. Proc. Roy. Soc. Edinburgh **41**:1–25. 1921.

[11] BURNS, W., and CHAKRADEV, G. M., An ecological study of Deccan grassland. Jour. Indian Bot. **2**:84–91. 1921.

possible that a more mesophytic grassland may be the climax, with the formation of a turf resisting erosion.

Investigations by BHIDE[12] during one of the worst droughts on record, in 1918–19, have taken into account some of the plants showing the most successful resistance to such arid conditions. Such data not only add to our knowledge of the existing vegetation, but furnish material for improving existing economic conditions in a region where grazing is of first importance.

The anatomy of many plants of the arid region is also being investigated by SABNIS.[13] The results of such efforts are certain to be valuable for India and interesting to botanists elsewhere.—G. D. FULLER.

Tension zone between forest and prairie.—Following an earlier study by WEAVER and THIEL, an interesting tension zone investigation has been carried on by POOL, WEAVER, and JEAN[14] in eastern Nebraska. Stations were selected at Peru, near the Missouri River, and at Lincoln, sixty miles west of Peru. By means of quantitative experimental study, striking contrasts between these two stations, due to both climatic and edaphic factors, were brought to light. The prairies and woodlands near Lincoln are much more xerophytic than those near Peru, in spite of the short distances involved between the two places. Available soil moisture during the summer of 1917 was exhausted on eighteen different days on a Lincoln prairie and on only four different days on a comparable Peru prairie. Many mesophytic woodland species pass out in traversing the area between these two places. The high saturation deficit and the low soil moisture content of the prairie sites in eastern Nebraska constitute barriers over which forest trees can scarcely pass. The authors feel that herein is the most ready explanation for the confinement of Nebraska woodlands to the moist slopes of narrow valleys and for the general treelessness of prairies. In the order of increasing mesophytism, the forests about Peru are as follows: bur oak-yellow oak, black oak-hickory, red oak, linden-ironwood, while the common forest type about Lincoln is that of the bur oak-hickory.—H. C. COWLES.

Composition of plants as affected by nutritive elements.—Growing the oat plant in analyzed quartz sand, DICKSON[15] has made a study of the effects of a deficiency of certain nutrient elements on the calcium and phosphorus

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[12] BHIDE, R. K., Drought resisting plants in the Deccan. Jour. Indian Bot. **2**: 27–43. 1921.

[13] SABNIS, T. S., The physiological anatomy of the plants of the Indian desert. Jour. Indian Bot. **2**:1–19, 61–79, 93–115. 1921.

[14] POOL, R. J., WEAVER, J. E., and JEAN, F. C., Further studies in the ecotone between prairie and woodland. Univ. Nebraska Studies **18**:1–47. *figs. 17*. 1918.

[15] DICKSON, J. G., The relation of certain nutritive elements to the composition of the oat plant. Amer. Jour. Bot. **8**:256–274. *figs. 2*. 1921.

content of the plant. The elements, the amount of which present in the nutrient solutions was varied, were calcium, magnesium, potassium, phosphorus, and nitrogen. The plan was to reduce the amount of these elements present to the lowest concentration that would allow the production of grain. This concentration would be below that required for the normal development of the plant, and it was thought that in such conditions the effect of the elements on the composition of the plant would be more strikingly brought out. Aside from the greatly reduced calcium or phosphorus content, when the amount of these elements in the respective nutrient solutions was reduced, the most striking results obtained were the low calcium content of the plants of the low phosphorus series and the low nitrogen series, and the high phosphorus content of the plants of the low calcium series and the low nitrogen series. Potassium and magnesium seemed to have little effect on the calcium and phosphorus content of the plant. Climatic factors, on the other hand, were shown to have a decided effect on the composition of the plant. A good bibliography is added.—S. V. EATON.

Alkali soils.—The hardening of irrigated land has been studied by SCOFIELD and HEADLEY,[16] who conclude that neutral salts of sodium as well as the carbonate, "black alkali," may produce hardening of the clay component of soils. The results are about the same, whether sodium-containing water is used on good soils, or whether pure water is used on salty soils; in either case the soil will harden seriously on drying, and become somewhat impervious to water. The theory advanced to explain the action of sodium on clay is that the sodium replaces other bases on the surface of the particles, and becomes in part sodium silicate. This hydrogel coating increases the effective size of each particle, reduces the porosity of the soil, and greatly retards water percolation through it. On drying, the colloidal gel cements the particles together, whence the hardness of these soils. If enough calcium or aluminium is present, however, the harmful action of sodium is obviated. When irrigation water contains more Na and K together than Ca and Mg, it is likely to cause hardening. The Colorado River and its lower tributaries contain too much of the hardening salts. The authors think injury to irrigated land may be avoided by treatment of irrigation water or land with soluble calcium or aluminium salts.—C. A. SHULL.

Nitrogen fixation by green plants.—WANN[17] presents some interesting results of experiments showing that members of the Chlorophyceae can utilize the uncombined nitrogen of the atmosphere. Seven species exhibited this

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[16] SCOFIELD, C. S., and HEADLEY, F. B., Quality of irrigation water in relation to land reclamation. Jour. Agric. Res. **21**: 265–278. 1921.

[17] WANN, F. B., The fixation of free nitrogen by green plants. Amer. Jour. Bot. **8**: 1–29. *pl. 1. fig. 1.* 1921.

power when grown on mineral nutrient agar containing a nitrate and glucose. There was no fixation when nitrogen was supplied in the organic form, and with a nitrate present but no carbohydrate, the amount of fixation was not marked enough to be conclusive. There seemed to be some fixation in the latter case, the lesser amount being due, perhaps, to the much decreased growth where the carbohydrate was omitted. One species seemed to have the power of denitrification as well as nitrogen fixation. The amount of nitrogen fixed by the algal species used compared favorably with the amount recorded by other investigators as fixed by the nitrogen fixing bacteria. The results recorded in this paper are contrary to the generally accepted view as to the ability of green plants to make use of free nitrogen. The possibility of green plants possessing this power of nitrogen fixation, however, is of such great interest both scientifically and economically that the work of WANN should be the stimulus for much more work along this same line.—S. V. EATON.

Variation in stomata and hydathodes.—In a study of the number of stomata per sq. mm. upon leaves of *Campanula rotundifolia* borne upon different parts of the same plant and upon the leaves of plants grown under different conditions of habitat, Miss REA[18] found some interesting variations. In general there was an increase in number from the lower to the higher position of the leaf upon the shoot, and an increase with conditions of increasing dryness. Such increase was least upon the under surface of leaves on different portions of the stem of the same plant. It is suggested that the increased number upon sun shoots compared with those developed in the shade is due to increased photosynthesis, although no causal connection is established. It would be desirable to know the connection between the size of the epidermal cells and the number of stomata, but this information is not given. Groups of hydathodes were found on the upper surfaces of all leaves examined, the number per leaf decreasing from the base to the apex of the shoots.—G. D. FULLER.

Water relations of Pinus and Leucadendron.—Following methods devised by FARMER, the water conducting power of the wood of *Pinus pinaster* and *Leucadendron argenteum* has been measured by AITKEN,[19] and a comparison instituted between transpiration and the rate of water transmission. The rate of transpiration was higher in *Pinus*, both per twig and per unit area, than in *Leucadendron*, as was also the ratio of transpiration to transmission. From the data obtained it would seem that the wood of *Pinus* is capable of transmitting a limited amount of water which it utilizes with a very small margin of surplus.—G. D. FULLER.

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[18] REA, MARGARET W., Stomata and hydathodes in *Campanula rotundifolia* L., and their relation to environment. New Phytol. **20**:56–72. *figs.* 6. 1921.

[19] AITKEN, R. D., The water relations of the pine (*Pinus pinaster*) and the silver tree (*Leucadendron argenteum*). Trans. Roy. Soc. So. Africa **10**:5–19. 1921.

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DEVELOPMENTAL SELECTION IN VASCULAR PLANTS[1]

JOHN T. BUCHHOLZ

(WITH TWENTY-EIGHT FIGURES)

In the numerous explanations and discussions of natural selection in the *Origin of species* and since the time of DARWIN, the process of competition has usually been regarded as taking place in the external environment. In striking contrast with this, developmental selection is characterized by the fact that it occurs between very minute or embryonic individuals whose struggle is limited to what might be termed an internal environment. It is well illustrated by the selection resulting from the polyembryony within the developing seeds of conifers and cycads, the embryonic selection in this case being a special form of developmental selection. The embryos of the latter are wholly surrounded by organic tissue; they are entirely inclosed within the ovule of the parent plant. Equivalent forms of developmental selection are found in ferns as well as in angiosperms, and it is intended to discuss briefly these internal selective processes in their relation to organic evolution.

Developmental selection is not to be confused with any of the older well known theories involving internal forms of selection. WEISMANN'S germinal selection is described as an internal process, but this is a supposed struggle between biophores within the germ cells; it is not even a competition between individual cells, and can be imagined only. It is based on a speculative hypothesis

[1] Presented before the Botanical Society of America, Chicago, December 29, 1920.

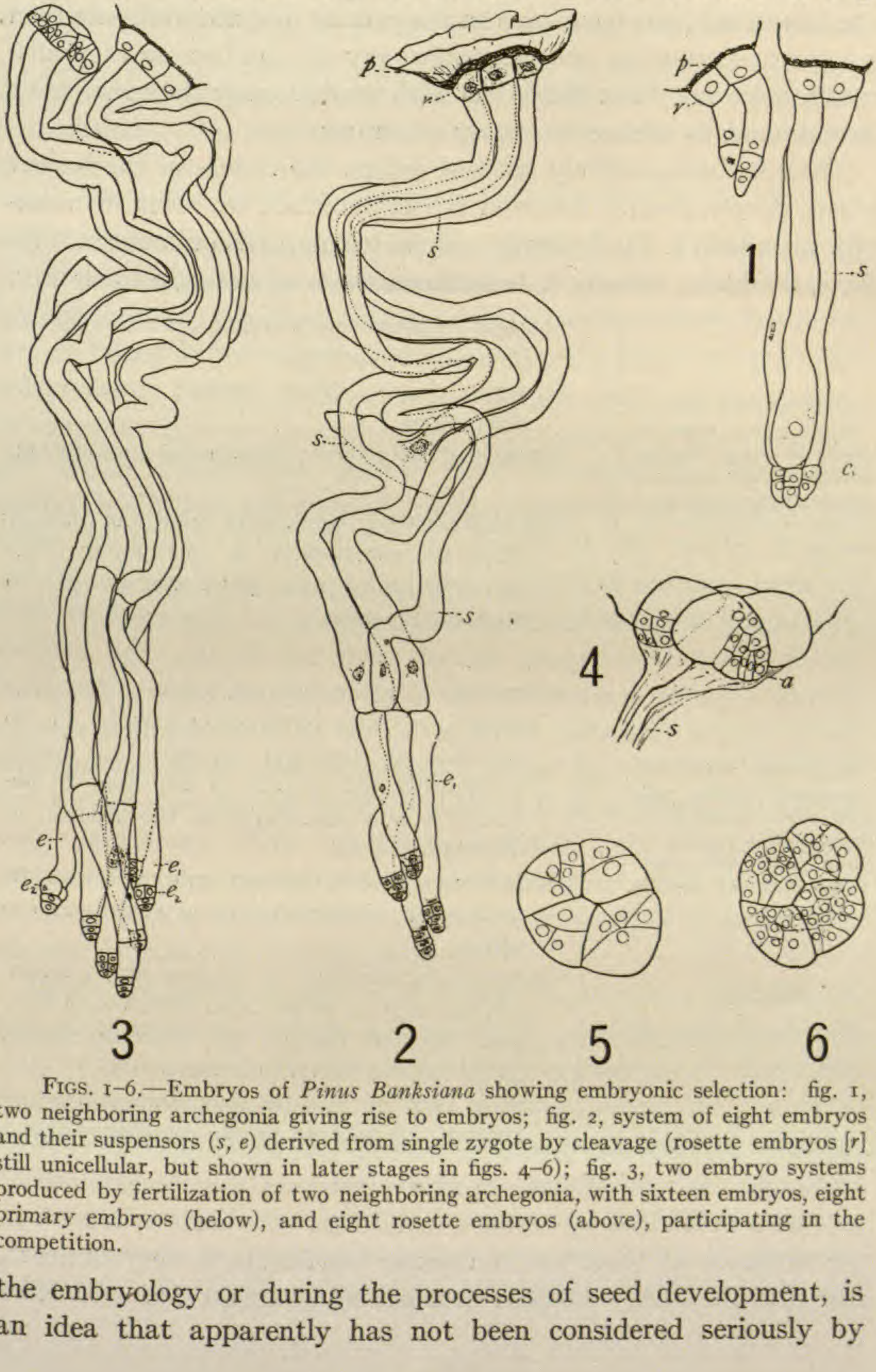
entirely incapable of experimental proof. Likewise ROUX's theory of intra-selection or the battle of the parts, a supposed struggle between the various organs of the body of a developing individual, is not a struggle between different individuals, but only between differentiating tissues and organs. Developmental selection, on the other hand, occurs between definite individuals which may be unicellular or multicellular, but the process is not intracellular. This can easily be demonstrated and is capable of being subjected to observation and experimental study.

The several isolation theories, as well as the other theories of species forming auxiliary to natural selection, have neglected any significant allusion to the type of illustrative material discussed in this paper. Developmental selection is different also from the theories of sexual isolation, physiological selection, or mechanical selection.

A definite rôle is not to be denied for natural selection, but it is not the only selective process. In developmental selection, we have a supplementary form of selection which occurs at other times during the life cycle, one which meets some of the most serious objections which have been raised against natural selection. It is capable of playing on mutations as well as other forms of variation, bringing about definite results in evolution. In fact, a real species forming rôle may be claimed for developmental selection, if we grant that such a rôle is to be found in any selective process. During the ontogeny of higher plants, therefore, there are a number of stages in the life cycle when a definite competitive selection between individuals occurs. In addition to natural selection, there is also this definite struggle between supernumerary gametophytes, when these are dependent on sporophytes, between supernumerary embryos as found in the polyembryony of gymnosperms and most ferns, or between excessive numbers of gametes.

In plants, natural selection, as it is ordinarily understood, occurs in the environment, when seeds or spores germinate in or on the soil, or when vegetative organs, such as roots, rhizomes, stolons, etc., from several neighboring plants of similar or different species give rise to new individuals in a crowded stand and in close competition. For animals, natural selection is usually understood to begin at birth, or when the young first come into contact with

the external environment. That a selection may occur during the earliest developmental stages of an individual, for instance, during



FIGS. 1–6.—Embryos of *Pinus Banksiana* showing embryonic selection: fig. 1, two neighboring archegonia giving rise to embryos; fig. 2, system of eight embryos and their suspensors (*s*, *e*) derived from single zygote by cleavage (rosette embryos [*r*] still unicellular, but shown in later stages in figs. 4–6); fig. 3, two embryo systems produced by fertilization of two neighboring archegonia, with sixteen embryos, eight primary embryos (below), and eight rosette embryos (above), participating in the competition.

the embryology or during the processes of seed development, is an idea that apparently has not been considered seriously by

students of biology, judging from the general neglect of the subject of polyembryony in the literature of organic evolution. Many of the important facts concerned in the process of embryonic selection in gymnosperms, as the polyembryony of conifers and cycads, which have not been linked up with evolutionary doctrine, have been known to science for nearly a century.

Besides being entirely inclosed within the tissues of the parent plant, developmental selection is characterized by being intraspecific or reflexive. It is always a definite competition between similar individuals; usually it is between those of a single fraternity.

NATURAL SELECTION

Environmental process occurring in external physical and biological environment of organism, where conditions of struggle for existence are very complex

- Struggle against unfavorable environment of physical surroundings.
- Struggle against other species; extraspecific competition.
- Struggle against fellows; intraspecific competition.

DEVELOPMENTAL SELECTION

Occurring during early embryonic or gametophytic stages within tissues of parent plant, under conditions uniform for competing individuals

- Selection between vegetatively branching parts of either the gametophyte or sporophyte; buds and branches of trees, which later give rise to reproductive parts.

- Interovular selection, between ovules within same ovary: (1) after fertilization, largely due to activities of contained embryos; (2) before fertilization, due in part to activities of contained female gametophytes, megaspores, or archesporial cells.
- Embryonic selection, between embryos within the same ovule, or within tissues of parent gametophyte.
- Gametophytic selection: (1) between male gametophytes, such as pollen tubes within carpellary and nucellar tissue; (2) between female gametophytes within the same ovule.
- Gametic selection: (1) between male gametes or sperms; (2) between female gametes or eggs.

Although it is realized that in many instances this developmental process is influenced by external conditions, it is clear at least that the influence of these environmental conditions is very indirect, and that the highly complex external environment does not exert

a differential effect on the competing individuals. The foregoing outline is suggestive of the general relation of the several forms of developmental selection to the general process of natural selection.

Developmental selection expresses itself in some form or other in the sexual reproductive cycle of practically all vascular plants. It is probably also involved in the life cycle of most of the cryptogamic forms, and is a factor to be reckoned with among animals as well. The main purpose in this discussion is to describe in a general way the various expressions of the principle of developmental selection as it applies to vascular plants.

The ordinary details of conifer embryogeny have been described (11) and may be assumed to be fairly well understood by botanists. It is generally known that in cycads or in such conifers as the spruce, for example, there are several embryos that engage in an intense life and death competition during their development. Only one embryo reaches its full term of growth to become the seed embryo, while the weaker individuals are aborted in the earlier stages. Only in extremely rare cases may two embryos be matured together in the conifer seed. In *Ginkgo* this happens rather more frequently, about 2 per cent of the seeds having been found with equally developed "twin" embryos (13). Even if several embryos should occur within the same testa, as in citrus seeds and several other angiosperms, these must necessarily come up so close together that a close competition between them will be inevitable after the seeds germinate. This competition which occurs after seeds germinate in the soil is environmental, however, and belongs to the categories of natural selection, where it remains as an intense intraspecific form of natural selection.

When pollination is successful enough to provide a plurality of male gametophytes, making polyembryony possible, practically all gymnosperms possess the feature of embryonic selection. Here the female gametophyte tissue is well formed before the embryos begin to develop, is somewhat firm and resistant, and it is only by a vigorous growth and rapid suspensor elongation, together with an abundant secretion of digestive ferments, that the successful embryo matures at the expense of its fellows and brings about their destruction. In angiosperms the endosperm within which the

embryo develops is much later in its origin, and is usually very soft and gelatinous in these early stages. This and the reduced suspensor of angiosperms may largely account for the fact that angiosperm polyembryony does not usually result in the definite selection of a single embryo before the seed is shed.

BROWN (1), who discovered polyembryony, pointed out that plurality of archegonia makes possible the fact of polyembryony among both cycads and conifers. It has been found also (5, 35) that in some conifers the zygote may undergo cleavage, resulting in several young embryos which compete with each other. Thus the fertilization of only one egg in *Pinus*, for example, results in the formation of eight embryos by cleavage (cleavage polyembryony), only one of which survives and completes its term of development (figs. 1–6). In other conifers, as in the spruce, the egg gives rise to only one embryo, but in any event the plurality of eggs makes possible simple polyembryony, in which a selection of embryos occurs.

A scheme of phylogeny, based in part on the character of polyembryony, whether simple or by cleavage, has been outlined in previous papers (5, 6). All the facts at hand seem to indicate that practically all conifers which do not possess cleavage polyembryony show structural evidence of having passed through this condition in their phylogeny. This indicates that either cleavage polyembryony originated among ferns, or it originated during the transition to the seed habit. All evidence is in favor of the latter alternative, and a definite hypothesis to account for the origin of cleavage polyembryony will be outlined in a later paper. In general, cleavage polyembryony is well developed among the more primitive conifers, and was eliminated sooner or later in all but one or two phyletic lines. Whether cycads passed through a similar stage of cleavage polyembryony is very uncertain. Nothing in the embryogeny of cycads thus far described appears to suggest this, but their simple polyembryony is doubtless of the same fern origin as that of conifers, that is, plurality of archegonia in the ferns from which cycads were derived.

Embryonic selection, either through cleavage polyembryony, plurality of archegonia, or a combination of both, is practically

universal among gymnosperms, and its character or type is of considerable importance in a study of phylogeny. For the origin of simple polyembryony, we must turn to a study of pteridophytes, as it is very evident that the plurality of archegonia in gymnosperm gametophytes was derived from a similar plurality among their pteridophyte ancestors.

Embryonic selection among pteridophytes

Many living pteridophytes have simple polyembryony, that is, a plurality of young sporophytes growing on a single gametophyte. Whenever the number of these sporophytes greatly exceeds the ability of the gametophyte to nourish all of them through their period of embryonic development, so that some of them are starved out in their early stages, a selection must occur among them. If this selection takes place in the earliest stages, before these young sporophytes are exposed to the external environment and become independent, we have embryonic selection as truly as that found in gymnosperms. When the several embryos in this competition are the result of a simultaneous fertilization, this embryonic selection has all of the advantages, as a measure of merit, that may be found in the gymnosperm polyembryony, and will be certain to result in the survival of the embryos that are strongest and most vigorous as determined by their actual performance. The facts that are definitely known concerning polyembryony and embryonic selection in various groups of pteridophytes may be considered as follows.

LYCOPODIALES.—The occurrence of several embryos per gametophyte in *Lycopodium* was definitely reported and shown by BRUCHMANN (3). Figs. 7–9 definitely show this plurality of embryos. These embryos do not all mature, but some of them remain in an arrested but viable condition for a considerable period, and are able to resume their growth if the larger sporophytes are injured by drought or otherwise. The smaller embryos probably fall into two groups, those which owe their origin to a fertilization simultaneous with that forming the successful embryo, and those which originate by a subsequent fertilization. It is very evident that the conditions for fertilization, even in these subterranean gameto-

phytes, are not continuous, but only occasional in occurrence. There is nothing that would hinder the fertilization of several or all of the archegonia which open on any occasion when fertilization takes place. A competition for food, together with a difference in the growth vigor of the embryos, probably determines which of several zygotes shall become the successful sporophyte. Some of the unsuccessful embryos doubtless abort and collapse in very

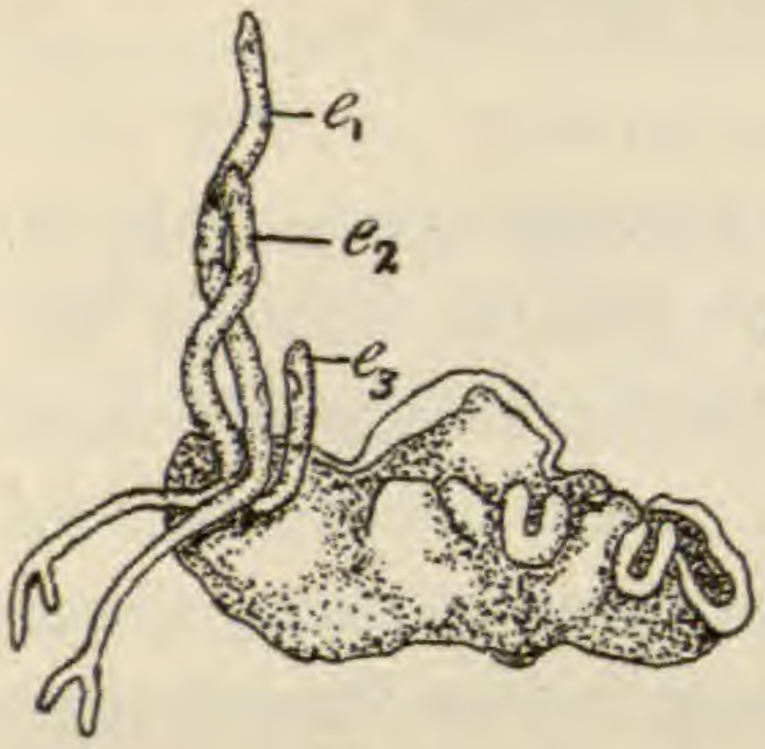


FIG. 7

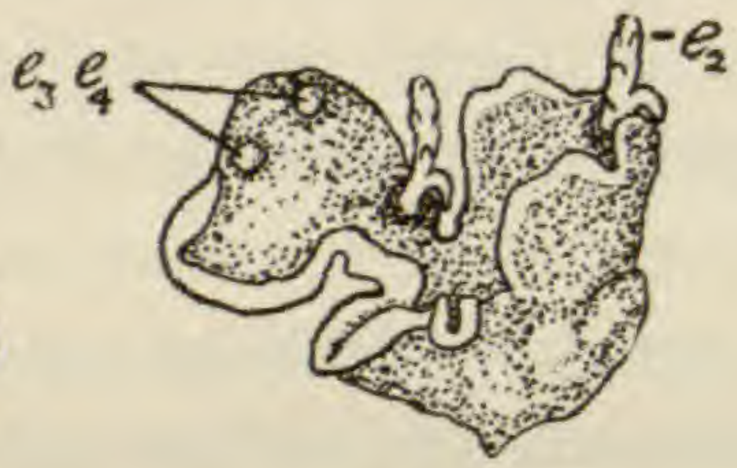


FIG. 8

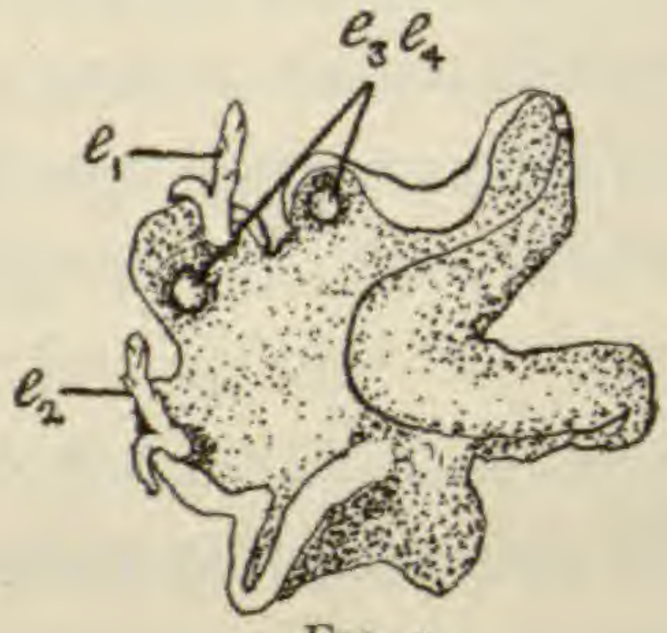


FIG. 9

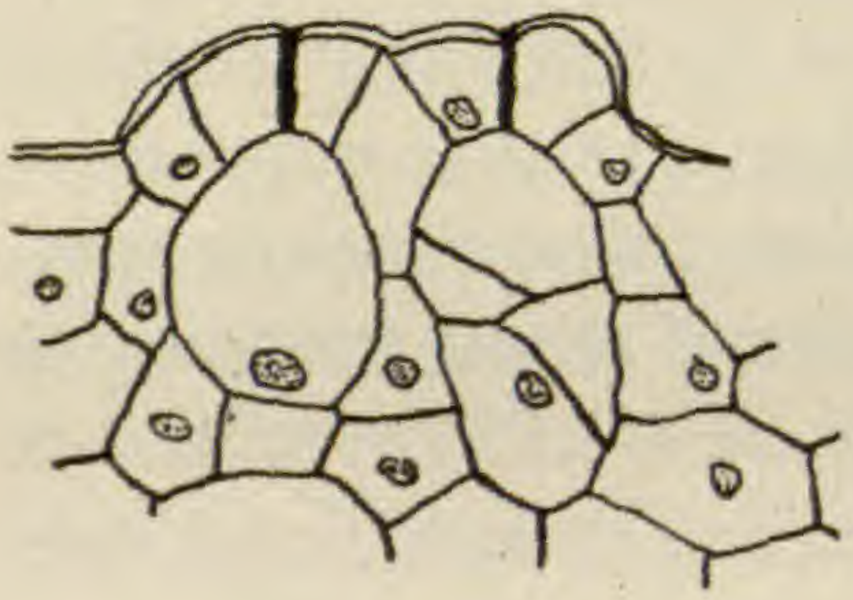


FIG. 10

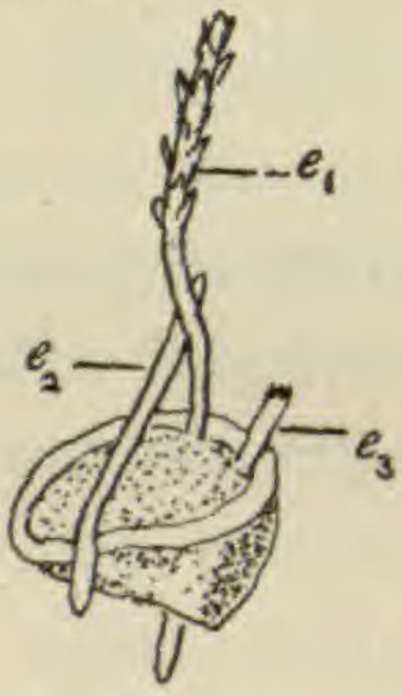


FIG. 11

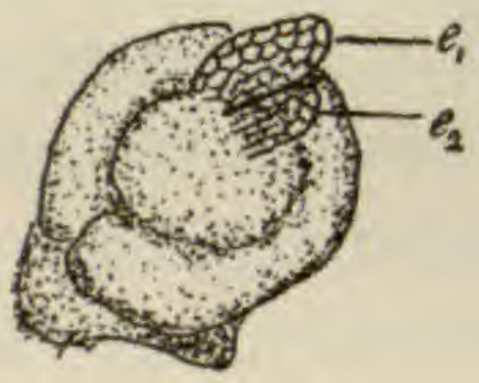


FIG. 12

FIGS. 7–12.—Fig. 7, gametophyte giving rise to several young sporophytes (e_1,e_2,e_3) of *Lycopodium annotinum*,×3; figs. 8, 9, gametophytes of *Lycopodium clavatum*, each with four embryos (e_1, e_2, e_3, e_4); after BRUCHMANN (3); fig. 10, section of gametophyte of *Tmesipteris* showing two neighboring embryos,×150; fig. 11, gametophyte of *Lycopodium volubile* bearing three sporophytes,×5; fig. 12, same with two very young sporophytes emerging,×8; figs. 10 and 11 after HOLLOWAY (23, 24); fig. 12 after CHAMBERLAIN (10).

early stages, soon becoming unrecognizable; while according to BRUCHMANN'S accounts, confirmed by subsequent observers, many of them remain in an arrested but viable condition for some time. These may doubtless be added to by subsequent fertilization of still other archegonia. It appears that among some *Lycopodium* species with large gametophytes several full fledged sporophytes may be produced.

CHAMBERLAIN'S (**10**) description of several New Zealand *Lycopodium* gametophytes includes two instances among his figures showing a plurality of young sporophytes. His figure of *L. laterale* shows one sporophyte with protocorm and two protophylls, while a second embryo of much smaller size has just broken through the gametophytic tissue. *L. volubile* (fig. 12) is also shown with two young sporophytes, one nearly twice the size of the other.

HOLLOWAY (**23, 24**) has made an extensive study of New Zealand *Lycopodium* and *Tmesipteris* gametophytes, and has described a number of them. *Tmesipteris* has polyembryony, it is figured twice with two very young embryos, one of which is reproduced in fig. 10, and *Lycopodium* species are frequently shown with several sporophytes per gametophyte (fig. 11). From HOLLOWAY'S letter, received in reply to an inquiry as to the occurrence of polyembryony among the New Zealand species of *Lycopodium* and *Tmesipteris*, the following paragraphs summing up these facts are taken:

> I have examined a large number of prothalli of *Tmesipteris* (most of them externally only), and have observed that not a few (I cannot say how many) bore two and even three well grown young plants on the same prothallus. These plantlets were developing healthily, and presumably would all continue to do so as the prothallus decayed away. Probably, of course, the time would then come when they would begin to crowd each other out. . . . . In the prothalli which I sectioned, I found two instances in which two archegonia side by side had been fertilized and were continuing their development. On one of these prothalli there was also the remains of the foot of an older plantlet. No other embryos were to be seen on these prothalli. . . . . Again, there were several instances found in which two young embryos (more advanced than those mentioned in the last paragraph) were developing side by side, no more embryos being present on these prothalli.
>
> The prothallus of *Tmesipteris* is of comparatively large size, and archegonia are present on most parts of it in great numbers, so that the examination of more prothalli in section should show that this form of polyembryony is by no means uncommon. Also I have noticed that the prothallus can continue growing in size after a plantlet has become detached from it. . . . . I have examined a large number of prothalli of each of the following New Zealand species of *Lycopodium*, both externally and in serial sections: *L. cernuum*, *L. laterale*, *L. ramulosum*, *L. Billardieri*, *L. Billardieri gracile*, *L. varium*, *L. volubile*, *L. fastigiatum*, *L. scariosum*. I can give the following facts: The prothalli of the first named three (*L. cernuum*, *L. laterale*, *L. ramulosum*) are comparatively small and short-lived. I have never observed on any of them

more than one young plant or embryo in fertilized archegonium. The prothalli of the next named three (*L. Billardieri, L. Billardieri gracile, L. varium*) are of the much branched epiphytic type, with a comparatively bulky central region on which the sex organs are borne. I have found that many well grown prothalli in all three species have two and even three healthy plantlets. Curiously enough, I have not found a single instance of two or more young embryos or fertilized archegonia existing on the same prothallus, although I have sectioned a large number.

The prothalli of the last named three (*L. volubile, L. fastigiatum, L. scariosum*) are large and deep living, the first two belonging to the *clavatum* type and the third to the *complanatum* type. I have examined a large number of each of these both externally and in serial section. These prothalli are of course all comparatively large in size, and they frequently have two or three developing healthy plantlets. From serial sections I have found on one prothallus of *L. volubile* one young plant and two embryos, and on another four embryos. The prothalli of *L. fastigiatum* supply the most noteworthy instances of polyembryony. One large prothallus showed no less than eleven embryos in different stages of development and three young plantlets, all of these fourteen being healthy. Other prothalli showed from three to five embryos. The archegonia in these two species are nearly exclusively on the liplike prominence which surrounds the top of the prothallus, so that the embryos and plantlets are generally quite close together. In *L. scariosum* I have also found two, three, and four embryos on the one prothallus.

From my own observations I can say that the large growing prothalli of certain *Lycopodium* types (as enumerated above), and also those of *Tmesipteris*, not uncommonly show polyembryony arising from the fertilization of several archegonia. I note, however, that CHAMBERLAIN shows two young plants on a prothallium of *L. laterale* which is of the small-growing form.

It is very evident that *Tmesipteris* and some of the Lycopodiales with large gametophytes may mature several sporophyte plants. Doubtless some kind of embryonic selection is found even among these, as it is highly probable that only a fraction of the zygotes produced attain their full term of embryonic development. Several young sporophytes, however, are usually produced on various parts of the tuberous gametophytes. If these arrested embryos remain healthy looking for a long period, a condition definitely reported for some, this fact would indicate a more primitive condition than that of their complete abortion.

Such a heterosperous form as *Selaginella* is much nearer to the condition from which the seed habit was derived. While some large vigorous gametophytes of *Lycopodium* may frequently give

rise to several sporophytes of some size with or without embryonic selection, these smaller female gametophytes of *Selaginella* that are contained within the megaspore coats do not produce more than one maturing sporophyte. There are, however, a number of archegonia per gametophyte in *Selaginella*, and several figures have been published showing two or three embryos in the same section. One of these is PFEFFER'S well known illustration of *S. Mertensii* (fig. 13). When we consider the occasional character of fertilization and some of the difficulties that usually attend this event for a land plant, it is very probable that the fertilization producing these several embryos occurred simultaneously.

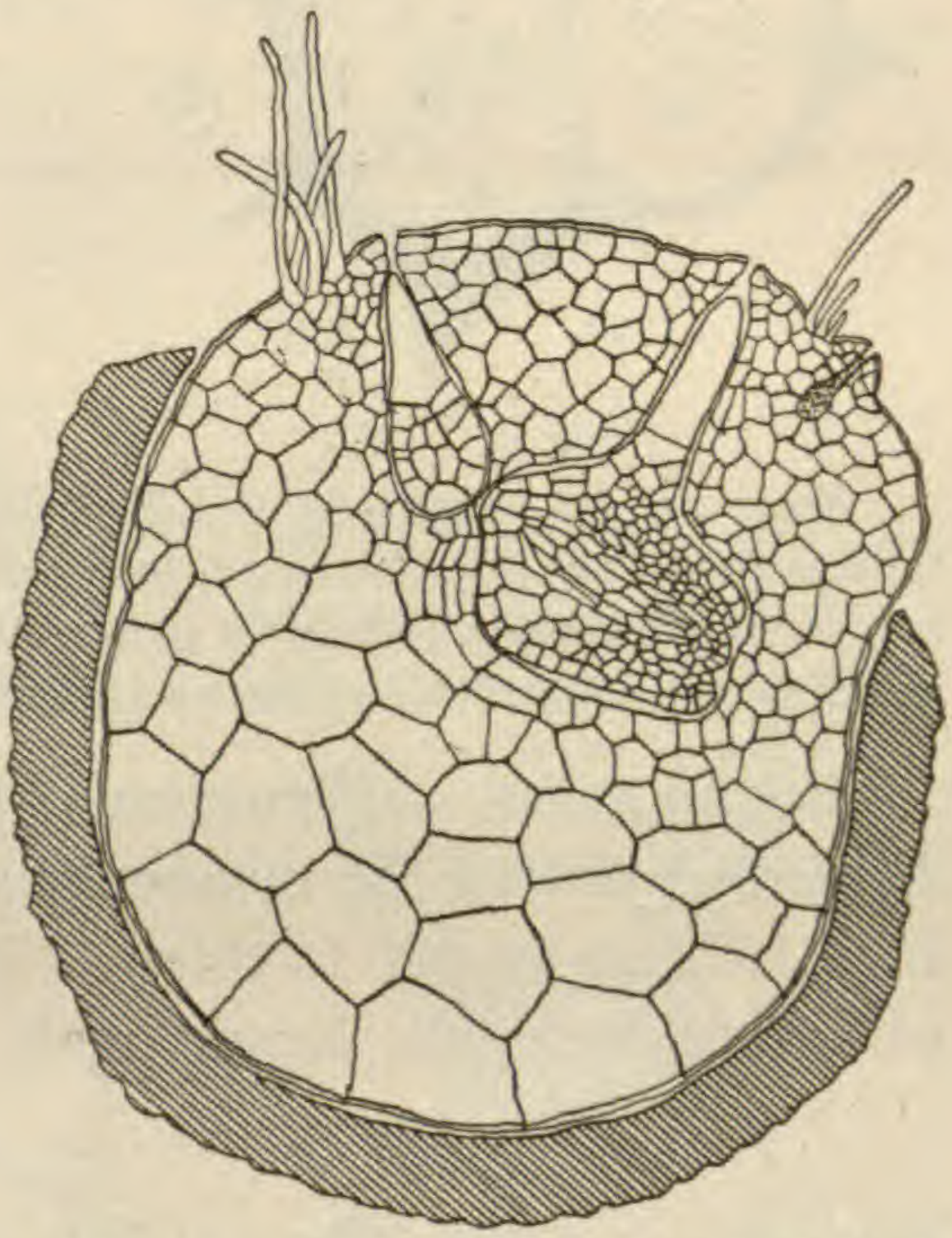


FIG. 13.—Female gametophyte of *Selaginella Mertensii* showing two embryos, ×160; after PFEFFER (33) as modified by GOEBEL (20).

BRUCHMANN (2) states in his monograph on *S. spinulosa* that although several embryos may start to grow, but one comes to maturity. Miss LYON (31), in her paper on *Selaginella*, also shows several instances of polyembryony. The one shown in fig. 14 is given as a possible fertilization of two eggs in one archegonium, or of the fertilization of the ventral canal cell and egg. It is also possible that these embryos were derived from neighboring archegonia, as fig. 15 (drawn to the same scale as fig. 14) would seem to indicate, the embryos having digested the single layer of gametophytic cells that separated their venters. In any event, this shows polyembryony. These embryos are still so small that they are evidently formed from a simultaneous or nearly simultaneous fertilization, and one has already begun to grow a little faster than the other, indicating that embryonic selection is taking place. It appears at least that in some species of *Selaginella* embryonic

selection may play a definite rôle under normal circumstances. In *Isoetes* there are usually several archegonia, so that it is possible for several eggs to be fertilized, at least occasionally, but nothing has been recorded concerning an actual plurality of embryos.

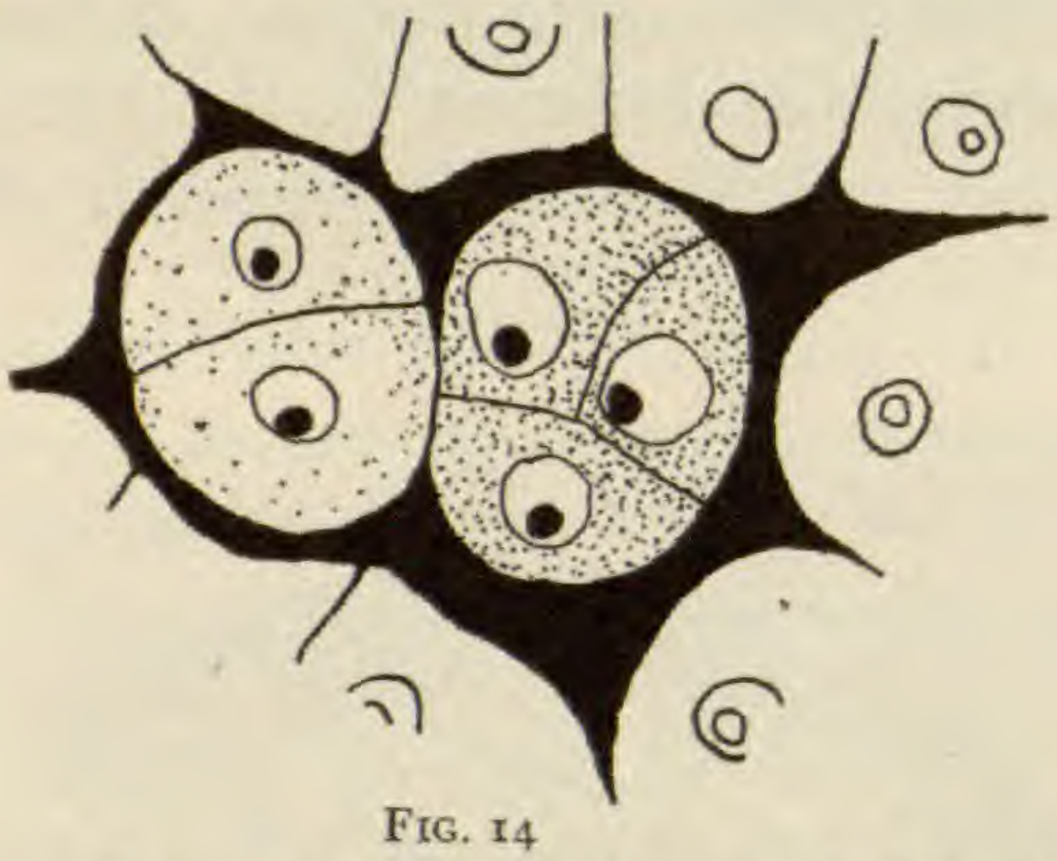


FIG. 14

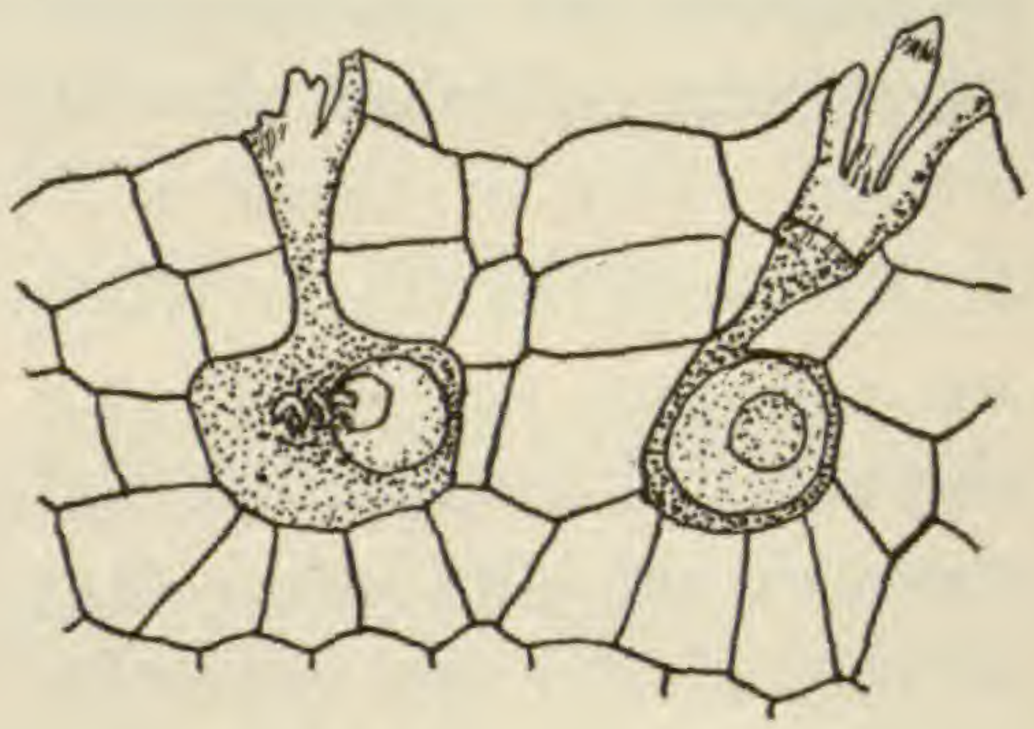


FIG. 15

FIGS. 14, 15.—Embryos of *Selaginella apus* showing polyembryony; fig. 15, fertilization of egg in archegonium beside another zygote; fig. 14 may represent two zygotes of neighboring archegonia such as those in fig. 15 (drawn to same scale) after gametophytic tissue between them was digested away; after LYON (**31**).

EQUISETALES.—HOFMEISTER (**22**) definitely states that in *Equisetum arvense* the number of archegonia of a vigorous prothallium is from twenty to thirty. It exceeds, therefore, the number of antheridia of the largest male gametophytes. As a rule more than one egg is fertilized. He counted as many as seven embryos on one such gametophyte. Fig. 16 shows *E. arvense* with two neighboring archegonia containing embryos in competition. In a more recent study by KASHYAP (**26**) on *E. debile*, the author states that in these vigorous gametophytes the number of archegonia may reach two hundred or more. Although the prothallus may bear only a single sporophyte, eight to ten young sporophytes on a single gametophyte are said to be very common. Under conditions of laboratory culture KASHYAP obtained fifteen or more sporo-

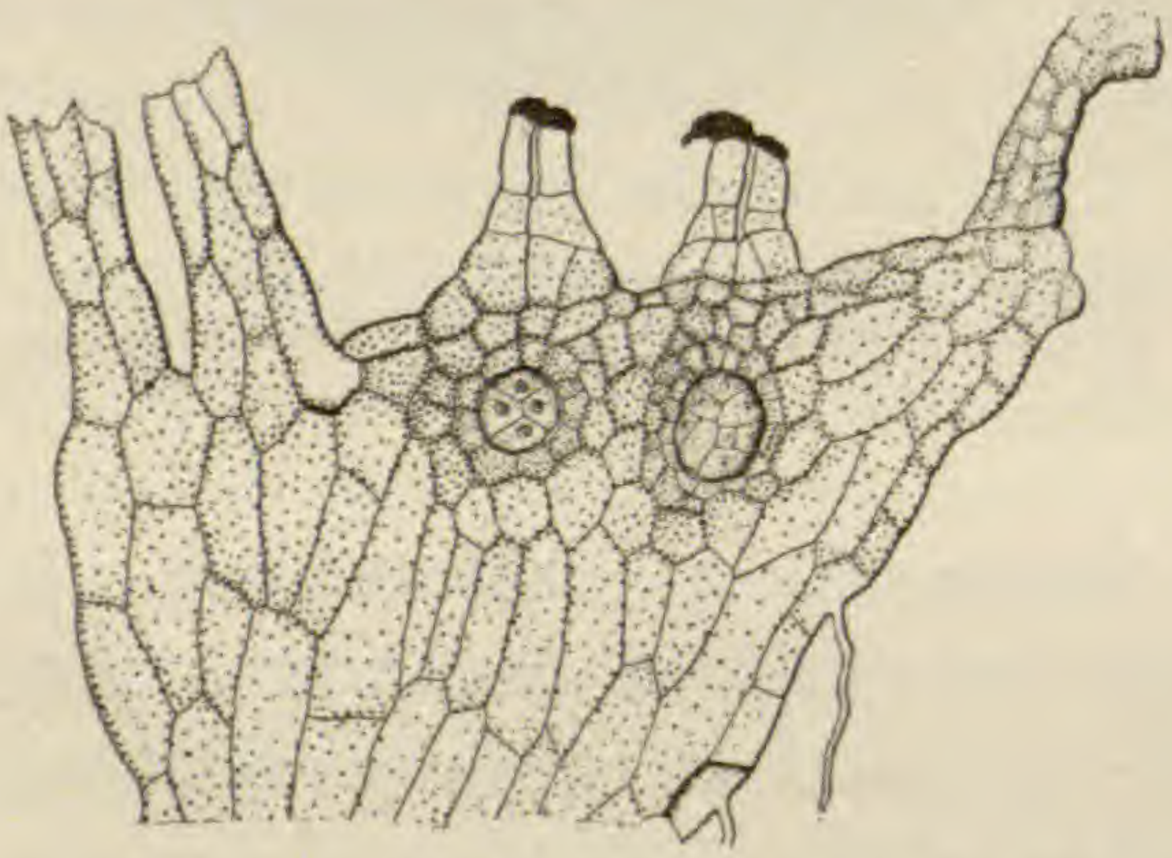


FIG. 16.—Polyembryony in *Equisetum arvense;* after HOFMEISTER (**22**).

phytes on a single gametophyte (fig. 17). It is difficult to understand how only one or a few eggs could be fertilized where hundreds of archegonia are found, even if they are of successive origin. The result of such a fertilization would produce dozens or at least quite a number of zygotes, a majority of which never develop beyond the stage of only a few cells, and many probably succumb in the struggle for nourishment in the one-celled stage, or before they divide many times. *E. laevigatum* was recently

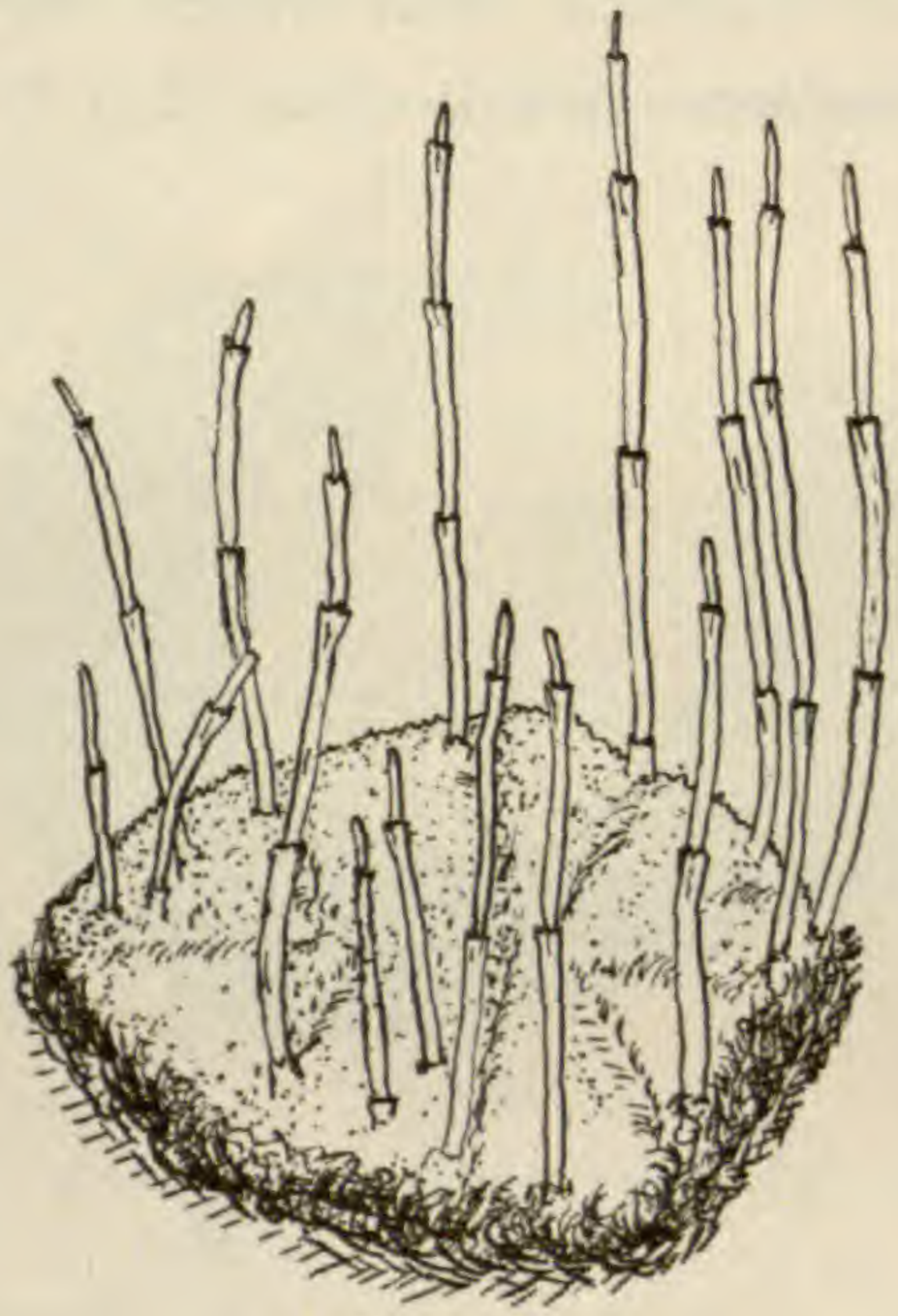


FIG. 17

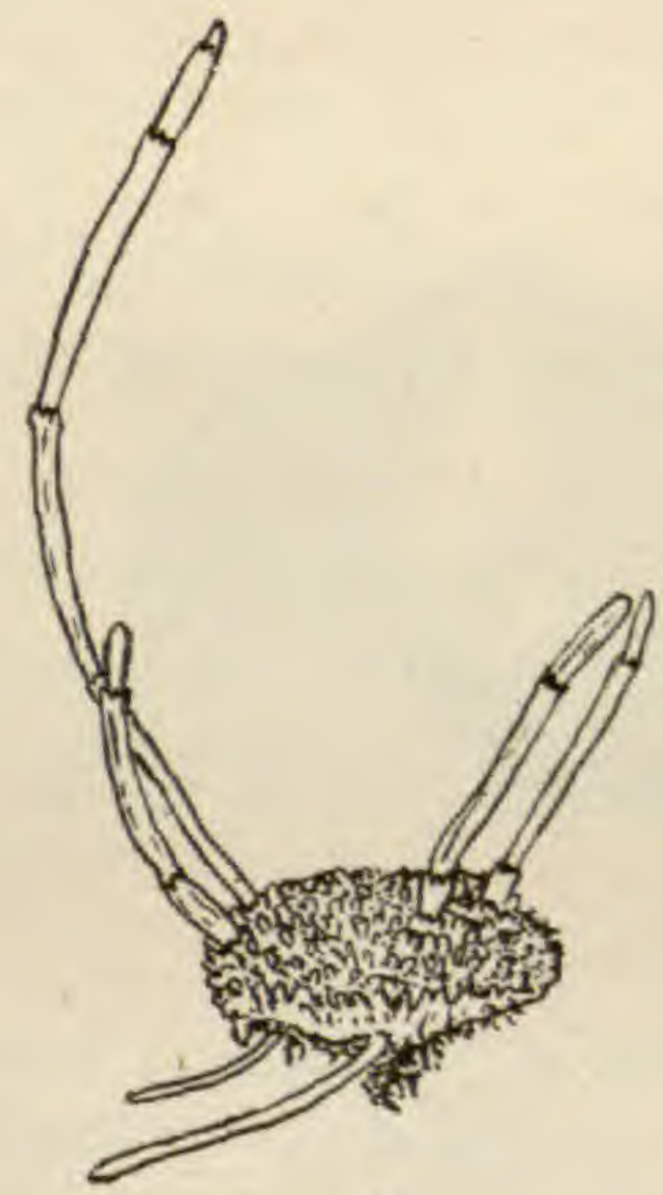


FIG. 18

FIGS. 17, 18.—Fig. 17, gametophyte of *Equisetum debile* giving rise to fifteen sporophytes under laboratory culture, ×1.5; after KASHYAP (**26**); fig. 18, *Equisetum laevigatum*, gametophyte bearing four sporophytes, ×2; after WALKER (**38**).

investigated by Miss WALKER (**38**), and this species likewise has a plurality of young sporophytes, four being shown in one case (fig. 18), and six in another. It is evident that embryonic selection plays a rôle in most, if not all species of *Equisetum*. Plurality of embryos seems to have been found in all carefully investigated species.

OPHIOGLOSSALES.—In his work on the gametophyte of *Botrychium virginianum*, JEFFREY (**25**) states and gives illustrations of the fact that one frequently finds two or more sporophytes on a single prothallium. BRUCHMANN found many very young

embryos in *B. lunularia*, but stated that never more than two could develop on the small gametophyte.

In his studies of *Helminthostachys*, LANG (**27**) found a plurality of young sporophytes, and also found and described many small aborted embryos, whose arrest in various stages of development was due to the supremacy of the larger successful sporophyte. Figs. 19 and 20 are from his figures, made by combining several sections of the series, and show the existence of embryonic selection in *Helminthostachys*. He also gives habitat data which may be taken as evidence that the fertilizations which gave rise to the

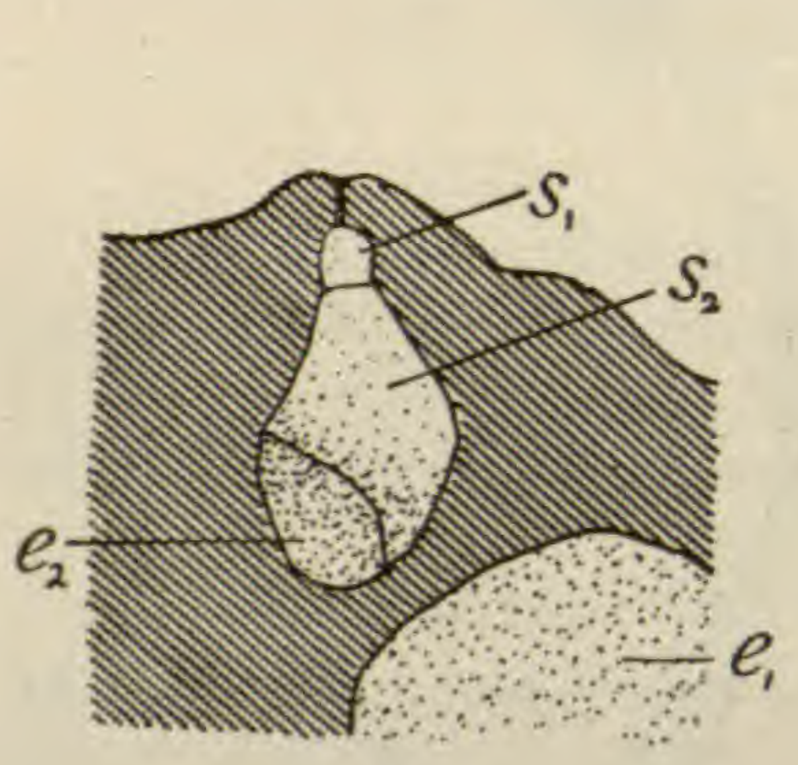


FIG. 19

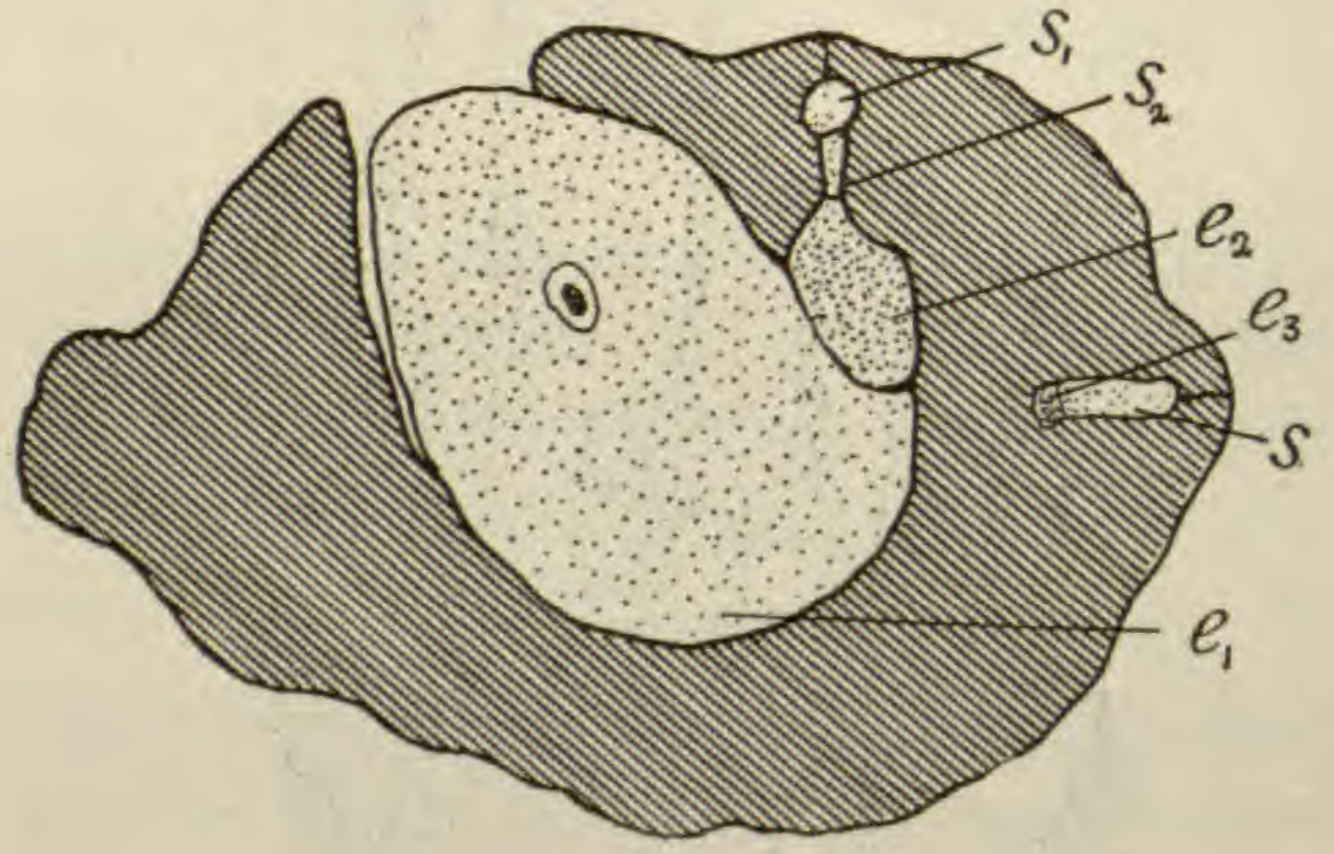


FIG. 20

FIGS. 19, 20.—Fig. 19, arrested embryo (e_2) of *Helminthostachys* beside foot of larger embryo (e_1); after LANG (**27**); fig. 20, two arrested embryos (e_2, e_3) beside larger sporophyte (e_1); s_1, s_2, tiers of suspensor; reconstructed from serial photomicrographs by LANG (**27**).

withered arrested embryos occurred at about the same time as that of the successful sporophyte plant, and that the aborted embryos were starved by the more rapidly developing sporophytes.

Botrychium obliquum has been studied more recently by CAMPBELL (**9**). Something definite concerning the occurrence of embryonic selection may be inferred from this statement in the following passage:

> Unicellular embryos are not uncommon, as several archegonia may be fertilized and begin to form embryos, but the later stages are not so easily found, and it was not possible to secure as complete a series as might have been wished. However, the essential points in the development of the embryos were made out, and there is no question as to the way in which the young sporophytes develop.

Of course embryonic selection would tend to make the later stages scarce, while the arrested unicellular and smaller embryos would be more frequent. Doubtless many of the latter are represented by the aborted embryos studied and mentioned by LANG and CAMPBELL.

LEPTOSPORANGIATE FILICALES.—Embryonic selection is also of common occurrence in many of the leptosporangiate ferns. In a paper on *Osmunda*, CAMPBELL (7) makes the statement, speaking of the *O. cinnamomea* gametophyte:

> Frequently more than one archegonium is fertilized as in the Gleicheniaceae (34), but as a rule only one embryo develops, although it is not at all uncommon to find several archegonia where the egg has evidently been fertilized, as is shown by its enlargement and investment with a cell wall. Only one case was met with where two larger embryos were present, but one of these was very much in advance of the other, and it is probable that the larger one would have ultimately starved out the other.

RAUWENHOFF (34) described the occurrence of several embryos in *Gleichenia* (fig. 22); and in *Vittoria* GOEBEL (19) found a similar

FIG. 21

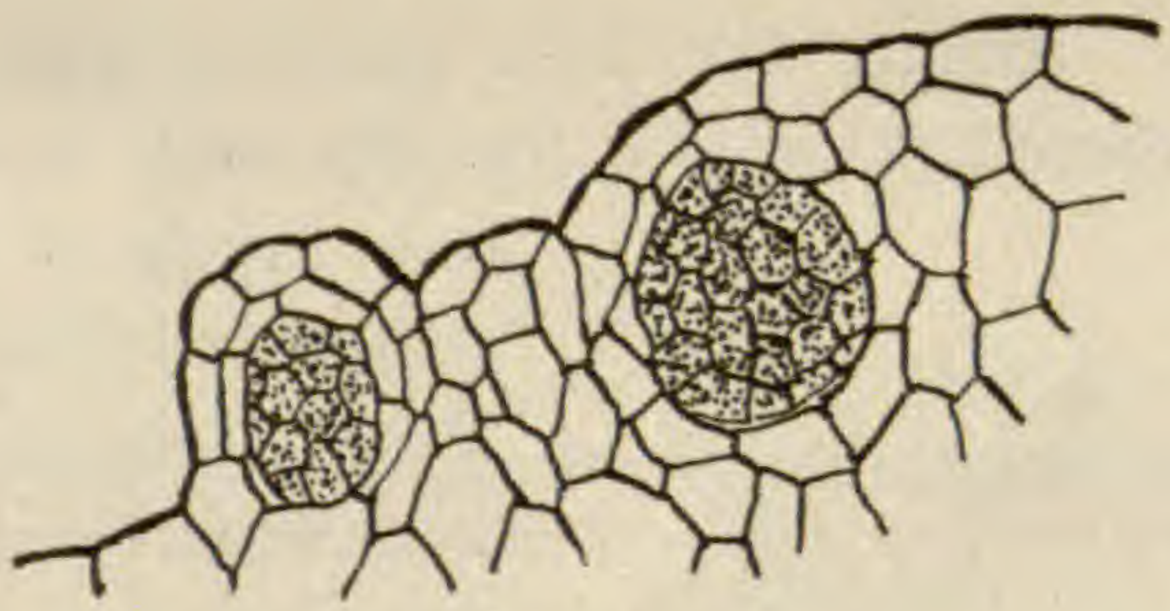


FIG. 22

FIGS. 21, 22.—Fig. 21, gametophyte of *Vittoria* with three embryos (*e*) on various parts of branching thallus: *ar*, archegonia; after GOEBEL (19); fig. 22, embryos of *Gleichenia* showing polyembryony; after RAUWENHOFF (34).

plurality of sporophytes and embryos. He states that he has no doubt that several sporophytes may come from a single prothallium; at least he frequently noticed several embryos in various parts of the prothallium (fig. 21), also prothallia on which there were still other embryos in addition to the larger sporophyte. GOEBEL states that it is dependent upon circumstances of nourishment whether or not these smaller embryos continue their development.

Among other Filicales this feature of embryonic selection is likewise to be found. MARKLE has prepared many slides of fern embryos in order to supply them for school use, and is therefore able to speak from considerable experience. Most of his material, which includes a variety of leptosporangiate species, was obtained from greenhouses. He has found that by carefully examining his sections in the paraffin ribbons before fixing them to the slides, he has usually been able to make more than one good preparation showing a one-, a two-, or a four-celled embryo on a slide, out of the ribbon obtained from a single large gametophyte. MARKLE states in a letter:

> I do not think I have ever seen more than one embryo on a gametophyte where each had reached the stage with the first leaf evident. I have, however, seen a number of instances where there were at least two or three, possibly four embryos in the two-celled or four-celled stage on one gametophyte. In sectioning material in which the largest embryo was in the stage where the four quadrants have their respective primary organs (foot, root, stem, leaf) well organized, I have seen other small embryos, very evidently suffering from the competition and losing out in the fight with the larger embryos, as was shown by the shrunken appearance of the cells.

Among the fern gametophytes of the preceding discussion, there are quite a few instances in which the several embryos are only those of the somewhat independent or remote archegonial cushions. For example, *Angiopteris evecta* (fig. 23) and *Vittoria* (fig. 21) both have the young sporophytes some distance removed from each other. This condition is found among ferns having large or branching gametophytes, which may have several archegonial groups more or less remote from each other. Among these, as well as among the ferns with large tuberous gametophytes, there is active embryonic selection only when two or more neighboring archegonia are fertilized. Likewise among leptosporangiate ferns the polyembryony has greatest significance when the competing embryos are near each other, as when they are on the same archegonial cushion. This form of embryonic selection, like that of conifers, only rarely produces

FIG. 23.—Gametophyte of *Angiopteris evecta* bearing two young sporophytes; after FARMER (18).

more than one sporophyte from a single gametophyte. This condition of polyembryony, which was casually mentioned by several students of pteridophytes, is well illustrated by fig. 24, which is a species of *Aspidium*, probably *A. Thelypteris*, collected in its natural habitat. While the larger embryo (fig. 24 *A*, e_1) has become multicellular, having approximately 25–30 cells, the second one (fig. 24 *B*, e_2), found on the remote side of the archegonial cushion, has remained unicellular, but has enlarged considerably.

Fig. 25 is from a gametophyte collected on the benches of a greenhouse whose species could not be determined, but could have been one of a half-dozen leptosporangiate species growing near by.

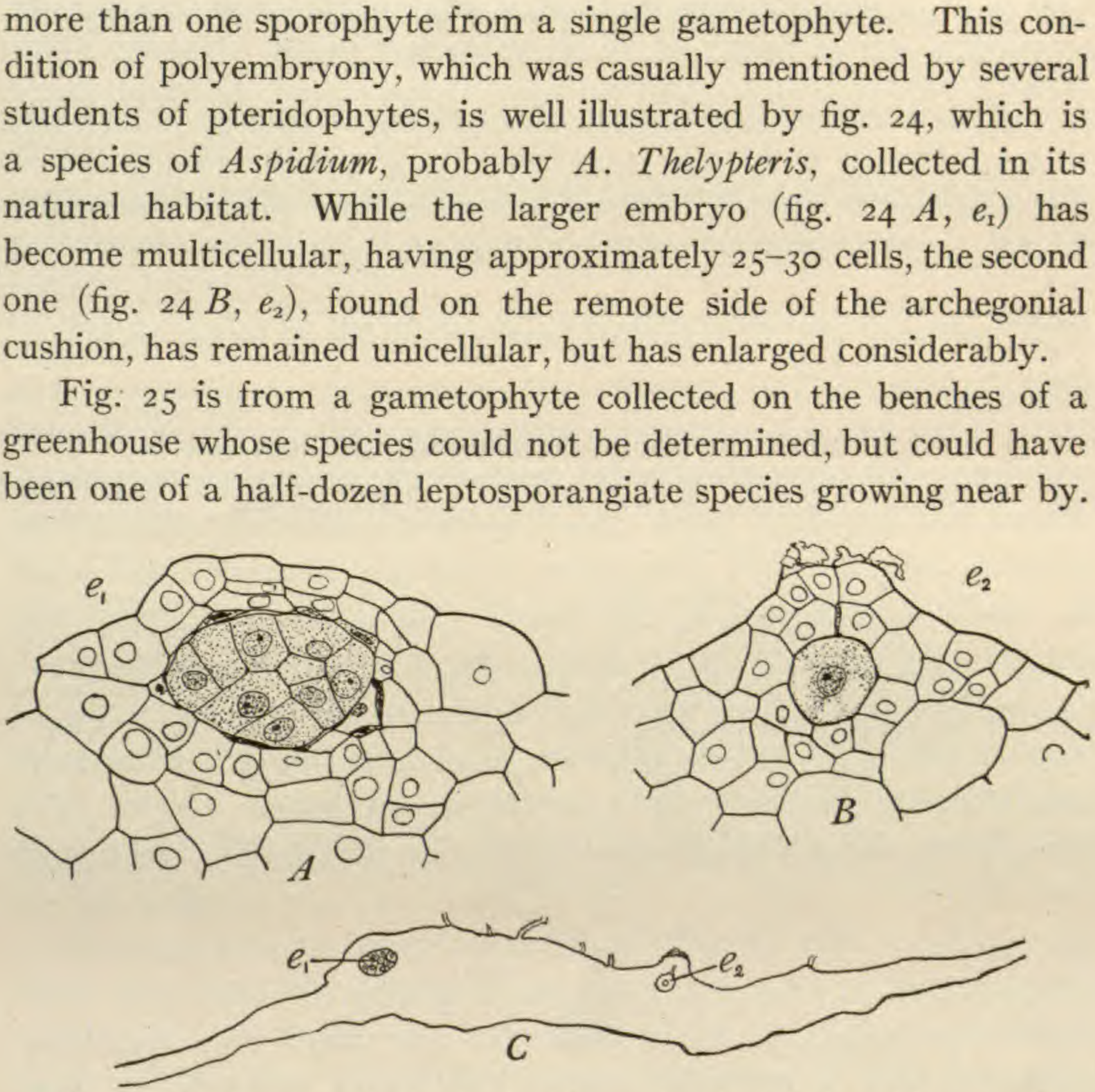


FIG. 24.—Embryonic selection in *Aspidium: A*, enlarged view of larger embryo of *C*, surrounded by calyptra; *B*, smaller unicellular embryo shown in *C* on same gametophyte, less than 1 mm. distant; (*C* reconstructed from several serial sections); *A* and *B*×200, *C*×42.

This is included here because it seems to show a stage slightly later than that of fig. 24, and indicates the fate of the smaller embryo of the latter. It is especially interesting to note the shrunken and starved appearance of the embryo in fig. 25 *B*. That the larger embryo starves the smaller is a very natural explanation; this is a factor which is very certain to be involved, but it is also possible that the excretions of one embryo tend to inhibit the development of the others. In ferns having large vigorous gametophytes with many archegonia, if the aborted embryos are not too quickly starved they should be subject to recall experimentally,

by any measures which would tend to prevent this embryonic competition.

From a careful examination of a number of species of leptosporangiate ferns, it is clear that there is a considerable period of enlargement of the egg following fertilization, before the zygote divides. It is obvious that the decisive part of the embryonic selection may transpire during this early period, so that most if not all of the zygotes are eliminated before they have undergone cleavage.

A few examples may be given showing the amount of increase in volume during the first stages of the embryogeny, calculated

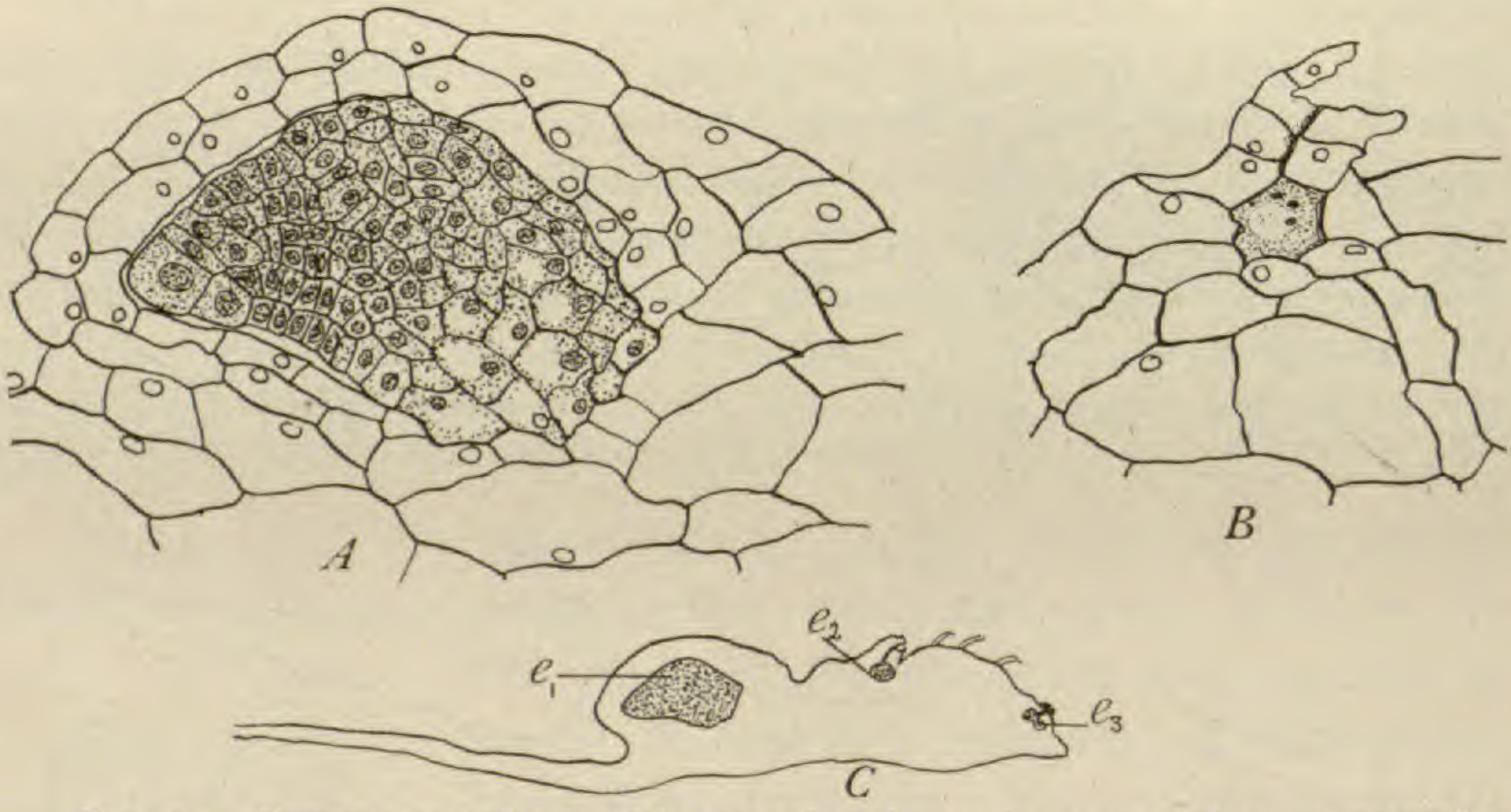


FIG. 25.—Fern gametophyte with several embryos in competition, representing slightly later stage than fig. 24; *A* is e_1 enlarged; *B* is e_2 (second unicellular embryo) with disintegrating nucleus, collapsing as it is aborted through embryonic competition; e_3 appears to be a third collapsed unicellular embryo (?); *A* and *B* × 200, *C* × 42.

from micrometer measurements. The average of several fertilized eggs in *Osmunda cinnamomea* measured 6000 cubic microns, while the zygote of the same species after only one cleavage measured 19,000 cubic microns. In *Adiantum* the diameter of the egg measured 17,000 cubic microns, while the two-celled zygote measured 65,000 cubic microns. In a species of *Pteris* the fertilized egg measured 33,000 cubic microns or less, while the two-celled zygote exceeded 195,000 cubic microns. Thus it appears that among leptosporangiate ferns there is a definite enlargement of the zygote of from 200 to 500 per cent during the first cleavages.

In eusporangiate ferns with well developed suspensors it is very much greater; hence the evidence to be found in the eliminated zygotes of this more active form of embryonic selection in ferns is not conspicuous. It occurs in the earliest stages, leaves only very small aborted embryos, and it is probably for this reason that embryonic selection has usually been overlooked as a normal process of the life cycle.

While many of the living ferns probably do not possess embryonic selection, at least as a very striking or prominent feature, practically all of them show good evidence of a derivation from forms possessing it; of having passed through this condition historically. During Paleozoic time, when pteridophytes constituted the dominant vegetation, embryonic selection was probably the prevailing condition. Even the environmental forms of competition were much more keen, as our vast coal deposits would indicate. There is little doubt that the early seed plants which were derived from these ferns retained embryonic selection, as it is a feature which has persisted until today in gymnosperms generally. The simple polyembryony of gymnosperms is therefore of fern origin.

SUPPRESSION OF EMBRYONIC SELECTION IN FERNS.—There are some pteridophytes among which there is a more or less complete elimination of embryonic selection. Such a highly specialized form as *Marsilia* presents a special variation in this direction, since only one archegonium is produced on the female gametophyte. Obviously there is no selection between two or more zygotes on the same prothallium. It appears that in *Marsilia* and other pteridophyte forms having only one archegonium, we have examples of the elimination of the embryonic selection, an advanced condition, doubtless the result of specialization. Leptosporangiate ferns, whose gametophytes are sometimes very much reduced in size, may perhaps provide additional interesting examples of the complete elimination of embryonic selection. Should this selection occur between archegonia, or between the eggs of neighboring archegonia during their development, it could certainly not be classified as embryonic selection; it may perhaps be designated as gametic selection, or in some other category of developmental selection.

It is needless to point out that morphological investigators have been concerned with only those stages in the development of the individual sporophyte or embryo which constitute the more important links of the life cycle, only occasionally illustrating or noting the occurrence of several embryos on one gametophyte. Sometimes an investigator illustrates a plurality of embryos without further comment, and LANG in his work on *Helminthostachys* reported a number of embryos only because he made use of the arrested embryos of one gametophyte in describing some of the missing stages of embryonic development.

STEPS IN EVOLUTION OF EMBRYONIC SELECTION AMONG PTERIDOPHYTES.—If definite steps in the evolution of embryonic selection among pteridophytes are recognizable, these may serve as a rough measure of their phylogenetic position, at least of the relative position within each of the several well recognized groups. It would seem that at least the following stages or steps in the evolution of embryonic selection may be recognized.

1. Many sporophytes are found on one vigorous gametophyte, a large portion of which reach maturity. Selection may finally occur under conditions of crowding in early or later stages, but this elimination occurs largely in the environment, and must then be recognized as natural selection, as, for example, *Tmesipteris* and *Lycopodium* with large vigorous gametophytes.

2. A few sporophytes appear above the soil or break through the tissues of the gametophyte, but a selection occurs among a much larger number during their embryonic stages; arrested embryos remain turgid for a considerable period, as for example *Lycopodium*.

3. One or only very few sporophytes break through tissues of gametophytes, but a selection occurs among a large number in their embryonic stages; arrested embryos are soon aborted and not easily recognized, as, for example, *Equisetum*, *Helminthostachys*, and *Botrychium*.

4. Normally only one sporeling sporophyte is produced, but several archegonia are fertilized, and selection between zygotes occurs in early embryonic stages; arrested embryos are usually soon aborted and not easily recognized, as, for example, *Selaginella*, *Osmunda*, *Aspidium*, etc.

5. Gametophytes are so reduced in size that only one archegonium is produced, making selection between embryos of separate fertilization impossible, as, for example, *Marsilia* and *Pilularia*.

Selection between gametes

Among all of these pteridophytes another form of developmental selection may be recognized. Doubtless a selection occurs among the male gametes as they swim to the archegonia. That the archegonia attract the sperms chemotropically has long been known. The gametic selection is therefore a measure of their response to this stimulus. While it may be largely a matter of chance which of the many sperms that reach the archegonium and swarm about its neck actually reach the egg to effect the fertilization, there can be no doubt that the less active sperms or those otherwise defective would be eliminated in the race to reach the egg. If only the most vigorous sperms take part in fertilization, and there seems to be very good ground for this, certainly a form of gametic selection is to be recognized. It may be noted in passing that with its return to aquatic life, the natural sphere of swimming sperms, *Marsilia* has exchanged one form of developmental selection for another. Embryonic selection was made impossible and lost through reduction of archegonia, but gametic selection was doubtless facilitated when this fern returned to the aquatic habit. Gametic selection is not a new suggestion, having been suggested by THOMSON (36) for animals, and it is probably in part along this line that the principle of developmental selection may be found to apply somewhat generally to the animal kingdom.

It must be remembered that no reduction divisions occur in the formation of sperms in ferns, nor is any special form of cell division known or recognized here which might bring about genetic changes. In animals the formation of sperms is accompanied by a chromatin reduction both equational and differential, a condition shared by some algae, notably *Fucus*.

OTHER FORMS OF DEVELOPMENTAL SELECTION.—There are still other forms of developmental selection which must be taken into account. One of these is illustrated by the selection which takes place at a certain stage of development in *Selaginella* between the

megaspore mother cells, a large number of which begin to appear only to degenerate when one of them is selected and enlarges to form the single tetrad of megaspores. This selection is neither embryonic, gametic, nor gametophytic, but belongs to a distinct category, somewhat similar to some other types of developmental selection which will be discussed later.

Developmental selection among spermatophytes

EMBRYONIC SELECTION.—Most striking of the forms of developmental selection of seed plants is the embryonic selection illustrated by the polyembryony of gymnosperms, which has already been described in a general way. The several embryos originating from the fertilized eggs engage in a competition in which the most vigorous individual is always the winner. Not only must the embryo rapidly become massive and multicellular, but it must also produce a stiffer and more vigorous suspensor, one which keeps the successful embryo in the commanding position. The winner is usually the foremost of the group of embryos, where the embryonal tubes of the elongating secondary suspensor are able to push the other competitors back, away from the most favorable position. This applies whether cleavage polyembryony occurs or not. The mature conifer seed has a single large embryo, but the remains of some of the other embryos participating in the competition can usually be found, crushed against the archegonial end of the embryonal cavity within the gametophyte (endosperm) by the suspensor or radical end of the successful embryo.

GAMETOPHYTIC SELECTION.—Another type of developmental selection is gametophytic selection. This is not intended to apply to the form of natural selection occurring between independent gametophytes, as those of liverworts or ferns, in the external environment, but rather to a plurality of male or female gametophytes which are dependent on a sporophyte, as they are in seed plants. It is well illustrated by the pollen tubes of a pine or other conifer whose competition predetermines in a measure which of the several archegonia shall first be fertilized. Although fertilization in conifers is almost simultaneous even in the various cones of the same tree, a fact first pointed out by HOFMEISTER (21), this

event does not usually occur absolutely simultaneously even in a single ovule. Some embryos are usually produced a little earlier than the others, and have a slight advantage, although this difference may represent only a few hours or minutes. The competition is continued between the several embryos in the ensuing embryonic selection, which becomes truly a struggle for existence, so that the resulting seed has only one embryo. Here in gymnosperms the developmental selection process is a sort of relay race between one pollen tube plus its embryo, and other pollen tubes plus their

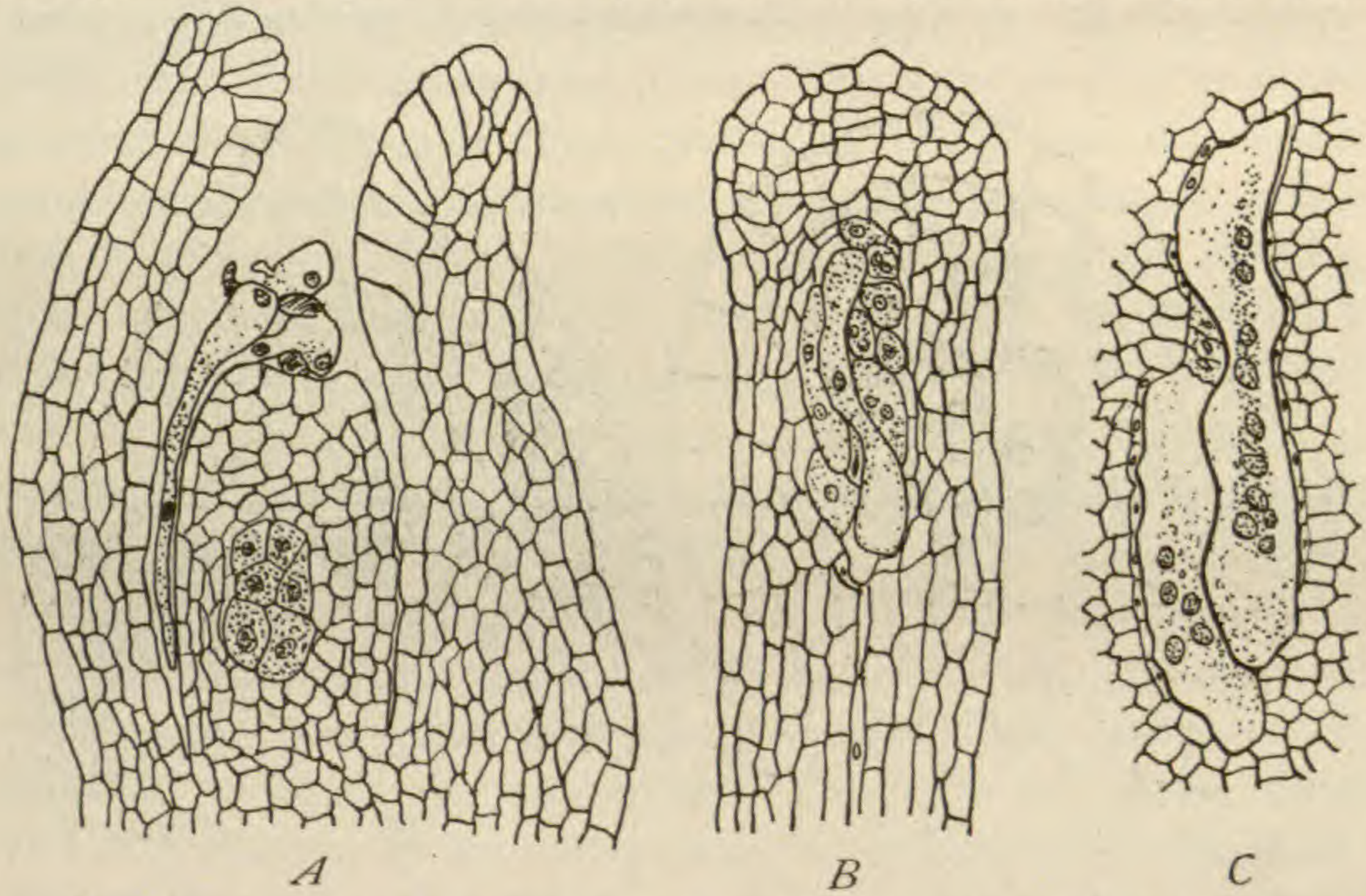


FIG. 26.—Sections of ovules of *Sequoia sempervirens*, showing selection between female gametophytes: *A*, ovule with six megaspore mother cells; *B*, numerous germinating megaspores; *C*, older stage showing two young gametophytes whose competition has persisted into multinuclear stage; after LAWSON (**28**).

embryos. The embryo of a gymnosperm seed is therefore the survival of a rapidly developing pollen tube combined with a very vigorous embryo.

Among conifers the male gametophytes are not the only individuals taking part in this competition. Female gametophytes may also undergo competition under normal conditions in some species. DUPLER (**16**), in his work on *Taxus canadensis*, showed that the existence of several female gametophytes arising from as many megaspores is quite the normal condition. LAWSON (**28**) found a similar situation in *Sequoia* (fig. 26), and apparently also

in *Cryptomeria* (29). The work of LOTSY (30) on *Gnetum* indicates the same thing, and occasionally slight evidences of this are found in *Pinus* and other conifers.

Even angiosperms sometimes have this competition between female gametophytes, which seems to occur as a functional form of developmental selection. This is especially true of some of the lower Archichlamydeae and Monocotyledons. *Casuarina* (37) has been reported to have as high as twenty or more megaspores, of which several enlarge considerably, but only one is functional. *Alchemilla* (20) has been observed with five or six ripe megaspores;

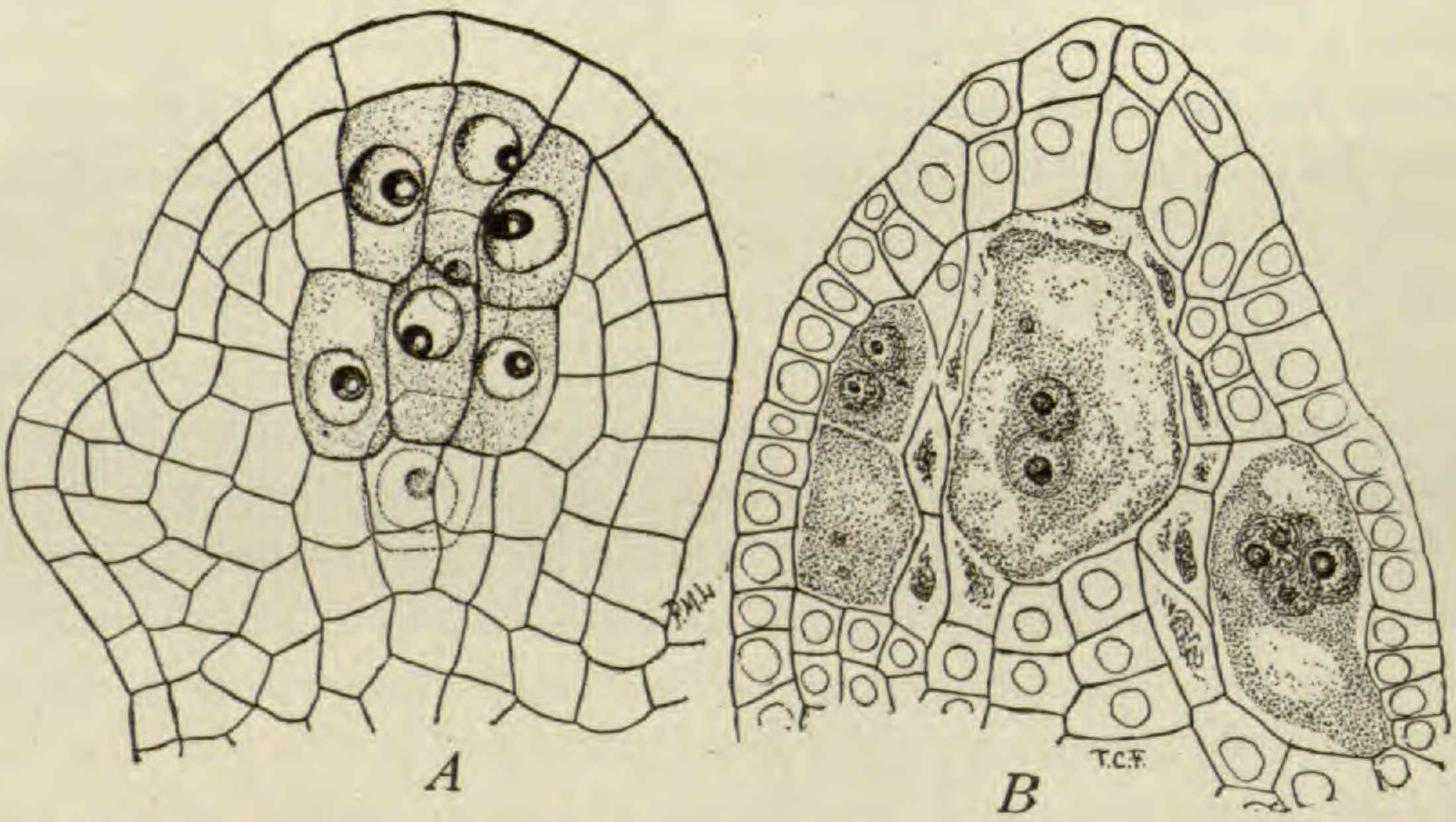


FIG. 27.—Selection between female gametophytes in *Ranunculus septentrionalis*: *A*, section of nucellus showing eight-celled archesporium; *B*, later stage showing several female gametophytes and aborting megaspores in early stages of development, ×400; after COULTER (12); cut lent by D. Appleton Company.

in *Arisaema* (8) something similar has been reported; and in *Ranunculus* COULTER (12) found as many as eight archesporial cells and three embryo sacs within the same ovule (fig. 27). Numerous additional examples are on record. GOEBEL (20), in discussing the many gametophytes of *Casuarina*, suggests that "biologically this repeats the case of the embryos of the Abietineae, where, of the many embryos which arise from one egg, only one develops." His interpretation of the significance of polyembryony in conifers is treated as a process of correlation, where he compares it to the

correlative "struggle" between vegetative parts, or flowers in a crowded inflorescence. It is evident that he recognized a significant similarity in these forms of developmental selection, although apparently he did not anticipate the significance of these facts in relation to the selective mechanism of evolution.

A very unique form of developmental selection is represented in *Welwitschia mirabilis* described by PEARSON (32). The female gametophyte gives rise to a number of nuclei, potentially eggs, which develop prothallial tubes that grow up into the nucellar tissue. When such a prothallial tube comes in contact with a pollen tube, fertilization takes place. The embryos may be found growing down through these prothallial tubes into the female gametophyte tissue. Although we have polyembryony, the selection is probably in part predetermined by priority of fertilization, which depends upon the pollen tubes and the prothallial tubes of the female gametophyte. Apparently the selection resolves itself, in part at least, into a competition between eggs, or prothallial tubes containing eggs, a form of selection between female gametes which is very rare in plants.

Megaspore tetrad formation and the abortion of the megaspores in angiosperms might suggest itself as a form of developmental selection, but the selection in this case seems to be largely one of position. It is not any megaspore of the group in a linear tetrad that may give rise to the embryo sac, but almost always the innermost of the four. This selection is not dependent on the physiological success of the megaspore, but is morphologically fixed, and therefore not properly included among processes of developmental selection.

Among angiosperms the selection between male gametophytes or pollen tubes represents the most important developmental selection machinery. In the pistil of the ordinary flower an excessive number of pollen grains may germinate on the stigmatic surface, but usually only a limited number of these can function in fertilizing the eggs within the ovules. Only one pollen tube is necessary to fertilize the single ovule in the pistil of maize, yet hundreds may fall on each stigma and germinate, producing pollen tubes of varying lengths. Fig. 28 represents the pistil of an

angiosperm, illustrating the important mechanism of this gametophytic selection. A mature cotton boll contains an average of from 30 to 40 seeds, yet hundreds of pollen grains may germinate on the stigma of the pistil. EAST (17) has determined by actual count that there are usually 1200 to 2000 pollen tubes in a single pistil in tobacco, sufficient to fertilize from four to six times the number of ovules in the ovary.

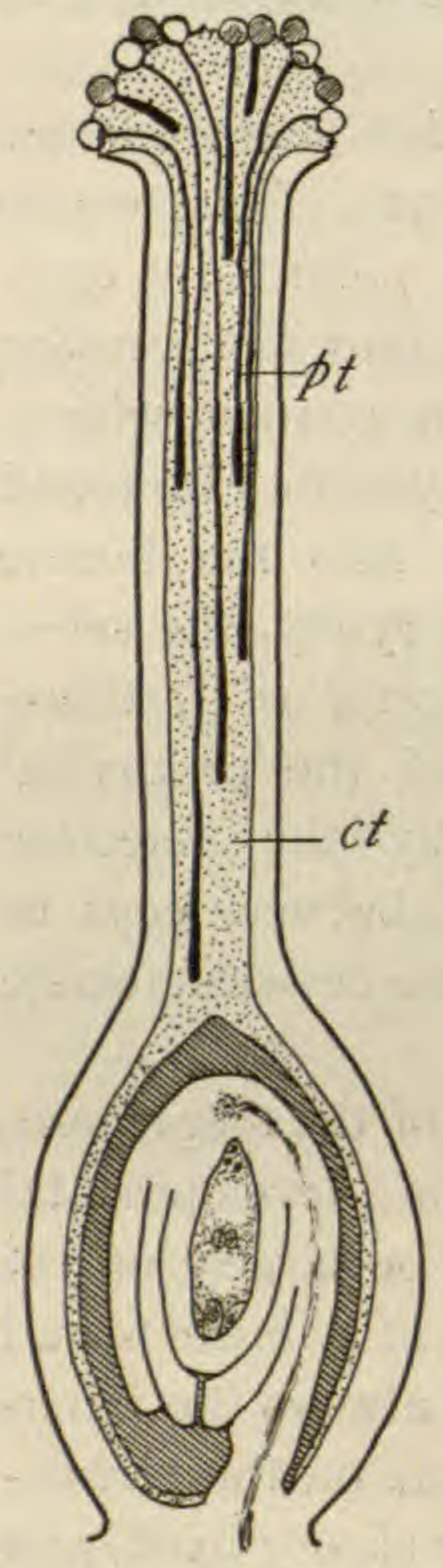


FIG. 28.—Diagram of pistil of angiosperm having one ovule, showing pollen tubes (*pt*) penetrating conducting tissue (*ct*) of style, engaged in competition to reach ovule, resulting in gametophytic selection.

Recent genetical studies have shown that this gametophytic selection in angiosperms represented by the pollen tubes has a significance of the first importance. For example, CORRENS (14, 15) has shown that in *Melandrium* there is a selection between the male gametophytes in their race to the ovules, that the female-producing pollen tubes are on an average decidedly faster in their growth than those carrying the factors which produce males. By applying much pollen so as to crowd the pollen tubes, he was able to increase the pollen tube competition in favor of the production of more females, and by sparse pollination he was able to stay this competition somewhat, resulting in the production of more males than under normal conditions. This may be taken as definite experimental proof of gametophytic selection in angiosperms.

Experimental studies of pollen tube competition in angiosperms have been in progress for some time by the writer, and will be published in separate papers. It may be stated here that gametophytic selection, as it affects the evolution of angiosperms, is a subject open to experimental study. It is already apparent that the genetic result of this gametophytic selection is a matter of the first importance if it relates to some of the heritable characters,

especially in plants with long styles. While this pollen tube selection in angiosperms is morphologically very different from the embryonic selection in gymnosperms, it is physiologically a very similar process. In the case of the embryonic selection the embryo sporophytes are digesting their way and pushing forward into gametophytic tissue, while pollen tubes represent gametophytic structures penetrating sporophytic tissue by what appears to be a very similar method.

An important difference, however, between the gymnosperm embryonic selection and the pollen tube competition of angiosperms should be noted. The embryos concerned in the competition are diploid individuals, while the pollen tube and other forms of gametophytic competition take place between haploid individuals. The recent work of geneticists shows that lethal factors may be present in one member of a pair of chromosomes apparently without serious consequences, as long as the same lethal is not present at the same time in both chromosomes. Factors lethal to the gametophyte could not be protected in this way by a homologous chromosome.

INTEROVULAR AND INTERFLORAL SELECTION.—Another form of selection, belonging more or less completely to the categories of developmental selection, is that occurring in angiosperms between the ovules within the same ovary. In species of *Quercus*, for example, there are six ovules within the ovary, although normally only one ovule with one embryo is found developed in the acorn which matures from this ovary. In *Fraxinus* and in the olive there are two ovules in each pistil, yet only a single seed with one embryo is matured. There are numerous similar instances in the plant kingdom, and whether the elimination of the unsuccessful ovules in such cases occurs as early as the stage when the megaspores or female gametophytes are developing, or only among the ovules containing zygotes after fertilization has taken place, remains to be determined. According to the published accounts of the morphology, the latter is probably what happens in *Quercus*. The selection between reproductive organs during early vegetational stages, as that previously described for *Selaginella*, should be included in this category of developmental selection.

Interfloral selection is a form that may occur between the individual florets of a crowded inflorescence, such as the head of Compositae, the spike of maize, or the umbelled cluster of flowers in *Asclepias*. It is to be referred back to a struggle between the embryos contained in the different flowers, but since this competition is indirect, it is not usually very decisive. The process is less secluded from environmental influences, and takes on a form somewhat similar to the selection between vegetative branches described later. Interfloral selection is of little consequence in evolution as a form of developmental selection, except probably in rare instances.

VEGETATIVE FORMS OF DEVELOPMENTAL SELECTION.—Thus far the reproductional phases of developmental selection have been the chief concern. These reproductional types of selection are by far the most important, since through sexual reproduction new zygotes combining diverse hereditary strains come into existence. The developmental selection which takes place during reproduction, therefore, is a kind which may occur between different phenotypes, and produces results that are genetically very significant.

That there are also vegetative forms of this developmental selection process should not be overlooked, but this vegetative selection remains within the same genotype, unless a vegetative mutation occurs. For example, the branches of a tree are in a state of competition for light and favorable exposure. A struggle for development was suggested long ago by MÜLLER, who pointed out that there are many times more buds on every twig of a tree than can possibly develop into branches. While external circumstances of exposure may largely determine the result of this selection, the merits of the individual buds and their branches are also responsible in part for the result. If bud mutations occur, this vegetative selection determines at once whether they shall survive to reproduce themselves later or be eliminated. The principle is very largely the same for any form of vegetative selection, whether in gametophyte or sporophyte, by the dichotomy of thallus, aerial branches, stolons, tubers, rhizomes, or roots. When practiced for the purpose of obtaining vegetative mutation, bud selection has been called clonal selection. Of course, it is evident that this selection

does not belong rigidly to the categories of developmental selection, for it is subject very largely to external environmental conditions. It is not entirely in the external environment, however, since internal physiological correlations are concerned. It is clearly a type of selection standing between natural selection and developmental selection, as previously indicated.

Summary

Embryonic selection and gametic selection represent the important forms of developmental selection found among pteridophytes. Among gymnosperms the gametophytes plus the embryo usually take part in this competition, and among angiosperms it is typically a competition between male gametophytes represented by the pollen tubes. The important reproductive developmental process is merely shifted to an earlier stage of the life cycle in passing from ferns to angiosperms, where vegetative and other less important forms of developmental selection are also found.

Developmental selection in relation to natural selection and mutation

It is very evident that a good selective process, whether developmental, artificial, or natural selection is meant, should meet at least the following four requirements. It should (1) start the competition simultaneously, (2) take place under uniform conditions, (3) measure comparable merit, and (4) rigidly eliminate the great majority that fall below the standard.

First it should launch the individuals who participate into the competition with an even start. This is a primary requirement in any kind of a performance race. Well fitted is the process of fertilization to launch the competition of embryos, as this is a relatively sudden event, one which may happen only occasionally, and may be simultaneous in producing several or a large number of zygotes. Similarly the shedding of sperms from an antheridium provides an even start in the competition of sperms, with a definite goal to be reached through their activities. Pollination, especially when the pollen is transferred in masses or clumps by insects, is another more or less sudden event, which launches the competition

of pollen tubes. All of these events which start the various processes of developmental selection are superior or at least equal to the equivalent initiatory processes of natural or artificial selection. Among the latter seed germination which, while it gives a fairly even start to many of the competitors, is a more variable process, easily modified by soil conditions or delayed and unequal germination; it is slower and it is not usually as efficiently simultaneous as pollination or fertilization.

Selection between vegetative parts is also initiated by the awakening of the buds in the spring, a process which may be more or less simultaneous and comparable with seed germination under the most favorable conditions. Birth and the hatching of eggs in animals is a process well fitted to initiate the competition of natural selection. It is apparent that the processes of fertilization, pollination, and the liberation of sperms are all very superior means of beginning a selective process, and that there are only a limited number of these events in the life cycle of an individual.

The second requirement, that the competition should take place under uniform conditions, is one in which developmental selection excels, while natural selection is very inefficient. Under the conditions of isolation in pure culture in artificial selection, the environmental conditions are made very uniform, but even here the conditions are not as isolated and insulated as they are within the ovule of the pine seed, or within the tissues of the stigma and style, where pollen tubes must carry on their competition. On the other hand, the external environment where natural selection occurs is exceedingly complex and diverse.

The third requirement, that selection should measure equal merits, is also one in which natural selection falls far short of providing the best possible mechanism. In the external environment not all of the competing individuals which are "saved" are required to go through exactly the same performance, at the same time, in the same place, and under the same conditions. So many and varied are the factors that might be used to determine survival, and so different are the responses of plants that might be made to them in obtaining survival, that the capacity for an equal performance of the same task under similar conditions is not measured,

but rather a general indefinite all round fitness. It has already been shown, however, how developmental selection sets the same task for all the competitors. Likewise artificial selection sets a uniform standard of excellence of performance for all participants, but a standard which is limited by the powers of discrimination of the breeder.

By providing a very uniform medium for the competition of embryos, pollen tubes, etc., and by forcing rigid elimination, developmental selection precludes indiscriminate survival. The surviving plant may owe its existence to the performance record of its parent pollen tube, or it may owe its existence to its own performance during embryonic development; but in any event, there are very definite measures of some kind of excellence to be lived up to on a competitive basis. The surviving individuals constitute a class, selected for their superiority among several, among dozens, or even among hundreds of other individuals which were destroyed in this competition. Developmental selection, therefore, is not open to this objection which has been urged against natural selection.

In the fourth requirement, developmental selection again excels, while natural selection is only feebly effective, for the defeated individuals are rigidly eliminated in developmental selection. The losers in the environmental competition are not always destroyed from reproduction; their progeny may only be diminished somewhat. Artificial selection also meets this requirement fully.

In connection with these special features of developmental selection, it is interesting to consider some of the objections which have been raised against natural selection. One of these concerns the chances of death, which have been ably discussed by several evolutionists, who point out that the destruction of individuals is very indiscriminate, that the fittest do not always survive, for many of them are destroyed. Likewise, the least fit do not always perish. Thus it has been urged that there is little evidence that natural selection actually selects any specific class of individuals in preference to others. In fact, so complex is the environment in which natural selection must sort out the superior, that accident and chance really play a major rôle.

Another of the objections which have been urged against natural selection is that this theory rests altogether too largely on an unwarranted analogy with the process of artificial selection, although this supposed analogy was a very convincing argument in the hands of DARWIN. This analysis shows how natural selection is weak in at least three out of four of the requirements in which developmental selection excels, and how a much closer parallel or analogy may be drawn between developmental selection and artificial selection.

That there are many details which do not permit a close parallel between natural and artificial selection may further be illustrated. For example, the breeder in practicing mass selection, plants a large number of seeds in a uniform soil, and seeks to eliminate all other environmental differences wherever possible. Pure breeding in isolated cultures is possible, and at some definite stage when the seedlings come up, or as they mature, they may be measured and selected by very nearly the same standard of size and growth vigor, color, size of fruit, disease resistance, etc. Natural selection must necessarily be a much less methodical process. In nature, survival must be determined on the basis of a total or all round fitness. Very often this survival is purely fortuitous. Naturally disseminated seeds are less likely to germinate simultaneously in a uniform environment than planted seeds in a cultivated soil. If seedlings do not get an even start, are not growing in a uniform environment, are not measured up to the same standard, and the unselected are not always destroyed, survival by chance plays a very important rôle, and their apparent competition cannot be one of the greatest consequence. This has been urged as a very serious objection to natural selection even as a highly efficient selective mechanism, aside from the question of its power in originating species. Obviously the mechanism of developmental selection is much better fitted to bring about a competitive form of selection. It may be considered more efficient even than artificial selection, where uniformity of environment is only approximate, and the standards of selection depend upon the discriminative powers of the breeder. Finally, developmental selection makes possible a very early decision, which is doubtless a most valuable

form of biological economy, another feature in which developmental selection excels.

A further objection which has been made to natural selection has to do with the difficulties that are involved in explaining how the first steps in any given variation may be of selective value. How can natural selection influence a structure whose advantage is to be reached only at some future time, after the results of the selection are achieved? This is asking natural selection to pass on a prophecy. Developmental selection, however, is a form of selection which can act on some very small quantitative characters. For example, by playing on such features as minute differences in suspensor length and rate of growth in gymnosperms, or a rapid pollen tube penetration in seed plants, developmental selection brings about a positive selection for minute differences in these particular characters. It may be asked how this selection for embryonic or pollen tube vigor could in any way affect a selection for other characters. The answer is found in linkage of characters. A sporophyte character of the mature plant must be linked with the factors producing either vigorous suspensors and embryos, or vigorous pollen tubes in the gametophyte stage.

CORRENS found just such a case in *Melandrium*, which was referred to previously, in which the female-determining pollen tube has the quality of growing slightly faster than the male-determining pollen tube. Judging from his account, this linkage is probably not an exceedingly close one, as he states that there are some male-determining pollen tubes that grow faster than the slowest female-determining pollen tubes. Such a condition may be due to the well known crossing-over phenomenon associated with linkage, between the sex factor and the gene or genes producing rapid pollen tube growth. If factors affecting the rate of pollen tube growth should become linked with quantitative factors, which are usually multiple factors affecting the size of an organ or part, it is easy to understand how developmental selection can play on them indirectly through their linkages. An accumulation of the effect of this selection would account for the building of a new structure. Of course natural selection can also play on such a character or structure when it or a factor linked with it has become

distinctly useful or harmful to the organism, but not before the structure is present.

DARWIN recognized such linkage, calling it correlative variability. He accounted for many useless characters by assuming their linkage with other factors which are sufficiently useful to have a survival value. It has already been shown, however, that there is great difficulty in definitely pointing out that certain characters are actually acted upon in the environment by natural selection on a consistent basis. In developmental selection there is no doubt about a definite selection taking place, and the experimental evidence that certain adult sporophyte characters are linked with the gametophytic or embryonic factors having definite values in selection is also available.

This will also explain the production of overdeveloped structures, or those having no advantage to the organism. Suppose the factors determining the length or shape of the spines in the ends of some pine cone scales, or other useless details of the plant, should become linked with the genes producing either the most vigorous pollen tubes or embryos, there is no doubt that the selection for the pollen tubes or the embryos would result in the selection of the other characters in the same linkage. The spines would then be selected in or out, as the case may be, by developmental selection. Details of variations or mutations, be they ever so small, may either tend to disappear or become fixed or overdeveloped, as they are affected by this internal selective mechanism. Such phenomena of determinate variations and evolution in definite directions have long been recognized, and have been attributed to a principle called orthogenesis. The mechanism of developmental selection described here looks very promising as a means of accounting for many orthogenetic phenomena.

It is probably needless to point out that many characters would not be linked with these factors involved in developmental selection at all. These would remain unaffected by the developmental selection processes. Among these most of the heritable characters that have been studied by the Neo-Mendelians are to be found. The genes of these characters segregate independently of the factors affected by this selective mechanism, and yield Mendelian ratios in the expected proportions. Among those which should probably be

classed as the ones affected by some form of developmental selection are many of the lethal factors that have been described in some plants. It has been shown by LITTLE that some kind of embryonic selection is responsible for the non-appearance of homozygous yellow mice whose inheritance was studied earlier by CUENOT. No attempt will be made to discuss here the lethal factors of *Drosophila* in this connection, but doubtless many things whose inheritance would follow the lethal type, could be caused by a kind of embryonic selection mechanism.

The origin of the variations or mutations is another problem. Chromosomal phenomena, such as gene mutations, the chromosomal mutation of non-disjunction, etc., are probably able to account for the actual origin of many variations. Similarly bud mutations and other heritable vegetative variations would also be accounted for by some type of nuclear or intracellular phenomena. They would be played upon by developmental selection even before their outward manifestations are recognized. These mutations may also be acted upon by natural selection if they possess some very marked advantage or disadvantage. It is in this connection that developmental selection has a very definite rôle in the origin and heritability of some mutations. Only those variations which are not affected by developmental selection could reappear regularly or give consistent Mendelian results. The outcome of developmental selection may be so decisively against a mutation that it may seldom recur. On the other hand, if the mutation is closely linked with a factor greatly favored by developmental selection, it may reappear as if fixed, even though the pollen produced is heterozygous for it. Thus we can understand how even the discovery and recognition of the mutations themselves depend upon whether they reappear in the next life cycle, and thus pass the censorship of the developmental selection machinery. Developmental selection is doubtless responsible for the recognizability of some variations as mutations, but we have no evidence that it could be held responsible for the chromosomal phenomena themselves.

Summary

1. The process of developmental selection is a normal event or succession of events in the life cycle of vascular plants, where it

assumes various forms, being represented chiefly by embryonic selection, gametophytic selection, and gametic selection.

2. Developmental selection differs materially from natural selection, germinal selection, the intraselection of ROUX, as well as the other selection theories.

3. Records of conspicuous cases of polyembryony in ferns are brought together. Original studies are added, constituting definite evidence that a selective plurality of embryos may normally exist even in the leptosporangiate ferns. Nearly all living ferns seem to have embryonic selection, or show evidence of having passed through a stage in which embryonic selection was the normal condition. The embryonic selection represented by the polyembryony of gymnosperms was derived from an embryonic selection habit in their fern ancestors.

4. Developmental selection in gymnosperms and angiosperms is not only represented by a selection among embryos, but also by a selection between female gametophytes and the male gametophytes represented by the pollen tubes.

5. A form of selection intermediate between natural selection and developmental selection may be recognized in the competition between buds and branches of a sporophyte or a branching thallus.

6. Developmental selection is a process which brings into play a definite internal competition between embryonic diploid individuals, as well as between the haploid sperms of fern plants, and the haploid male and female gametophytes of gymnosperms and angiosperms. On the other hand, natural selection usually acts on the diploid generation in these plant groups, or on the haploid fern gametophytes, where selection may take place in the external environment.

7. The discussion seeks to show why the process of developmental selection is not open to the more serious objections which have been urged against natural selection, and on what basis it equals or excels the latter as an effective selective process.

8. The discussion also shows how developmental selection may account for some of the phenomena of orthogenesis on a mechanical basis.

9. Developmental selection is not responsible for the origin of the chromosomal or other intracellular phenomena involved in

mutation, but it is a powerful mechanism whose censorship may determine whether or not any particular intracellular phenomena causing mutation may complete the life cycle to be heritable, and therefore recognizable as a mutation.

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BIOCHEMISTRY OF PLANT DISEASES

III. EFFECT OF SCLEROTINIA CINEREA ON PLUMS[1]

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(WITH SEVEN FIGURES)

From the viewpoint that a fungus attacks a host plant, not to destroy it, but to gain a livelihood, it becomes of interest in the study of the chemistry of resistance to discover why a fungus can parasitize some varieties of a host and not others. There may be several bases for this difference: (1) the structure of the resistant host may offer mechanical difficulties to the entrance of the parasite; (2) the host may contain or produce repellent substances, such as tannins, acids, antienzymes, and antibodies; or (3) the host may fail to furnish the proper kinds and amounts of nutrients for the normal development of the fungus.

Each of these possibilities has received some attention at the hands of investigators; but the two latter, constituting what may be called the biochemical basis of resistance and susceptibility, have received the least. It was decided, therefore, to attack the problem of resistance and susceptibility in plants from the standpoint of the nutrition of the parasite, using the brown rot organism of stone fruits, *Sclerotinia cinerea*, as the experimental organism. The first paper in this series dealt with the vitamine requirement of the fungus (**52**), the second with its relations to the pectic substances of the host (**53**); the present paper deals with the composition of certain varieties of plums, and the changes in composition brought about during the process of rotting by the fungus.

Previous work

Cook and Taubenhaus (**16, 17**) found that a great many fungi are very sensitive to tannin, and they believed that this could be a limiting factor in their ability to attack certain plants. In general, parasites are more sensitive to tannin than saprophytes.

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They demonstrated an oxidizing enzyme that produces tannin from gallic acid, and found that the abundance of this enzyme in fruits is correlated with their resistant properties. BASSETT and THOMPSON (3) studied this enzyme still further. KNUDSON (37) found that *Aspergillus* and *Penicillium* can utilize tannin as a source of carbon, by means of the enzyme tannase. That genera and even species within a genus vary greatly in their sensitiveness to tannin was shown by COOK and WILSON (18) in studying the chestnut blight. In the case of *Sclerotinia*, VALLEAU (46) failed to find any correlation between tannin content and resistance in plum varieties.

In recent years considerable attention has been given by investigators to the relation between the H-ion concentration of the soil and that of the plant, and between the latter and its resistance properties. In 1912 COMES (15) announced that the wheats that were more resistant to rust had more acid saps, and that fertilizers which would increase the acidity of the sap would convey added immunity to the plant. Although others have since failed to corroborate these statements, positive correlations have been established between the acidity of the soil and the occurrence of potato scab (23, 24), between the acidity of grape saps and their resistance to disease (2), between the H-ion concentration of soil and that of the plant juice (14, 45), and between spinach mosaic and the PH value (27). On the other hand, a lack of correlation between the acidity of the host and its resistance properties has been found in the case of potatoes toward *Pythium debaryanum* (32), *Phytophthora infestans* (35), and *Chrysophlyctis endobiotica* (50). WAGNER (47, 48) noticed that certain plants increased their acidity when infected with bacterial pathogens. The acidity returned to normal after a brief period, unless the plant were unable to withstand the attack, in which case there took place a sudden fall in acidity much below normal, the death of the tissue, and then a post mortem rise in acidity. The relation of H-ion concentration to the metabolism of fungi and bacteria has received some attention (25, 38, 49). SCHMIDT and HOAGLAND (41) give an extensive bibliography on the relation of bacteria to the reaction of the medium. In general the fungi have been found to be less sensitive than bacteria to the reaction of the medium, and hence fewer

instances are known where the reaction of the sap is a controlling factor in a fungus disease. In the case of *Sclerotinia cinerea* no measurements have been made of its relation to the P_H value of the medium, except some rough titrations made by COOLEY (**19**) on cherry juice, which showed that this fungus can grow and sporulate through a considerable range of acidity and alkalinity.

Many investigators have sought for the mechanism by which fungi penetrate through the tissues of the host plant. Without going into the voluminous literature on this subject, it may be said that several different ways have been found: (1) by mechanical pressure, (2) by enzymes which dissolve either the cell walls or the middle lamellae (**12, 13, 19, 46, 53, 54**), and (3) by toxic substances other than enzymes, especially oxalic acid (**6, 7, 9, 12, 13, 19, 42, 46, 53, 54**). COOLEY found oxalic acid produced by *S. cinerea* in small amounts, and VALLEAU demonstrated that solutions of this acid would disintegrate tissues, probably by removal of calcium from the pectic material of the middle lamella.

The changes in composition which tissues undergo when rotted by organisms, and the differences in composition between resistant and susceptible varieties, have received considerable attention. One of the earliest of such studies was by BEHRENS (**4**), who used a number of fungi, among them *Sclerotinia fructigena*, on apple. REED (**39**) found that *Glomerella* not only decreases the acidity of apples and of synthetic media, but actually makes the latter alkaline. HAWKINS (**28**) found that *Glomerella cingulata* grown on peaches could hydrolyze and assimilate the pentosans, as well as utilize the monosaccharides, but that *S. cinerea* could not utilize the pentosans of the peach (**29**). In the latter case there was an increase in titratable acidity during the rotting. In the case of potato tubers infected with various species of *Fusarium* (**31**) there was a decrease in sucrose, reducing sugars, pentosans, galactans, and dry matter; an increase in crude fiber, due to its formation in the hyphae of the fungi; and no change in the starch and methyl pentosans. BISBY (**6**) and EDSON (**21**) also reported no effect on the starch of potato tubers by certain fungi, and suggest that potato starch could be made from rotted tubers. VALLEAU (**46**) examined many plum varieties as to their content of tannin, but

could find no correlation between this factor and their resistance to brown rot. CULPEPPER, FOSTER, and CALDWELL (**20**) made detailed analyses of apples infested with *Sphaeropsis malorum*, and found that the rotted fruit had undergone considerable loss in dry matter, a loss in alcohol-ether-water-alcohol extractives, an increase in protein nitrogen and in protein phosphorus, a transfer of minerals from the insoluble to the soluble fraction, a loss in total sugars, mostly in the monosaccharides, a decrease in titratable acidity, and a marked increase in alcohol. Starch was not affected.

STEVENS and HAWKINS (**43**) adopted a procedure that elucidates the progressive changes during rotting, by analyzing (1) the fresh strawberry fruits, (2) the sound fruit after storage under the same condition as the inoculated fruit, and (3) the fruit inoculated and rotted by *Rhizopus nigricans*. These three samples show the parallel changes in sound and infected fruit. They found that the acids in the sound fruits decreased, probably due to respiration, and that the acids decreased to a less extent in the rotted fruit. The authors believed this to be due to an interference with the tissue respiration by the fungus and not to the production of ammonia. Sucrose, reducing sugars, and dry matter decreased more rapidly in infected than in sound fruit. The fungus causes the tissue to soften and to become watery, but whether this is due to the death of the cells or to an anesthetic effect is still an open question. STEVENS and MORSE (**44**) reported that in the end rot of cranberries there is a marked decrease in sugars, while the proximate constituents remain fairly constant. The protein, fiber, and ash, however, show such relative increases as would be expected from the loss of dry matter by respiration. GIDDINGS (**22**) and RUSSELL (**40**) reported the inauguration of studies on apple leaves and on potato tubers, respectively, to determine the chemical basis of resistance, but they offer no conclusions as yet.

Recently several papers have appeared which deal with the nitrogen distribution of diseased plant tissues, and which promise to furnish a new line of attack on these problems. BONCQUET (**10, 11**), working with the mosaic disease of tobacco, *Streptococcus solani* on potato, and *B. morulans* on beet leaves, reported that nitrites and ammonia were invariably found in diseased but never

in healthy parts. The amount of nitrites was proportional to the intensity of the pathologic condition. The reducing organisms were mostly in the vascular tissues. The reduction of the nitrates brought about nitrogen starvation, with a consequent yellowing and distortion of the affected tissue. In a field where potatoes had been grown continuously for fifteen years, nearly every vine was affected with nitrogen starvation, although the soil contained an abundance of nitrates. In tobacco mosaic it was observable that the plants tended to oppose these chemical forces both by morphological and by physiological means; thus the secondary organs were reduced in size, more water was transpired, and the oxidizing enzymes showed greater activity.

JODIDI, MOULTON, and MARKLEY (34) made a detailed dissection of the nitrogen constituents of spinach mosaic, and found evidences of denitrification, due to the production of nitrites and their subsequent action on amino nitrogen groups. In cabbage mosaic (33) a similar condition was found, hence nitrogen starvation is believed to be the cause of the abnormal appearance of the leaves in these diseases. It is to be regretted that BONCQUET and BONCQUET give none of their methods of analysis, nor any data whatsoever in their papers.

Material

Five varieties of plums, grown at the University Fruit Breeding Farm at Excelsior in 1920,[2] were selected for the work. Three of them show marked resistance to the attacks of the brown rot fungus, while the other two are very susceptible. Samples were picked at three stages of growth: (1) when half grown, (2) when fully grown and just beginning to ripen, and (3) when fully ripe, but still on the tree. In most cases each sample was divided into three portions. One portion was analyzed immediately, another was inoculated with a pure strain of *Sclerotinia cinerea* and placed in a moist chamber to rot, and a third portion was placed in a moist chamber without inoculating and left for the same length of time as the corresponding inoculated portion. The inoculations were made by injecting a suspension of spores with a hypodermic syringe into the

[2] Acknowledgments are due to Dr. M. J. DORSEY for assistance in obtaining the material.

tissues of the plums, after the latter had been sterilized with mercuric chloride. The plums were left to rot as long a time as was practicable, which was usually from five to seven days after all the tissue had turned brown to the stone. The same degree of rotting was not obtained in all cases, since this cannot readily be judged.

Methods

PREPARATION FOR ANALYSIS.—In preparing the samples for analysis the stones were removed, the pulps frozen in an ice and salt mixture for three hours, ground in a food grinder, and pressed in a hydraulic press. All manipulations were maintained as uniform as possible throughout the series. The expressed juice was then used for all the subsequent analyses. HARVEY (**26**) has shown that, in order to obtain the true P_H of a juice, the latter should be expressed without freezing, since the freezing precipitates certain proteins and thus changes the H-ion concentration. This fact had to be ignored in the present instance, however, since the determination of the other solutes must be made on juice from frozen tissue, and since the amount of material available was not large enough to admit of two samples of juice being taken in each case. It might be of interest to record the results of a single test of the effect of freezing. The material was some seedling plums about one-third grown.

	Frozen	Unfrozen
Percentage of pulp obtained as juice	70	60
P_H	1.67	1.48

SPECIFIC GRAVITY.—The specific gravity was obtained by means of a Westphal balance, after the juice had stood at least an hour.

TITRATABLE ACIDITY.—Because of the high pigmentation of the juices soon after expression, titration by means of an indicator was impossible; hence the electrical conductivity method was employed. The peak of the curve could be read with an error of about ± 0.3 cc. 0.1 N NaOH.

HYDROGEN-ION CONCENTRATION.—The electrometric method was used for determining the P_H of the juices. Considerable trouble was experienced with the poisoning of the electrode by the

juices, but with care in the renewal of the platinum an accuracy of 0.3 millivolt was obtained.

TANNIN.—The Procter-Löwenthal method as detailed in the Official Methods (1) was used.

OXALIC ACID.—In the case of the juice, 40 cc. were treated with 80 cc. of 95 per cent alcohol, filtered, washed, and made up to 200 cc. Aliquots of this were used for oxalic and for malic and tartaric acids. For oxalic the alcohol was evaporated, the material neutralzed with ammonia, acidified with acetic acid, filtered if necessary, treated with calcium acetate in the hot, the calcium oxalate filtered off, and titrated with permanganate. The precipitate no doubt was impure, as it adsorbed some coloring matter, but a satisfactory end point was obtained, and the results are at least comparative. In the case of the residue, 10 gm. was digested with 75 cc. of 0.8 per cent hydrochloric acid for one hour, to liberate any oxalate existing in the residue in the form of the calcium salt. The extract was treated as in the case of the juice.

NITRITES.—The qualitative test with α-naphthylamine and sulfanilic acid was used.

PROTEIN AND NON-PROTEIN NITROGEN.—Trichloracetic acetic acid was used as the protein precipitant. BLISH's (8) copper hydrate method was tried in comparison with the trichloracetic acid. Since the latter gave the same results and is simpler to use, it was adopted. Twenty cc. of juice plus 20 cc. of 25 per cent trichloracetic acid were allowed to stand overnight, and the nitrogen in a filtrate determined. In the case of the residue a weighed sample was extracted with boiling water, and the filtered extract treated with the protein precipitant. It was realized that in the case of the residue two factors for precipitating proteins were used, heat and the trichloracetic acid. This fact might give incomparable results on the two sets of samples, but there was no other apparent way of getting the data on the residue.

MALIC AND TARTARIC ACIDS.—The optical method (51) for obtaining these two acids in the same solution was used, since it was thought possible that both were present, although BIGELOW and DUNBAR (5) found only malic. If only one of the two acids be present when this optical method is used, the rotation readings

should indicate zero for the one absent. In applying the method to the plum samples, the results in general did give zero values for tartaric. In some cases, however, positive values were obtained, and in others even negative values. Since these anomalies indicated the presence of some interfering substance, it was found necessary to reject all the data on these two acids; hence they are not presented here, although it was deemed well to record this failure of the double polarization method on plum juices.

Experimental data

CHARACTERISTICS OF ROT.—In table I are listed the varieties of plums used, the abbreviations for these varieties used throughout this paper, the dates of taking samples, and the length of time required for rotting in each case. Probably the same degree of rotting was not obtained in all cases, but the relative rates of progress of the fungus attacks are no doubt fairly well indicated

TABLE I

SAMPLING DATA FOR VARIETIES OF PLUMS USED

VARIETIES	ABBREVIATIONS USED	STAGE I, HALF GROWN		STAGE II, FULLY GROWN*		STAGE III, RIPE	
		Picked	Days required to rot	Picked	Days required to rot	Picked	Days required to rot
Resistant							
Burbank × Wolf 9.....	B × W9 or 9	July 12	15	Aug. 25	17	Aug. 31	15
Burbank × Wolf 16.....	B × W16 or 16	July 12	13	Aug. 20	18	Aug. 28	15
Abundance × Wolf 30.....	A × W30 or 30	July 12	13	.....	.....	.....	.....
Susceptible							
Compass.....	C	July 12	9	Aug. 3	11	Aug. 20	12
Sand Cherry × Formosa...	SCF	July 12	10	Aug. 3	13	Aug. 11	12

by the figures. It is clear that the varieties listed by the horticulturists as resistant are considerably more slowly rotted than are the susceptible varieties. There is more difference in this respect in the earlier than in the later samples. This is in accordance with the commonly observed characteristics of fruit diseases, that in general they become less resistant as maturity approaches. Because of this, the first set of samples was observed more keenly than the following ones, and some interesting points were noted. In connection with the rate of spread of the rot, the time required for

the surface of the fruits to become brown was recorded, as shown in table II. These figures show the same slower rate of rotting of the resistant varieties.

TABLE II

BEHAVIOR OF VARIOUS PLUM VARIETIES TOWARD ROTTING ORGANISM AT FIRST STAGE OF GROWTH

VARIETIES	DAYS REQUIRED FOR SURFACE TO BECOME BROWN	DAYS REQUIRED FOR COMPLETE ROTTING	RELATIVE ABUNDANCE ON SURFACE OF	
			Hyphae	Spores
Resistant				
Burbank×Wolf 9.....	13	15	++	+
Burbank×Wolf 16.....	7	13	+++	+
Abundance×Wolf 30.....	6	13	+	+++
Susceptible				
Compass.....	5	9	+++	+++
Sand Cherry×Formosa..	5	10	+++	+++

FIG. 1

FIG. 2

FIGS. 1, 2—Fig. 1, dish of Burbank×Wolf 16 plums at end of rotting period in half-grown stage of growth (table II), showing abundance of hyphae on surface and relative scarcity of spore tufts; fig. 2, dish of Abundance×Wolf 30 plums at end of rotting period in half-grown stage of growth (table II), showing abundance of sporulation and scarcity of hyphae on surface.

Another varietal difference was the character of the aerial portions of the fungus, especially as to the relative abundance of spore tufts and of hyphae. These data are recorded in table II. Although the differences among the varieties were well marked, they are not correlated in any striking way with resistance

characters. The susceptible varieties show more abundant aerial growth of both sorts than do the resistant. Possibly the amount of surface growth is dependent on the vigor of the subsurface growth, and the latter is no doubt less in the resistant varieties (figs. 1, 2).

Another varietal characteristic was the relative firmness of the rotted fruit. The sound fruit of the resistant varieties was somewhat more firm than that of the susceptible, particularly in the ripe stage. After rotting, the differences were even more marked. Although this fungus causes what is usually called a firm rot, the rotted fruit of the *C* and *SCF* varieties was almost watery in some instances; while the resistant varieties maintained a firm or even hard texture. This phenomenon may have to do with the character of the pectins, as has been suggested by others. The pectin relations of hosts and parasites offer a fruitful field of investigation (53).

Chemical analyses

Instead of presenting the analytical results in tabular form, which would be rather involved and cumbersome, they are given in the form of charts (figs. 3–7). The data could conceivably be grouped in many different ways, so as to show (1) the comparison of the fresh fruit of the several varieties; (2) the progressive changes during the ripening process; (3) the changes involved during storage in the laboratory, both with and without the action of the fungus; and (4) the effect of the rotting process. This would mean four different groupings of the data in four sets of charts. It was decided to limit this to two groupings. The first set, figs. 3 and 4, bring together side by side the data showing the change in composition of the samples during the storage and rotting in the laboratory. The fresh samples in each case are designated *a*, the sound samples stored in the laboratory without inoculation *b*, the rotted samples *c*. In these charts it is easy to follow the changes brought about by the rotting, and the changes taking place during the three stages of growth, by comparing all the *a* samples, the *b* samples, and the *c* samples in each variety.

The second set, figs. 5–7, bring together side by side the data for comparing the various varieties, that is, the *a* samples for all

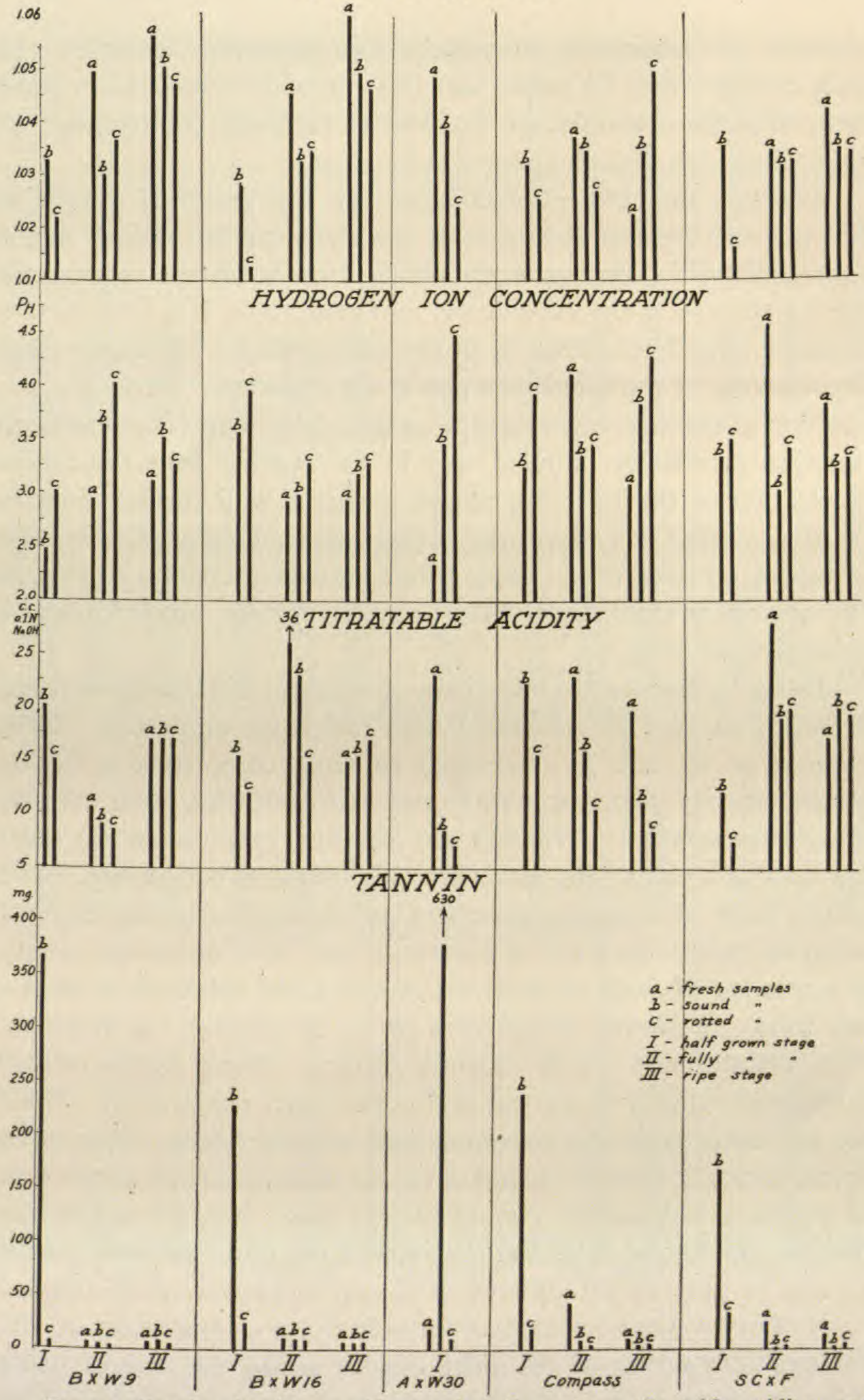

FIG. 3.—Graphs showing specific gravity, P_H values, titratable acidity, and tannin content of juices of all plum samples; figures for titre and tannin are on basis of 10 gm. of juice.

varieties are assembled, then the *b*, and then the *c* samples. In each case the three (in some cases two) resistant varieties are given first, then the non-resistant, in order to facilitate comparison. A brief discussion of each factor will be given.

SPECIFIC GRAVITY.—Referring to the top group of graphs in fig. 3, it will be seen that in most cases the specific gravity of the expressed juice decreases from *a* to *b*, that is, in the sound fruit during storage in the laboratory, and that there is a still further decrease from *b* to *c*, that is, in the rotting fruit. In many cases the decrease in the rotted samples is very marked. Probably respiration consumes sugar in the sound fruit with a consequent decrease in density of juice, and in the rotting fruit the added respiration of the invading fungus causes a still further drop in density. STEVENS and HAWKINS (43) noted a similar phenomenon in rotting strawberries. There is one marked exception to this, in the case of the third stage of the Compass variety, but this may be an analytical error.

In fig. 5 there are indications of varietal differences in juice density that may be correlated with resistance properties. Thus varieties 9, 16, and 30 (resistant) in most cases have a higher specific gravity than varieties *C* and *SCF*, and this holds even in the rotted samples. Whether the osmotic pressure of the host sap may be a controlling factor for this fungus is not known. The writers know of no measurements of its tolerance to strong nutrient solutions except the work of HAWKINS (30), who tested the ability of a number of fungi to grow on concentrated solutions of sugars and salts. *S. cinerea* would grow on 2.4 M glucose, 1.4 M potassium nitrate, and 0.6 M calcium nitrate. These figures would correspond roughly to 43, 14, and 9 per cent, respectively, which are far higher than any concentrations of fruit juices. It is to be noted, however, that HAWKINS gives no information as to the rate of growth at the various concentrations used; hence it is possible that the differences found in the saps of the plum varieties might account in part for the differences in rate of growth of the fungus.

HYDROGEN-ION CONCENTRATION.—In fig. 3 a comparison of the changes in reaction of the juice can be made, the values being given in terms of P_H. No consistent and striking differences are

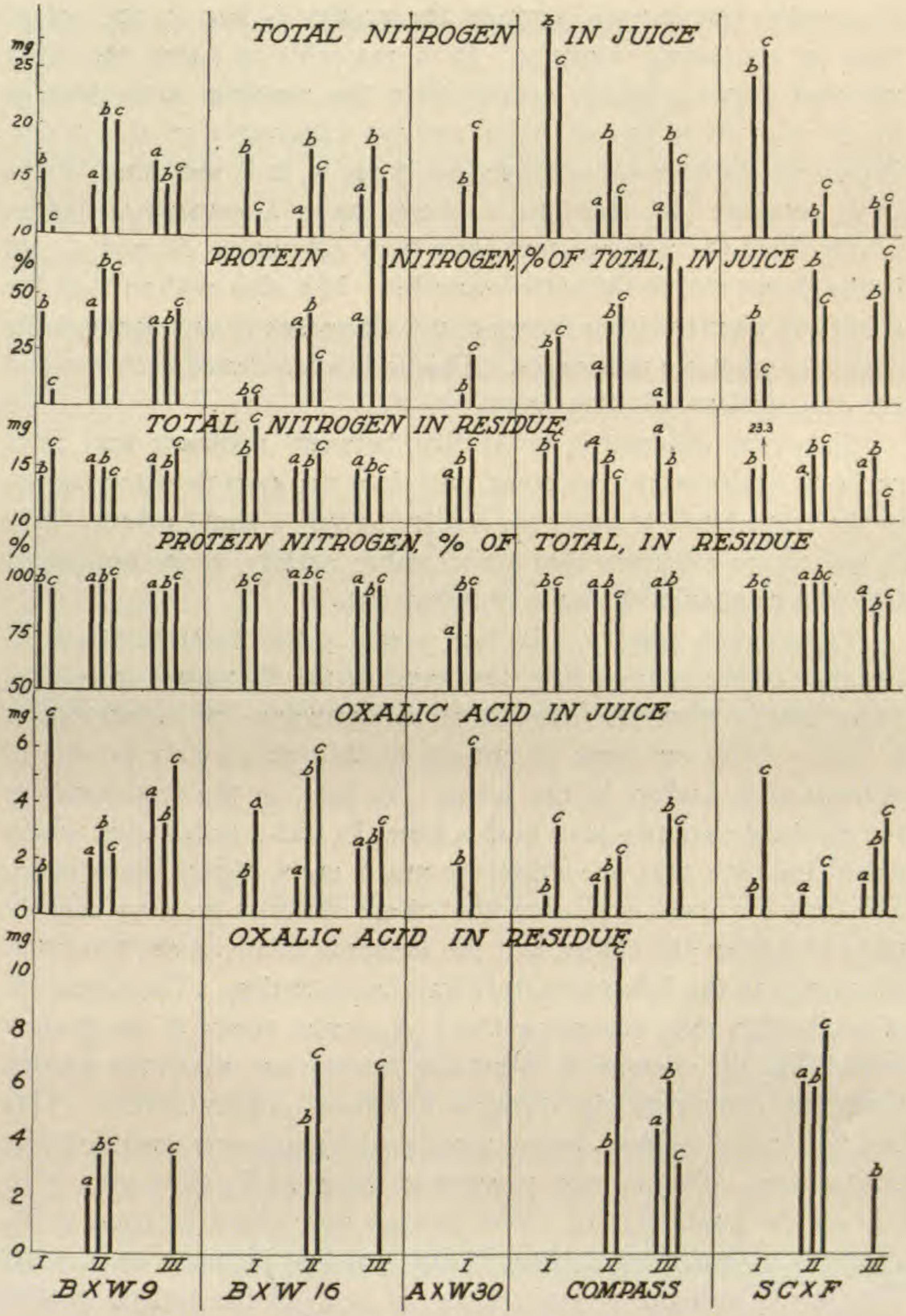

FIG. 4.—Graphs showing total nitrogen, protein nitrogen, and oxalic acid in juices and residues of all plum samples; figures are on basis of 10 gm. of juice and 1 gm. of residue.

discernible, but in most samples the acidity is less in the rotted than in the sound samples. In a majority of cases the fresh material shows a higher acidity than the material after storage in the laboratory, but the data are not conclusive on this point. When the analyses are arranged as in fig. 5, it is seen that in the fresh samples the resistant varieties have a somewhat higher acidity that the non-resistant, but such differences do not obtain in the stored and in the rotted samples. It is also evident that the acidity in plums neither increases nor decreases to any appreciable degree as ripening progresses. This is in accordance with most of the observations on other fruits.

Since the differences in acidity between resistant and non-resistant varieties are not great, and since the growth of the fungus in the tissue tends to lower the acidity to only a slight extent, there is not much evidence that unfavorable acidity is an important factor in resistance of plums to brown rot.

TITRATABLE ACIDITY.—In figs. 3 and 5 the determinations of the titre of the juices follow the trend of the P_H values in reverse order, that is, when the titre is high, the hydrogen-ion concentration is high. There are some exceptions to this, which may be due to differences in buffers in the juices. In fact, in the fresh samples the resistant varieties have both a lower P_H and a lower titre, which would indicate that the acids are much more highly dissociated. The data for oxalic acid show that these varieties do have slightly more of it than the others, but the amounts involved are too small to amount to the differences in H-ion concentration. The character of the buffers may determine this. A careful study of the graphs shows that the changes in titratable acidity are relatively greater than the corresponding changes in H-ion concentration. This becomes more evident when numerical values are used for the comparison. The average percentage decrease in titre from *b* to *c* in all the samples is 17. The average percentage increase in P_H values is 9 (assuming a theoretically possible increase to $P_H = 7$). This would indicate a consumption of acid by the fungus, rather than a production of buffer, in modifying the reaction during rotting. It was hoped that the malic acid determinations would give direct evidence on this point. These determinations will be

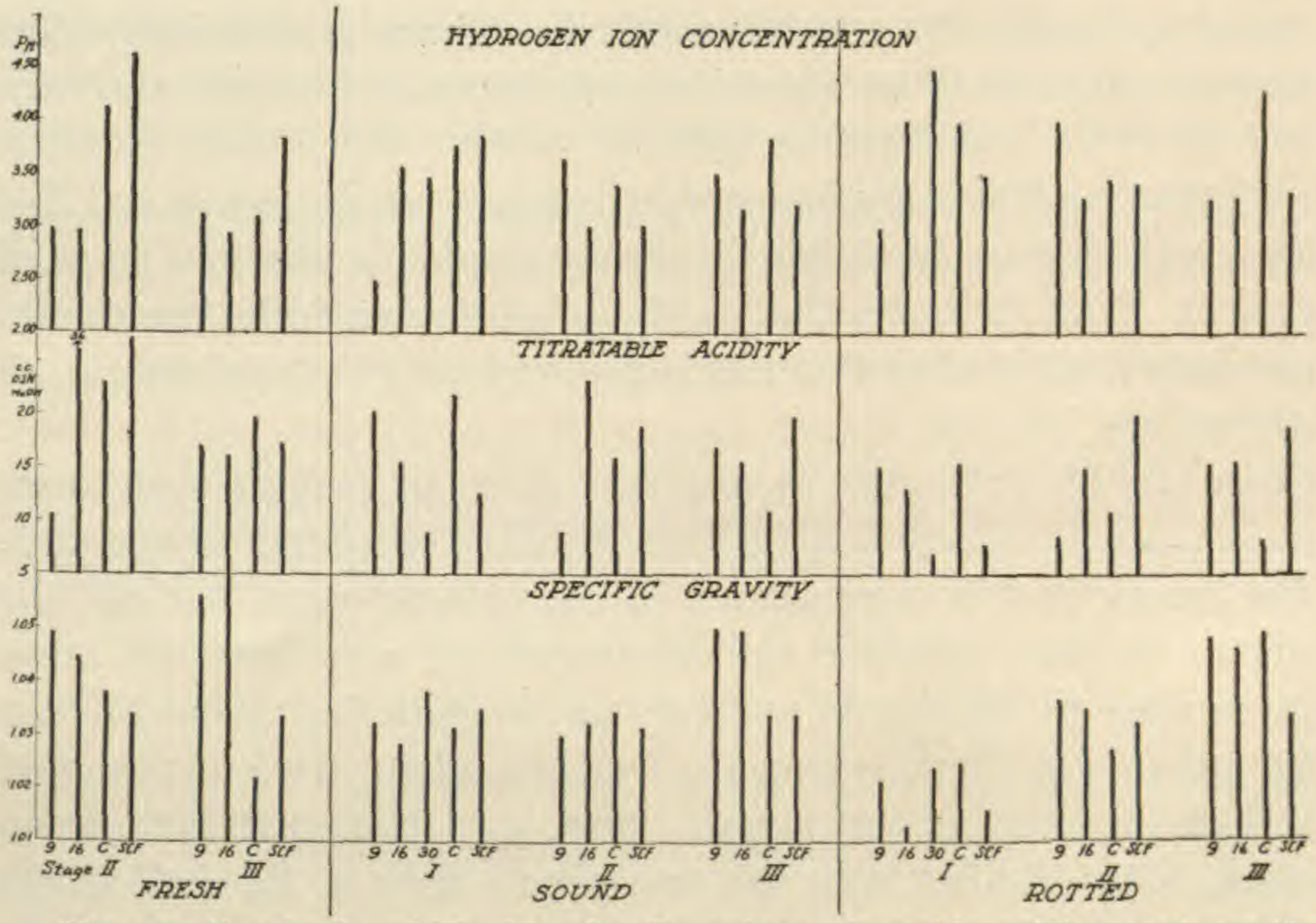

FIG. 5.—Graphs in which data for P_H values, titratable acidity, and specific gravity are assembled to bring together resistant and non-resistant varieties of plums for direct comparison; varieties 9, 16, and 30 are resistant (see table I and fig. 3).

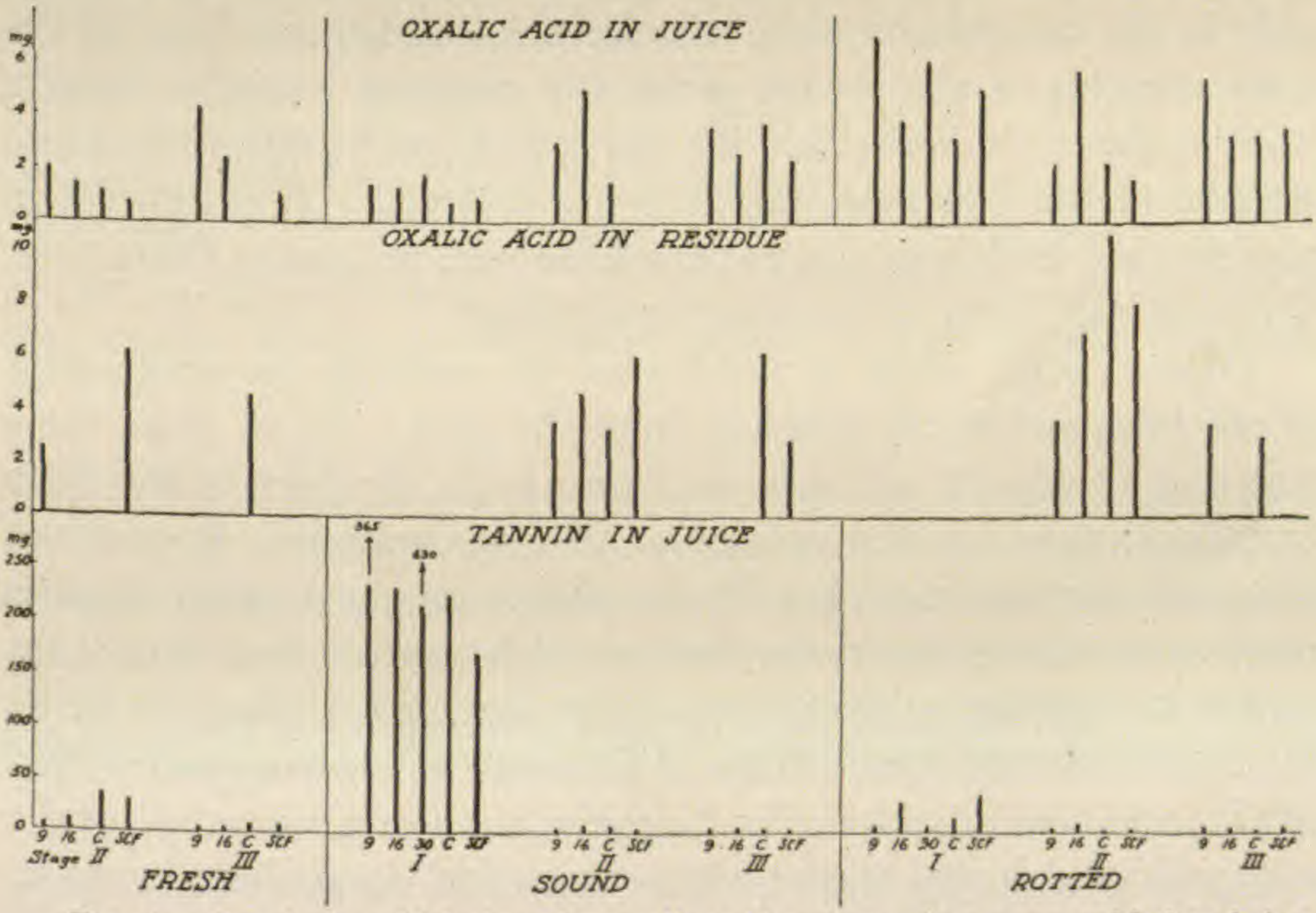

FIG. 6.—Graphs in which data for oxalic acid and tannin are assembled to bring together resistant and non-resistant varieties of plums for direct comparison (see table I and figs. 3, 4).

attempted again by another method. Citrus mottle leaf offers another example of an interesting discrepancy between the titre and the H-ion concentration (**36**).

TANNIN.—The conspicuous fact brought out in figs. 3 and 6 is the great increase in tannin in the *b* samples in the first stage of growth. This indicates that in these half-grown fruits the tannin increases rapidly after the fruit is picked from the tree, but that if the fruit is infected by the fungus the tannin does not increase. COOK and his colleagues (**3, 16, 17**) report an enzyme that forms tannin rapidly either when the fruit is picked or when it is wounded. The former fact is corroborated in the present work, but not the latter. In fact, infection by the fungus not only does not cause an increase in the tannin content, but in varieties *C* and *SCF* in fig. 3 there is a decrease over the fresh samples. Two facts should be kept in mind in this regard: first, that the great increase in tannin after picking from the tree occurs only in the half-grown stage of growth; and second, that the decrease in tannin in the rotted samples is noticeable only in the two later stages of growth, since unfortunately in the first stage the fresh sample was analyzed only in the case of $A \times W30$. In fig. 6 it can be seen that in the fresh samples of the second stage the resistant varieties have a lower content of tannin than the susceptible, and that in the sound samples of the first stage the facts are reversed. It is difficult to perceive any facts that can be correlated with resistance characters. VALLEAU (**46**) came to the same conclusion.

OXALIC ACID.—Figs. 4 and 6 give the analyses for oxalic acid in the juice and in the residues from the juice. In all cases there was a small amount of oxalic acid present in the juice of the fresh fruit, as judged by the reduction of permanganate. It does not average over 0.02 per cent of the juice. In most cases there is more oxalic acid in the *c* than in the *a* or *b* samples, indicating that during the rotting a production of the acid takes place. This is in accordance with the findings of COOLEY (**19**), who reported that oxalic acid was produced by *Sclerotinia*. The amount of oxalic acid produced in the rotted plums, however, seems insufficient to exert any very marked solvent power on the tissues. Although the data for oxalic acid in the residues are very incomplete, they indicate

the same general trend as do those for the corresponding juices. There are some indications in fig. 6 that the resistant varieties have a higher oxalic acid content than the susceptible, both with and without fungus action in the tissues. If this is found to be the case in future analyses, it may constitute some new evidence on the question of resistance properties.

NITROGEN DISTRIBUTION.—In figs. 4 and 7 are presented the results of the analyses for total and for protein and non-protein nitrogen. There is great irregularity in the quantities of total nitrogen in the juice in the three groups of samples, so much so

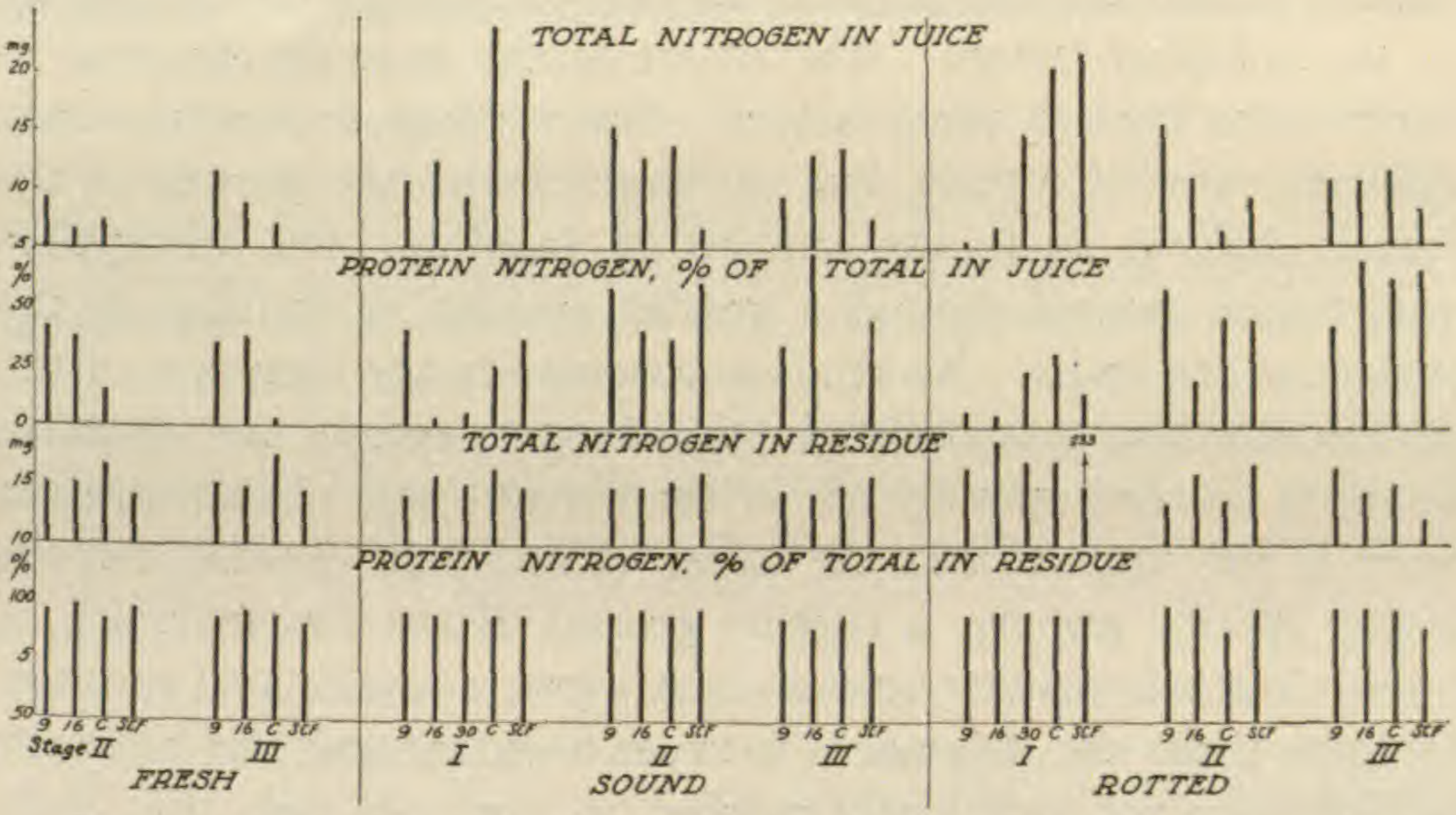

FIG. 7.—Graphs in which data for total and protein nitrogen are assembled to bring together resistant and non-resistant varieties of plums for direct comparison (see table I and fig. 4).

that it is difficult to see any definite trend to the graphs in fig. 4. In the case of the residue, there is some evidence that the rotted samples (*c*) have a greater amount of total nitrogen than the sound (*b*). This is no doubt due to the facts that a far greater proportion of the nitrogen is in protein form in the residue, and that the building of fungus protein makes this protein nitrogen still higher in the residues of the rotted samples. No definite trend nor significance can be seen in the data for the protein nitrogen in the juice.

NITRITES.—No test for nitrites was obtainable in any of the samples. The disturbance of the nitrogen nutrition of the host

cannot be a factor in this disease as it seems to be in others (**10, 11, 33, 34**). No varietal differences in the nitrogen content and forms of nitrogen are discernible in fig. 7.

Discussion and summary

The laboratory inoculations recorded in this paper corroborate the field observations on plum varieties as to their relative resistance to the brown rot fungus, *Sclerotinia cinerea*. In the field an important factor in resistance is the thickness of the skin. In the present studies this was eliminated by injecting the spores into the tissues, so that the differences in the rate of rotting were due mostly to physiological factors. The object of the investigation was to throw some light on these factors. The varieties showed not only different rates of rotting, but the character of the growth of the fungus differed as to the amount of fruiting. The susceptible varieties in general showed a greater amount of fruiting on the surface of the fruits. No study is recorded in the literature of the factors affecting sporulation in this fungus, except the vitamine relations touched upon by one of the writers (**52**). In the present work it was noticed that the juices of resistant varieties have a higher specific gravity, a slightly greater H-ion concentration, a lower titratable acidity, and a slightly greater oxalic acid content. In these items the differences between resistant and non-resistant varieties are not sufficiently marked to convince one that they constitute the chemical basis of resistance. Culture work with *Sclerotinia*, using fruit juices in which the various factors can be varied and controlled, will no doubt throw considerable light on the question.

When the fungus rots the plum, some well marked changes in composition take place in the tissues. The juices show considerable decrease in specific gravity, a decrease in true acidity, a decrease in titratable acidity that is of greater magnitude than the decrease in true acidity, and an increase in oxalic acid content. The fungus in some way prevents the production of tannin that takes place in the green fruit after it is picked from the tree. The fungus converts a portion of the non-protein nitrogen of the host into protein nitrogen in its own mycelium.

Nitrites could not be detected in any of the samples. They are probably not a product of the rotting by this fungus. No hypothesis can be suggested as yet for the chemical and physiological basis of resistance in the brown rot of stone fruits.

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VARIATIONS IN CYTOLOGY AND GROSS MORPHOLOGY OF TARAXACUM

I. CYTOLOGY OF TARAXACUM LAEVIGATUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **293**

PAUL BIGELOW SEARS

(WITH PLATES IX, X)

Introduction

These investigations are the outgrowth of a study, begun in 1914 at the instance of the late Professor C. E. BESSEY, of parthenogenesis in *Taraxacum vulgare* (Lam.) Schrk. and *T. laevigatum* (Willd.) DC. These two species are the common ones in central United States, being respectively designated as *Leontodon Taraxacum* L. and *L. erythrospermum* (Andrz.) by BRITTON and BROWN (3). In 1917 the existence of ameiotic parthenogenesis in both species was confirmed (**23**) and certain pollen abnormalities briefly described. These abnormalities have invited more critical analysis as a means of throwing light upon certain phases of variation and degeneracy, and likewise upon the problem of synapsis. The study of non-cytological variations has been directed largely to leaf characters. This is due to frequent references in the literature of *Taraxacum* to "polymorphy" and to the wholesale erection of species (cf. Index Kewensis **10**).

Maturation phenomena in embryo sac and pollen have been homologized and found to be highly fluctuating. The fluctuations, instead of being anomalous, seem plainly to indicate variations in the duration and relative intensity of (1) chromosome individuality, (2) sex, (3) polarity.

Synapsis in the sense of chromosome pairing is expressed with varying degrees of vigor and quite without reference to doubleness of the spireme thread.

Leaf variation within the species is shown by quantitative studies to be a matter of senescence and rejuvenescence. The rôle of environment is secondary. The interplay of senescence and

environment is sufficiently potent to have produced from a few valid species transient forms fitting many published descriptions given specific or varietal rank. Attempts to correlate degree of leaf dissection with internal anatomy have not succeeded, but senescence and dissection are accompanied by an increase in carbohydrates as compared with nitrogenous substance.

Numerous colleagues, and in particular Professor JOHN H. SCHAFFNER of Ohio State University, have given generous help whenever called upon. It seems proper also to express appreciation of the difficulties confronting earlier workers whose conclusions, and in some cases whose observations, have not been confirmed here.

IDENTITY OF SPECIES.—This plant, known as the "red-seeded dandelion," is without doubt cosmopolitan. It is listed as *T. erythrospermum* Andrz. by GRAY (19), and as *Leontodon erythrospermum* (Andrz.) Britton by BRITTON and BROWN (3). SEARS (23), as well as SHERFF (25), for reasons that will appear later, has accepted the decision of HANDEL-MAZETTI (6) with respect to nomenclature.

BRITTON employs the generic name of *Leontodon* upon the authority of LINNAEUS' Sp. Pl. 798 (14). HANDEL-MAZETTI in his monograph presents the tabulation and critique of authorities upon which he bases his selection of the name *Taraxacum.* It is convincing. Incidentally he makes clear why *Leontodon Taraxacum* L. cannot stand as a valid species name because of incomplete diagnosis.

The species with which we are concerned here is discussed by the same authority as follows:

> Was den Namen anbelangt der bereits auf die diversisten Pflanzen angewendet wurde, so konnte ich mich an dem schön fruchtenden Originalexemplar Willdenows von seiner Bedeutung überzeugen. . . . . *Leontodon laevigatus* Willdenow, 1800; *Taraxacum laevigatum* De Candolle, 1813; *T. erythrospermum* Besser, 1822.

The last citation (quoted from the tabulation of synonyms on p. 109) is doubtless the one upon which GRAY and BRITTON base their specific nomenclature. BRITTON'S citation for the species is ANDRZEJOWSKY in Bess. Enum. Pl. Vilh. 75. 1821. For further critical discussion the careful paper by SHERFF (25) may be cited.

REPRODUCTION.—The more important studies of reproductive physiology and morphology in *Taraxacum* are so well known and so frequently cited that they require only brief comment here.

1896. SCHWERE (**22**): embryo arises from egg in sac of typical appearance.

1900. ANDERSSON and HESSELMAN (**1**): pollenless arctic specimens produce fruit, parthenogenesis suspected.

1903. RAUNKIAER (**18**): "Species Danicae Taraxaci castratione agamice propagari demonstratum est; species omnes Taraxaci semper parthenogenetice propagari verisimile est."

1904. KIRCHNER (**13**): pollen of *Taraxacum* never found germinating upon *Taraxacum* stigmas, although often abundant.

1904. MURBECK (**15**): confirms findings of SCHWERE, RAUNKIAER, and KIRCHNER. Finds embryos in unopened, pollenless flowers.

1904. JUEL (**11**): embryo sac maturation reduced to single division. Apparently no reduction, although prophase resembles heterotypic.

1905. JUEL (**12**): compares maturation phenomena of sexual *Chicoraceae* with those of *T. officinale* (*vulgare?*). Notes double thread in prophase of former but not in latter; former shows haploid number of bivalents in diakinesis, while *Taraxacum* shows diploid number (24–26) of univalents. These facts believed to favor the parasynaptic view of reduction. Following diakinesis in *Taraxacum*, nucleus is believed to elongate and chromosomes to split temporarily, the sequence being regarded as a shift from heterotypic to homotypic prophase. Pollen goes through reduction forming 12 or 13 bivalents in first prophase, but doubleness of spireme not observed.

1907. HANDEL-MAZETTI (**6**): noteworthy monograph of genus. Emphasizes genetic importance of parthenogenesis, but believes RAUNKIAER'S conclusion too sweeping. Shows clearly that pollen development must be highly variable.

1907. DAHLSTEDT (**4**): notes presence of numerous sterile seeds and surmises that normal eggs may be found in otherwise parthenogenetic heads. Also believes sexual species likely to exist.

1909. ROSENBERG (**20**): compares chromosome conditions of *T. officinale* (*vulgare?*) and *T. confertum*, finding in the latter a typical reduction from 16 to 8.

1910. IKENO (**9**): reports *T. platycarpum* Dahlst. to be sexual, while *T. albidum* Dahlst. (white-flowered) is not.

1912. SCHORBATOW (**21**): confirms previous findings for *T. officinale* Wigg. Takes liberal cognizance of cytological variation.

1913. OSAWA (**17**): compares in detail cytology of species studied by IKENO, agreeing in general with JUEL's conclusions. Finds a variable degree of pairing in pollen diakinesis of *T. albidum*, and besides normal maturation of tetrads, the formation of diads by "homotypic" division. Notes amitosis and supernumerary nuclei in pollen; also 16 and 8 chromosomes in sexual, 36 to 40 in parthenogenetic species. Parthenogenesis probably due to hybridization.

1917. SEARS (**23**): *T. laevigatum* as well as *T. vulgare* shows ameiotic parthenogenesis. The former generally gives higher percentage of sterile fruits, and both exhibit pollen abnormalities, including extrusion of chromatin, amitosis, and defective spindles.

1920. STORK (**26**): *T. laevigatum* is ooapogamous, and embryo sac maturation agrees in general with accounts of JUEL and OSAWA for other ooapogamous forms. Also 26 to 30 chromosomes found, but said not to split during the elongated phase which is believed to follow diakinesis.

RELEVANT CYTOLOGICAL PROBLEMS.—As suggested earlier, those of chief interest in connection with the present study are (1) the mode of synaptic pairing, and (2) cytological variation.

SYNAPSIS.—The conclusion of workers already quoted (**12, 17**), who have compared ameiotic species of *Taraxacum* with related sexual species, favors the parasynaptic interpretation of reduction division. Such conclusion is doubtless justified if the observations of prophase conditions upon which it is based are unexceptionable.

The development of thought upon the subject of synaptic pairing has been fully treated by numerous workers, the present trends of botanical opinion being fairly crystallized in papers by DIGBY (**5**) and SHARP (**24**). It is unfortunately true that various questions involved hinge upon observations made near the limit

of resolving power of the microscope. This introduces what should frankly be recognized as a potential source of error, and dealt with accordingly. In undertaking this study it was hoped that the more obvious sequence of events in a parthenogenetic plant might afford a check upon observations necessarily made under conditions of optical difficulty. This hope has at least been partially justified.

VARIATION IN CELL PROCESSES.—Cytologists have, through no fault of judgment, been so charged with the duty of learning the normal sequence of events in plants that as a rule they have given little attention to "anomalies." Even the most conservative theories of the cell as a physico-chemical mechanism must admit the likelihood of considerable fluctuation in its processes. WASIELEWSKI (**27**) emphasizes the phylogenetic continuity between mitosis and the types of amitosis produced by chloroforming meristem. Moreover, the results obtained by NATHANSOHN (**16**) in producing abnormal division types by the use of ether are highly suggestive, when viewed either in the light of modern theories of anaesthesia or of such work as that of BONNS (**2**). The latter has clearly shown a marked increase in proteolytic enzyme activity as a result of etherization. The experiments of HOTTES (**8**), demonstrating powerful effects of temperature change upon the spindle mechanism, are likewise significant. They become peculiarly so in connection with the intimate relation of temperature to enzyme activity.

Careful study and classification of variations in cell behavior have already yielded data of interest in genetics, and they may afford the clue to an isolation and analysis of the factors involved in cell behavior, which are now known, so far as they are recognized, by terms so general as to be noncommittal.

Procedure

All material studied was collected from plants which had been identified after fruiting. A wide range of fixing reagents was tested, including mixtures of absolute alcohol and glacial acetic acid in various proportions. The best results were obtained with a solution of two parts of alcohol and one of acid. Beautiful

preparations were obtained by the use of Flemming's stronger fixing fluid. In comparison with acetic alcohol material these showed little difference in nuclear condition, but inspection of the cytoplasm made it very evident that the electrolytes contained in Flemming's solution had caused violent coagulation of certain cell colloids. This circumstance should militate against its use in critical work. Acetic alcohol kills almost instantly, and contains two components whose effects are mutually corrective. From the theoretical standpoint of modern colloid chemistry it ought to be a very desirable reagent. Numerous experiments, as well as the variety of formulae which have been recommended by different workers, suggest strongly that failures with it have often been due to use of unsuitable proportions of the components.

Sections were cut from 6 to 12 μ in thickness, and were stained with iron-alum, alone and with counterstain, and with Flemming's triple solution. Drawings, unless otherwise noted, were made with Spencer camera lucida through Bausch and Lomb binocular equipped with no. 12 compensating oculars and 1.9 fluorite objective.

It should be noted that the use of the word synapsis in this paper has been limited to the matter of synaptic pairing. The term synizesis is used throughout to designate the balling of chromatin in early prophase.

Observations

SOMATIC DIVISIONS.—These were observed very frequently in all stages, in nucellar and other meristem. So far as can be determined, they present no unusual features. The chromosomes segment as curved rods from a fairly thick and quite uniform spireme, and are certainly about twenty-six in number.

MATURATION DIVISIONS.—The earliest stages that could be identified were marked by an enlarged nucleolus and not more than thirteen paired centers of chromatin aggregation. These are shown in figs. 1 and 2, and can fairly be construed as prochromosomes. JUEL (12) does not mention them, while OSAWA (17) describes them for both the species he studied as originating by an increase in size and number of the granules upon a linin meshwork,

that is, from a resting nucleus as conventionally described. Observations here lead to agreement with SHARP (**24**), as follows:

> While one easily gains the impression of separate chromatin granules connected by threads with another substance . . . . it seems more probable that the "chromatin granules" are merely the heavier portions of the alveolated and reticulated chromosomes, and that the lighter "supporting network" consists simply of the thinner portions of the same, together with the delicate anastamoses.

In portions of the thread as it enters synizesis, a curious partly paired, partly vacuolate-split appearance is discernible (fig. 3). It is stages of this sort which lend themselves as conveniently to one philosophy of nuclear division as another, and which should not be taken as pivotal until all other means of explanation have been exhausted. The optical difficulties attendant upon the close state of aggregation are very great here of course, necessitating thin sections and special technique.

Synizesis culminates in an extremely dense ball whose components are without doubt filiform. The embryo sac mother nucleus in fig. 4 is typical in every respect save that of size, being larger than usual. The loosening thread (dolichoneme) is fairly uniform at first (figs. 5, 6), and JUEL (**12**) is doubtless right in stating that any apparent nodes are optical effects due to foreshortening or crossing.

After the thread becomes rather evenly distributed through the nuclear cavity, its uniform appearance is altered by the advent of changes which are hard to explain except as fissions. Certainly they are quite different from (1) accidental or other juxtaposition of whole threads, or (2) the twisting together of limb and bight into a loop. Both (1) and (2) are to be seen in figs. 7 and 8, where they may be compared with the seeming fissions. Whatever the change that gives this appearance of duality, it is clearly not simultaneous throughout the thread. A priori, is there any reason why it should be? The unevenness of its origin perhaps may explain the failure of other students of parthenogenetic species of *Taraxacum* to observe anything suggesting a "double" thread. Careful inspection of OSAWA'S fig. 46 (**17**) shows that the phenomenon is by no means precluded there.

Instead of occurring uniformly, thickening of the thread seems to be accompanied by the beginnings of its segmentation. Figs. 9 and 10 show nodes whose structure is seemingly homogeneous. On the other hand, it is always possible to find some showing clearly a longitudinal duality, even culminating in a divergence or forking of the two attenuate ends. If this appearance of doubleness were visible in every node and the divergent internodes were not visible, one would be justified in seriously questioning the validity of the interpretation. It might then be simply the lateral shadowing normal to translucent cylindrical bodies. If, however, there is really duality, the separating plane in certain nodes must lie more or less parallel to the section and hence not be visible. This would account for those nodes whose appearance is homogeneous.

The nodes rapidly shorten and become truly homogeneous, only the bifurcate internodes remaining as evidence of the double origin of each chromosome. As in all chromosomes, there are occasional lateral projections in addition to the forking internodes, due doubtless to imperfect retraction of pseudopodia at some time during aggregation. The papers already cited (**12, 17, 26**) evince little proof of close attention to this stage, a circumstance doubtless due to its transient character. Figs. 11–13*b* show it in varying aspects. Fig. 12 suggests a rough correspondence between this phase and the so-called second contraction. Certainly the thread shortens greatly, and the chromosomes as they first cut apart are no longer peripheral, but in the nuclear interior.

The bifurcations at each end of the chromosome are not retracted at once, but may shift slightly in position. This gives the appearance of pseudopodia, generally four in number. STORK, OSAWA, and JUEL have all more or less plainly figured but not accounted for these pseudopodia. Fig. 14*b*, as those of the authors cited, shows that after the chromosomes drift to the nuclear membrane and become peripherally oriented, the quadruple projections tend to move to the side of the chromosome away from the membrane. These, with other irregular projections of earlier or later origin, may constitute the "fringe" referred to by JUEL and figured by OSAWA.

Counts at this stage, and also at the somewhat later one resembling diakinesis, show twenty-six chromosomes. This agrees

with JUEL'S (12) counts for *T. officinale*. The count registered by STORK for the species now under discussion is twenty-six to thirty. Since the latter takes no cognizance of fission at any time before metaphase, it is possible that his higher estimate is due to reckoning separated halves as units. It will be recalled that the somatic number is about twenty-six, and that there are about thirteen pairs of prochromosomes. Fig. 22 shows a normal reduction division of thirteen univalents at the homotypic plate. These facts all give the necessary assurance that in prophase we have the origin of the diploid number of univalents, unpaired, from a dual and therefore a split thread.

To summarize developments thus far, there is first the appearance of approximately thirteen (the haploid number) pairs of prochromosomes. The thread entering synizesis shows in places a doubleness unexplainable at present. The thread emerging from synizesis becomes very evenly distributed through the nucleus, and then shows what is interpreted as non-simultaneous splitting. By the time segmentation is reached splitting becomes indubitable, and the formation of twenty-six univalent chromosomes occurs by the lateral refusion of the two halves previously split apart.

In contrast with these findings it should be noted that JUEL, OSAWA, and STORK, working on parthenogenetic species of *Taraxacum*, all expressly state that the postsynizetic thread is single and remains so, and that the univalent chromosomes are single in composition. JUEL and OSAWA, working on sexual plants of the same or nearly related genera, report an obvious doubleness of the thread. Since there is no question of the duplex nature of bivalent chromosomes in sexual plants, these investigators conclude that the doubleness noted is due to synaptic pairing. The three workers cited agree that diakinesis is followed by the greatly elongated nucleus as mentioned. STORK, however, considers the chromosomes here to be unpaired, that is, unsplit because "there are certainly not upward of sixty." Accepting his maximum count of thirty as correct, one would scarcely expect to find more than sixty halves.

Comparison of figs. 12–15 with figs. 36–41 strongly suggests that the elongated nucleus is not the outcome of diakinesis, but a

divergent form of it. The chromosome forms in the two sets of figures show no interrelation, but a common origin at segmentation.

It will particularly be noted that connections between chromosomes persist in the beginnings of the elongated stage but not in diakinesis, making it quite unlikely that the former is a derivative of the latter, but not militating against the idea that both are derivable from late segmentation.

The nucleus in the elongated stage is often lobed, moreover, as in fig. 40, and constricted and binucleolate as in fig. 41. In short, the elongated nucleus with about twenty-six *X* and *Y*-shaped chromosomes must be regarded as part of a distinct sequence

TABLE I

Stage	Sequence *A*	Sequence *B*	Sequence *C*	Sequence *D*
Prochromosome	13 pairs	13 pairs	13 pairs	13 pairs
Synizesis	Normal	Normal	Normal	Normal
Loose skein	Normal	Normal	Normal	Normal
Splitting	Visible	Visible	Visible	Visible
Segmentation	26 cuboids	26 cuboids	26 cuboids	26 *X*'s and *Y*'s; nucleus long
Synapsis	Prompt	Slow	Slow or none; nucleus long	None
Orientation	Compact	Loose	Irregular	None; nucleus lobing
Spindle	Fibers to bivalents	Fibers to univalents	Defective	None
Metaphase	Qualitative, narrow	Quantitative, broad	None or very irregular	Amitosis
Second division	Quantitative, homotypic	Quantitative, somatic (or none)	None, irregular or amitotic	None or amitotic

arising from segmentation and culminating in amitosis, and not as a curious step in the normal maturation process. This amitotic type of development may be designated as sequence *D* (table I). It is illustrated in figs. 36–42.

Returning to diakinesis with its twenty-six cuboid chromosomes, this stage may develop further in any one of the three ways outlined in table I, and designated as sequences *A*, *B*, and *C* respectively. Type *A* is illustrated in figs. 16–22. Here pairing is end to end, following diakinesis, and is both prompt and complete. Orientation is uniform and compact, resulting in a heterotypic metaphase

with thirteen bivalents. This sequence has been completely followed in pollen through heterotypic mitosis and the ensuing homotypic division where (fig. 22) thirteen univalents show. In the embryo sac it has only been traced with definiteness through the compact orientation stage (fig. 17), while fig. 21*a*, *b* represents a badly masked anaphase showing thirteen chromosomes at each pole. In addition, it is likely that the metaphase shown by STORK in his fig. 16 is heterotypic, since it agrees with the general aspect of such a stage as found frequently in pollen. Whether the second (quantitative or homotypic division) can occur in the embryo sac as it does in the pollen maturation, giving true reduction, is not known. Inspection of hundreds of embryo sacs failed to disclose tetrad formation, and yet the large numbers of empty fruits in *T. laevigatum* may eventually be explained by occurrence of reduced embryo sacs, never fertilized, quite as much as by the occurrence of amitosis of sequence *D*.

Sequence *B* is shown in figs. 23–30*b*. It is ostensibly the sequence which results in reproduction, inasmuch as it is the only mechanism found which in the absence of fertilization insures preservation of the constant chromosome equipment characterizing the species. In this sequence the nuclear membrane disappears before synapsis and orientation are complete. Synaptic pairing is end to end, but takes place so slowly that spindle fibers become attached to each of the halves of each univalent instead of to the univalent as a whole. In consequence the pairs come to metaphase thirteen in number, but with components still end to end and transversely oriented. The resulting spindle (figs. 27, 28) is much broader than that of sequence *A*, and the division is quantitative instead of qualitative, if ordinary canons be right. The partial or delayed pairing here was noted by OSAWA in the pollen of *T. albidum* only, did not attract the attention of STORK, and seems to have been interpreted by JUEL (12) as a splitting. HOGBEN (7) has described similar phenomena (delayed synapses) in parthenogenetic animals, while the present observations are amply verified by numerous counts made throughout the sequence. Sequence *B* in pollen seems to result in diads of fairly uniform nature, which as a rule do not undergo further growth. In the embryo sac it likewise

produces a diad. One of the cells, usually the apical, disintegrates, while the other develops into an eight-nucleate sac by regular vegetative mitoses. A prophase of the first of these mitoses is shown in fig. 30*a*, *b*, with twenty-six somatic chromosomes segmented.

Sequence *C* is illustrated in figs. 31–35. It comprises a rather wide range of gradations in behavior, completely bridging the gap between types *B* and *D*. Following segmentation, the nucleus elongates, and the membrane disappears, with the twenty-six cuboids widely scattered and quite unpaired. As the spindle fibers appear, orientation and pairing are quite variable in their degree of perfection. In fig. 32 spindle, synapsis, and orientation seem rather perfect, excepting that chromosomes from the extreme ends of the nucleus have been caught at the poles and will doubtless remain there. In other cases pairing cannot be detected, and the majority of the cuboids may be caught at the poles, only a few or none reaching metaphase position. These latter constitute the "delayed" chromosomes familiar in descriptions of pollen abnormalities, although actually the lagging ones are those at the ends. Obviously it is but a short step from this condition, where no cuboids reach metaphase, to amitosis as already described for sequence *D*.

Sequence *C* is best exemplified in the pollen. In the embryo sac it has been traced through orientation. With amitosis it shares most of the responsibility for pollen abnormalities recorded in a previous paper. The chromosomes which never reach metaphase position are likely to be reorganized into nuclei before those at the center reach the poles. These latter "delayed" chromosomes then reorganize as supernumerary nuclei. Additional causes of supernumerary nuclei are (1) irregular lobing during first amitosis, (2) a second amitotic division, (3) extrusion of chromosome substance and formation of membranes about it.

It should be understood that the four sequences described intergrade almost insensibly. It should also be noted that sequence *D* in its extremest fluctuations shows nuclear elongation and amitosis beginning so soon after synizesis that the chromatin has no opportunity to organize beyond the condition of a granular thread.

Discussion

The more important implications of these findings fall under (1) relation to previous hypotheses explaining maturation in parthenogenetic species of *Taraxacum;* (2) effect upon interpretations of normal reduction division (particularly as to synapsis) which have been based upon comparisons of sexual and parthenogenetic species of *Taraxacum;* (3) elucidation of the findings themselves in terms of the fundamental cell activities involved.

1. JUEL'S (12) hypothesis, accepted in more or less modified form by subsequent workers, is that maturation in *Taraxacum officinale* begins as a heterotypic and shifts to a homotypic division. As previously stated, this is based upon his belief that the elongated nucleus with *X*-shaped chromosomes follows diakinesis and precedes spindle formation. Since, as has been indicated, the elongated nucleus is a member of a distinct sequence, the hypothesis is placed upon the defensive. Barring this discrepancy, however, the type of division described by JUEL is essentially that of sequence *B*, the type effective in reproduction. It might appear that this is virtually homotypic, since quantitative, and therefore mainly if not in detail in agreement with JUEL'S theory. Possibly this is true, but number and character of chromosomes do not correspond with those usual in homotypic divisions. Sex as a factor is completely absent in homotypic division, while here it is present, in abeyance of course, but potential. This is evidenced by (*a*) chromosome number, (*b*) pairing of prochromosomes, (*c*) synaptic pairing (albeit delayed) of the cuboids, (*d*) occasional cases of true reduction in pollen and presumably in embryo sac. It seems, therefore, that the designation "ameiosis," or "amiosis," proposed by SEARS in 1917 (23), and indicating a type of maturation which obviates necessity for subsequent fertilization, is to be preferred to "homotypic mitosis," a term of very explicit implications.

2. The parasynaptic interpretation of reduction division, so far as normal sexual species of *Taraxacum* are concerned, was favored by the work of JUEL and OSAWA, since both workers noted a duality of spireme thread in meiotic and none in ameiotic plants. Closer scrutiny previous to segmentation has revealed a duality in the segmenting thread, while precise counts have indicated that this

duality represents a splitting and not a pairing. These facts seem sufficient to warrant more critical comparison of prophases in sexual and parthenogenetic species of *Taraxacum* before deciding that parasynapsis is actually the source of duality in spireme threads of the former. Moreover, the completed synapsis in sequence *A*, as well as the delayed pairing in sequence *B*, is end to end, rendering any assumptions still more difficult.

3. We have seen that ameiosis does not involve the complete elimination of sex. Rather it involves a retardation and partial inhibition of sex expression. The least degree of inhibition gives us sequence *A*, practically a normal reduction division with synaptic mates pairing only a little more slowly than is usually the case. A greater degree of inhibition obviously occurs in sequence *B*, the delay being more marked. Whatever the ultimate cause of such delay, there can be no question that it amounts to a persistence of chromosome individuality, which at segmentation supersedes the individuality of the nucleus as a dominant phase. The nature of sex inhibition in sequence *C* is more complex. Synapsis is slow and of varying perfection. It is marked by an elongation of the nucleus, clearly indicating a premature expression of polarity. We may conclude, therefore, that encroachment upon sex is progressively increasing.

Type *D* is readily interpreted, in view of these intermediate conditions, as the still earlier and more powerful expression of polarity at the segmentation stage. Not only does the nucleus become greatly elongated and eventually pulled apart, but the spireme split begun in prophase is never even temporarily overcome by the forces making for chromosome individuality. This is evidenced by the presence of *X* and *Y* forms, already noted. Such interpretation of the amitosis in sequence *D* by no means vitiates any possibility that it may be a matter of emulsification, as suggested by coupling the work of NATHANSOHN and that of BONNS. It merely involves a third, and not unreasonable factor, enzyme action, as a means of upsetting the delicate balance between the forces which we ignorantly know as individuality, polarity, and sex. That the dominance of polarity is not likely to be perfect seems probable from the nature of the factors which it overrides.

We have therefore a theoretical right to expect such phenomena as chromatin extrusion, irregular lobing, etc. The supernumerary nuclei produced by such means are thus quite a secondary phase of pollen degeneracy.

Summary

1. Maturation in *Taraxacum laevigatum* differs from that previously described for parthenogenetic species of *Taraxacum* in early prophase, chiefly by showing a split thread from which twenty-six univalent chromosomes segment.

2. Following segmentation, there may be any of four intergrading sequences instead of a single uniform sequence as described for other parthenogenetic species of *Taraxacum*.

3. These sequences are: *A*, almost typical reduction division characterized by perfect end to end pairing of the univalents; *B*, a qualitative division resulting in diads from which the functional embryo sacs arise and for which the term "ameiosis" is proposed, and in which sequence the univalents are slow in pairing; *C*, a more or less irregular division in which pairing of univalents is variable, accompanied by premature elongation of the nucleus and defective orientation; *D*, amitosis in which the nucleus elongates very prematurely and the split thread persists after segmentation, giving twenty-six *X* and *Y*-shaped chromosomes. There is no spindle.

4. These variations are not anomalous, but are traced to an increasing degree of inhibition of sex by other forces, to wit, chromosome individuality and polarity.

5. JUEL'S (12) interpretation of maturation in *T. officinale*, that it begins as heterotypic and switches to homotypic, does not apply in the present case.

6. Evidence for parasynapsis in Chicoraceae, so far as predicated upon the presence of a dual thread only in sexual species, must be reexamined.

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EXPLANATION OF PLATES IX AND X

All figures show a magnification of about 1600, excepting fig. 25, which is about 1200.

FIG. 1.—Complete view of very early E.S.M.C., showing about thirteen paired centers of chromatin aggregation.

FIG. 2.—Partial view of similar nucleus.

FIG. 3.—E.S.M. nucleus entering synizesis, showing curious partly paired, partly vacuolate appearance of chromatin masses.

FIG. 4.—Unusually large E.S.M.C. at climax of synizesis.

FIG. 5.—E.S.M.C. emerging from synizesis, showing uniform character of thread.

FIG. 6.—The same, somewhat later.

FIG. 7.—The same, thread becoming less homogeneous.

FIG. 8.—The same, thread showing dual character in places.

FIG. 9.—The same, segmentation beginning, thread thicker and dual character obvious in most nodes.

FIG. 10.—P.M.C. showing segmentation and dual nodes.

FIG. 11.—E.S.M.C., chromosomes becoming homogeneous, duality mainly visible at internodes.

FIG. 12.—The same, showing origin of cuboid chromosome form; thickening of thread producing contraction of mass toward center.

FIGS. 13*a*, *b*.—Complementary sections of same E.S.M. nucleus, with twenty-six (diploid number) chromosomes, cuboid and with traces of dual internodes still showing.

FIGS. 14*a*, *b*.—The same, somewhat later, showing various retractions and shiftings of quadruple internodal traces.

FIG. 15.—Part of P.M.C. at same stage as preceding.

FIG. 16.—Complete P.M.C., showing beginning of end to end synaptic pairing of twenty-six univalent cuboids.

FIG. 17.—Late orientation stage in sequence *A*, E.S.M.C. showing synapsis completed.

FIG. 18.—Same for P.M.C.

SEARS on TARAXACUM


23


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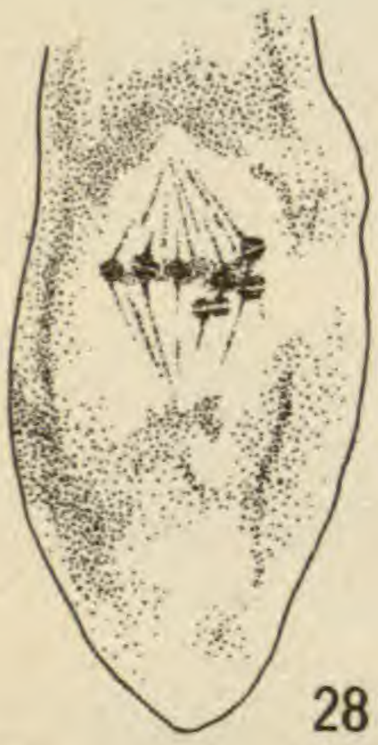
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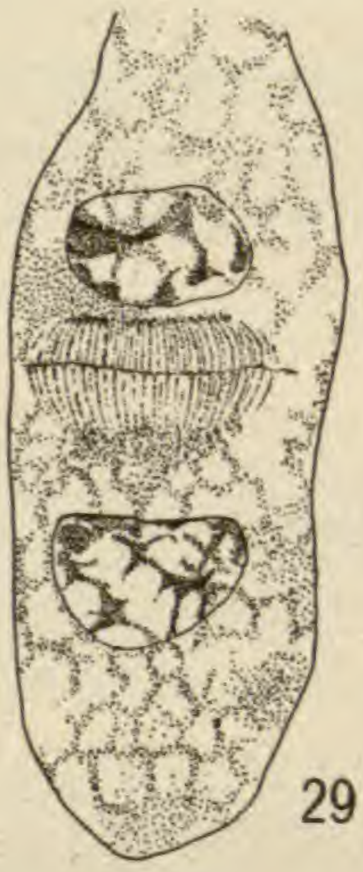
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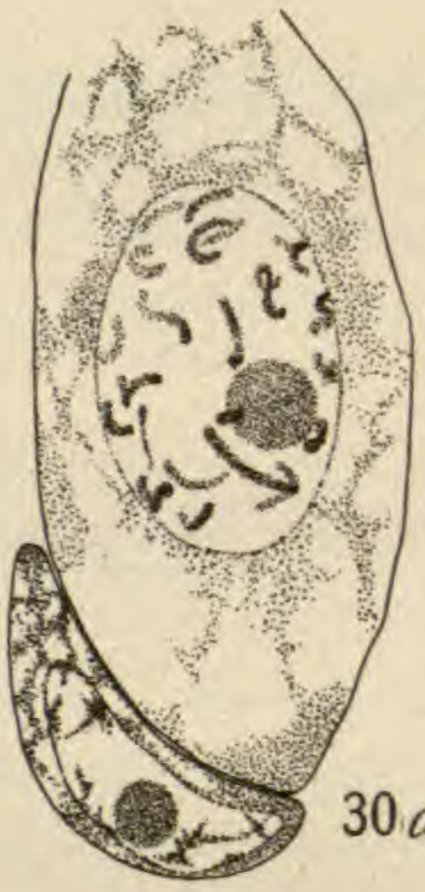
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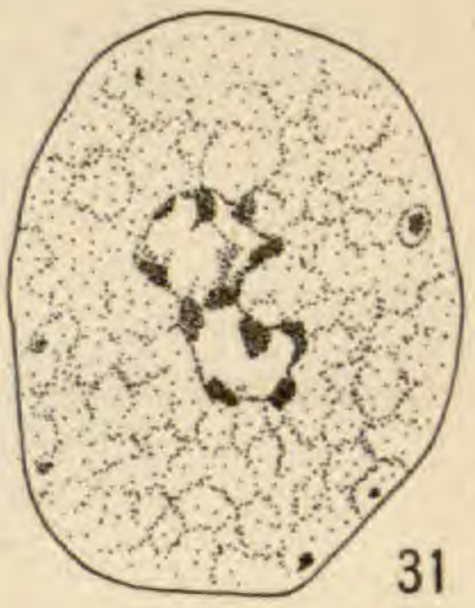
30*b*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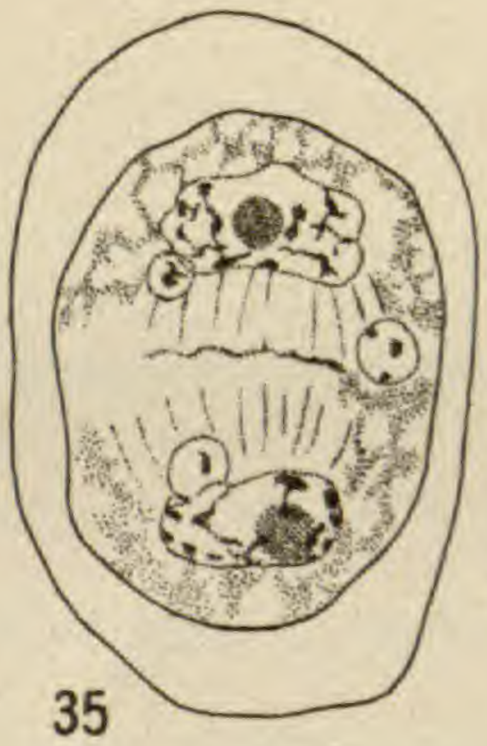
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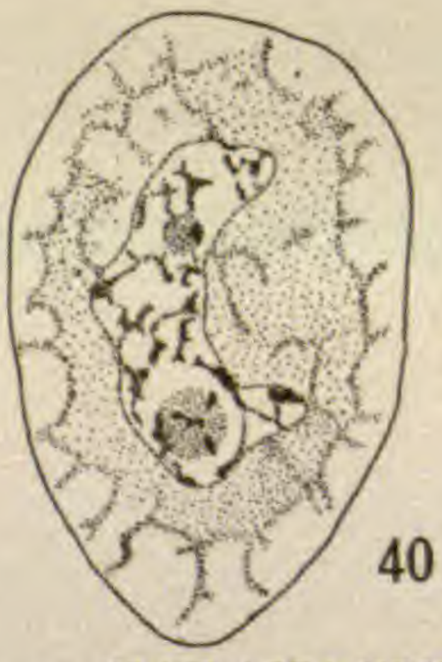
36


37


38


39*a*


39*b*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42

SEARS on TARAXACUM

FIG. 19.—P.M.C. at metaphase, sequence *A*, showing narrow heterotypic spindle.

FIG. 20.—P.M.C. showing heterotypic anaphase of sequence *A*.

FIG. 21*a*, *b*.—Complementary sections of E.S.M.C. anaphase, masked but doubtless sequence *A*.

FIG. 22.—Second, true homotypic metaphase of sequence *A* in P.M.C., showing thirteen chromosomes at plate.

FIG. 23.—P.M.C. orientation in sequence *B*, pairing somewhat delayed; nuclear membrane disappearing.

FIG. 24.—P.M.C. showing beginnings of delayed synaptic pairing in sequence *B;* membrane quite gone (cf. fig. 16).

FIGS. 25, 26.—Two different E.S.M.C.'s somewhat further along than fig. 24; such stages grade insensibly into sequence *C*.

FIG. 27.—P.M.C. showing wide metaphase spindle characteristic of sequence *B*.

FIG. 28.—E.S.M.C. in ameiotic metaphase of sequence *B;* synaptic mates end to end, perpendicular to spindle axis, giving quantitative division along line of prophase split.

FIG. 29.—Telophase of first E.S.M.C. division, probably sequence *B*.

FIGS. 30*a*, *b*.—Second division, purely somatic, following ameiosis in E.S.M.C; note breakdown of apical daughter and presence of about twenty-six homogeneous segments.

FIG. 31.—Portion of P.M.C. showing disturbance in orientation due to premature elongation of nucleus; beginning of sequence *C*.

FIG. 32.—Similar portion, further along; pairing about complete, but spindle defective (cf. fig. 25).

FIG. 33.—P.M.C. in sequence *C*, synapsis virtually complete but chromosomes unequally sequestered at poles without orientation or metaphase.

FIG. 34.—P.M.C. in sequence *C*, chromosomes sequestered with little or no synapsis.

FIG. 35.—P.M.C. in irregular telophase, probably of sequence *C*, showing organization of small supernumerary nuclei.

FIG. 36.—E.S.M.C., late segmentation stage to show gradation into sequence *D* or amitosis.

FIG. 37.—E.S.M.C. showing beginnings of nuclear elongation during late segmentation.

FIG. 38.—The same, showing persistence of internodes between *X*-shaped chromosomes and origin of latter by persistence of prophase split.

FIGS. 39*a*, *b*.—Complementary E.S.M.C. sections, showing more than twenty split chromosomes.

FIG. 40.—Beginning amitotic division (sequence *D*) in P.M.C.

FIG. 41.—Amitotic constriction of sequence *D* in E.S.M.C.; note chromosome form.

FIG. 42.—Amitotic irregularities in P.M.C., showing also chromatin extrusion.

ANNULARIA WITH PALEOSTACHYA FRUIT

EDA M. ROUND

(WITH TWO FIGURES)

Among the most common fossil plants in Rhode Island are Annulariae, thought to be allied to the modern *Equisetum*. While the Annulariae or leafy shoots are seldom found attached to their supposedly Calamitean stems, the trunks of these ancient cryptogams are often seen in the coal strata and occasionally prove to be of large size. Much more rare, however, are the fruiting stalks of these primitive plants, one specimen of which has appeared from the coal shales of Rhode Island. This fossil is regarded by the writer as a new species of *Annularia*, both from the character of its foliage and the nature of its fruit.

Annularia clarkii, n. sp.—In considering the affinities of the leafy shoots of *Annularia clarkii* one may cite *Asterophyllites lentus* Dawson.[1] The Canadian material, however, is so fragmentary that correlation therewith is questionable. The fertile stalks of *Annularia clarkii* resemble in many ways *Paleostachya* (*Volkmannia*) *gracilis* Renault,[2] especially in the position of the sporangia, which appear to be borne in the axils of the leaves. The sporangiophores of the European species, however, are shorter and less stout than those of the Rhode Island plant (figs. 1, 2).

In a recent publication[3] statements are made to the effect that Calamariae, to which the Annulariae supposedly belong, show four main types of fruiting. The first includes *Calamostachys*, forms in which the cones are made up of fertile and sterile parts, the sporangiophores being placed midway between the leafy bracts. The second or *Paleostachya* type consists of cones, the fertile parts of which are borne in the axils of the sterile bracts. The third or *Cingularia* type is characterized by sporangiophores borne just under

[1] Bull. Nat. Hist. Soc. N.B. **6**:247. 1910.

[2] RENAULT, B., Autun **2**:75. *pl. 29. figs. 1–7.* 1893.

[3] SCOTT, D. H., Studies in fossil botany. Ed. 3. Vol. I. p. 43. 1920.

the sterile whorls. The fourth has a sporangiophore without bracts, or arranged as in the modern *Equisetum*. The same author states

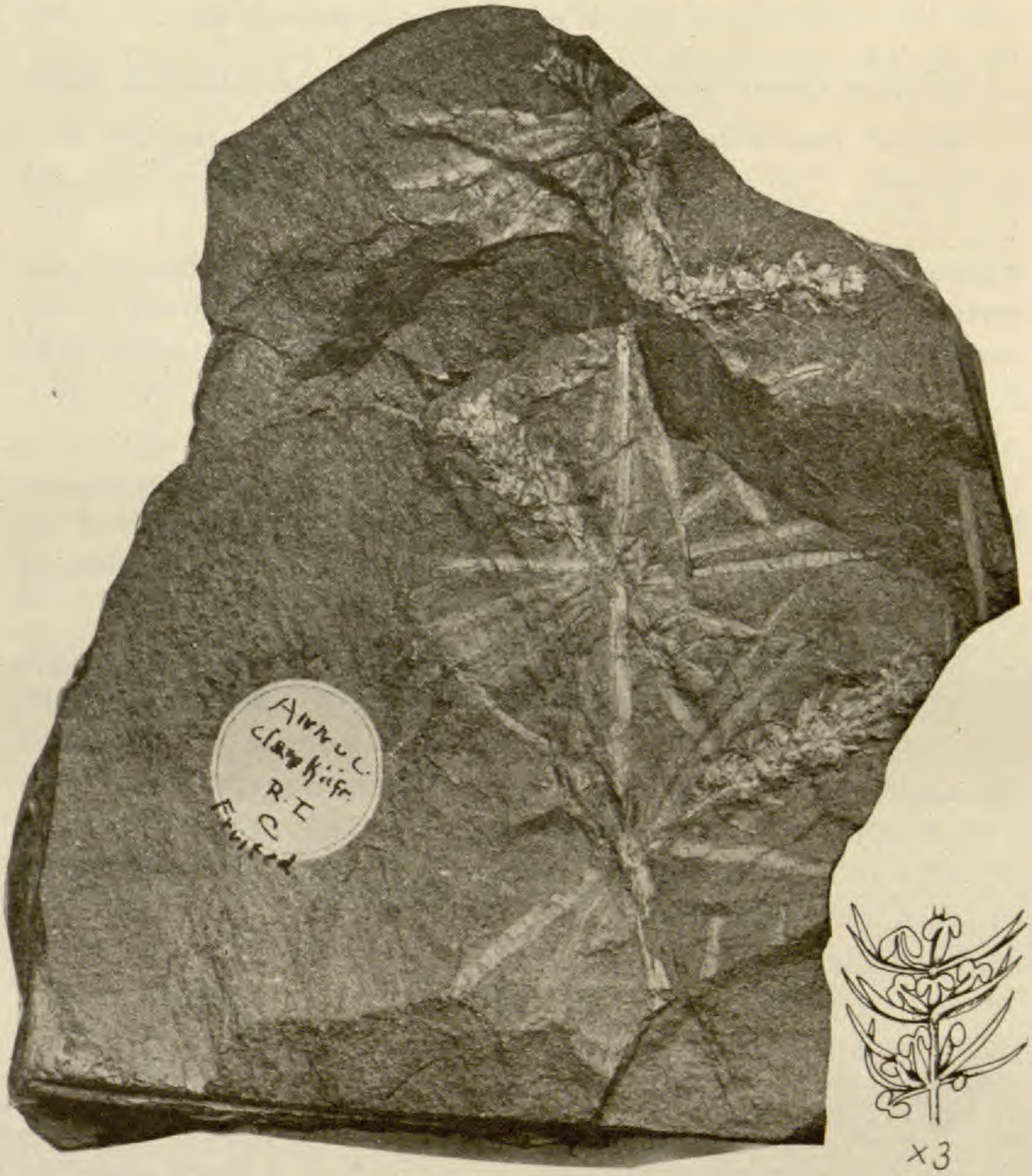

FIG. 1

FIG. 2

FIGS. 1, 2.—Fig. 1, *Annularia clarkii:* photograph, natural size; fig. 2, *Annularia clarkii:* drawing, ×3.

further that "in all cases where fructifications have been referred to *Annularia* . . . . they have been proved to be of the *Calamostachys* type."

Another English author[4] of earlier date, however, states the situation less sweepingly, as follows:

> It would appear . . . . that the *Annularia* type of branch usually bears cones which conform to the genus *Calamostachys* (*Stachannularia*); . . . . But this rule is not constant, and we are not in a position to speak of cones of a particular type as necessarily characteristic of definite types of Calamitean shoots.

BERRY[5] has stated the problem in a still more positive way as follows: "Some of the *Paleostachya* cones are of large size and they are usually associated with *Annularia* type of foliage." This opinion is further borne out by the characteristics of the present species, *Annularia clarkii*. The illustrations of this fossil reveal several fruiting cones attached to their leafy shoot, the lower right hand one being sufficiently distinct to show that the sporangiophores spring from the axils of the leaves. This fact places the specimen in the *Paleostachya* group, and refutes the contention by SCOTT that Annulariae have always been characterized by *Calamostachys* types of fruiting. The following description of *Annularia clarkii* is offered:

> Verticilli 10–12 foliorum circa quemque nodum; quodque folium linearium 4 cm. vel longum paullum, apices acuti, mediae costae apparent; fructus ramus quemque nodum quasi 4.5 cm. longus, sporangio-phoreae quasi 1 mm. longae. Duae fruges annectae sunt alterno lato stili curvati. Hic ultimus in axilla curtae bractae lineariae est, quae fructum superarcuant. Plurimi stili sporosi ex unico nodo enascuntur.

> Whorls of 10–12 leaves at each node; each leaf linear, 4 cm. or less in length, apex acute, midrib present; fruiting branch at each node about 4.5 cm. long, the sporangiophore about 1 mm. long. Two spores are attached on either side of a curved stalk. The latter is in the axil of linear bracts which overarch the fruit. Several spore-bearing stalks seem to spring from a single node.

Sterile fossils of this species are very common from the Pawtucket, Valley Falls, and Portsmouth sections of Rhode Island, and may be found in the Brown University collection. The fruited specimen used as the basis of this article is now a part of the Roger Williams Park Museum collection, Providence, Rhode Island.

[4] SEWARD, A. C., Fossil plants. Vol. I. p. 364. 1878.

[5] BERRY, E. W., Paleobotany: A sketch of the origin and evolution of floras. p. 319. 1920.

CURRENT LITERATURE

NOTES FOR STUDENTS

The unarmored Peridiniales.—Excepting only the diatoms, no group of unicellular organisms is of such fundamental importance in the biology of the seas as the peridines, and there are few groups concerning which our knowledge is so unsatisfactory. This is particularly true of the unarmored forms, which are not only apt to be ruined in collecting, but, if they survive the plankton net, are destroyed by the preservatives used and usually remain recognizable for only a short time under the conditions speedily developed in a jar of sea water or on a microscopic slide. For these reasons all who have occasion to study the minute life of the ocean will heartily welcome the appearance of the splendid monograph by KOFOID and SWEZY.[1] About one-fifth of the text is devoted to topics of a general nature, the remainder to the detailed descriptions of genera and species. Among the topics discussed are the general and comparative morphology of the group, life cycles, physiology, including short but pregnant discussions of nutrition and luminescence, and finally evolutionary development and distribution. The authors take marked exception to WEST's statement that over 80 per cent of the peridines are "true vegetable organisms with a holophytic nutrition," declaring on the contrary that "the number actually containing chromatophores is relatively small throughout the entire Dinoflagellata." In spite of this, however, the great abundance of some of the forms characterized by undoubted holophytic nutrition insures that the group will continue to be of as great interest to the botanist as to the zoologist.

The authors regard the Peridiniales as a monophyletic group, derived from a cryptomonad ancestry, and describe a new genus, *Protodinifer*, which is one of the simplest known peridines. One line of development, starting near some such form, may have given rise to the *Haplodinium*, *Exuviella*, *Prorocentrum* series; another would lead to the simpler Gymnodiniaceae, from which coordinate lines of development lead on the one hand to the higher unarmored forms, culminating in such elaborately organized genera as *Pouchetia* and *Erythropis*, and on the other hand to the thecate forms, such as *Ceratium*.

The systematic treatment includes descriptions of 223 species, distributed among 16 genera, of which *Protodinifer*, *Gyrodinium*, *Torodinium*, *Pavillardia*, *Protopsis*, *Nematodinium*, and *Proterythopsis* are new. Of these, *Torodinium*

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[1] KOFOID, C. A., and SWEZY, OLIVE, The free-living unarmored Dinoflagellata. Memoirs Univ. Cal. 5: viii+562. *pls.* 12 (colored). *figs.* 388. Univ. Cal. Press. Berkeley. 1921.

and *Protopsis* include in part previously described species, and *Gyrodinium* replaces SHÜTT's genus *Spirodinium* which is preoccupied zoologically. The other new genera are established to contain newly discovered species.

A few minor typographical errors occur, but in general the work is remarkably free from them. The text figures are irregularly distributed and frequently many pages from the references to them. It would greatly facilitate the use of the book if the references were by page instead of merely to the figure. These are trifling defects, however, in a work of such unusually permanent value. It is to be confidently expected that its publication will prove a distinct stimulus to further study in a field where there is still much to be learned.—G. W. MARTIN.

A new type of nuclear division.—CHATTON,[2] in a brief but significant paper throws new light on the peculiar nuclear phenomena in the Peridiniales described by LAUTERBORN, JOLLOS, BORGERT, and in his own earlier contributions. His studies were made on species of *Syndinium*, which live parasitically in the body cavity of copepods, especially favorable material because of the small number of chromosomes. These have previously been described as ten somewhat curved filaments from a single pole like the ribs of an umbrella. Further study has shown that this structure is in reality composed of five V-shaped chromosomes with very sharp bends, with the points of the V's converging at the pole. Cleavage takes place throughout the length of the chromosomes, so that ten V-shaped daughter chromosomes are formed. Five of these remain grouped about the original pole; the points of the remaining five become centered about a new pole which is at first close to the original one, then gradually moves away. In some species the cleavage is completed at once; in others the daughter chromosomes remain united at their tips and turn as upon a hinge, so that a bipolar spindle-shaped structure (not a true achromatic spindle) is formed, composed of the two series of V-shaped chromosomes converging at either end. The chromosomes then break apart, and it is this separation which was formerly interpreted as a transverse division of chromosomes, when it is in reality merely the final separation of chromosomes previously formed by longitudinal fission. Nothing resembling a true spindle was seen.

Ordinarily no resting stage appears to occur, but it was observed in certain cases where the development of the peridine had been inhibited by some factor, as, for example, the presence of another parasite. In such cases the nucleus appeared to be composed of a large number of microsomes grouped around a central nucleolus.

A division of this kind, while distinctly simpler than one taking place in connection with the usual achromatic structures, would appear to be quite as

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[2] CHATTON, EDOUARD, Sur un mecanisme cinetique nouveau. La mitose syndienne chez les Peridiniens parasites plasmodiaux. Compt. Rend. Acad. Sci. **173**: 859–862. *fig.* 1. 1921.

effective in securing an exact distribution of the chromatin. The author proposes for it the special term "syndinial mitosis," and expresses the opinion that further study of the free peridines will show that nuclear division in such forms is also of this type.—G. W. MARTIN.

Cytology of Porphyra.—Since a cytological study of the Bangiales might throw light upon the much discussed but little investigated problem as to whether this group is primitive or reduced, whether it should stand at the beginning or at the end of the red algae, ISHIKAWA[3] fixed material and has described several critical stages in the life history of *Porphyra tenera*, a characteristic genus of the order.

The cell wall shows no cellulose reaction, but responds to tests for pectic substances. The large stellate chromatophore contains one conspicuous pyrenoid which has often been mistaken for a nucleus, the real nucleus (only 1.5–2 μ in diameter) being hard to detect in living material, although easy to see in well stained preparations, where it appears as a black globule with no structural differentiation. At division the nucleus elongates, and splits by longitudinal fissures into three filaments which constrict in the middle, so that three pieces go to each pole. The process looks like that described for some Cyanophyceae, especially *Synecocystis*. The figures show neither nucleolus nor nuclear membrane, so that the type appears to be very primitive. The antheridium consists of 64 or 128 cells, and the spermatium has a chromatophore and a group of three chromosomes without any nuclear membrane. The carpogonium is slightly prominent at both ends, the prominence constituting a rudimentary trichogyne. Spermatia were found attached to the trichogyne, but the actual process of fertilization was not observed, nor was there any study of the first and second divisions of the zygote. It seems reasonable, however, to suppose that reduction of chromosomes occurs during these divisions, as in many other algae.

ISHIKAWA would regard Bangiales as a connecting link between the Cyanophyceae and the Florideae, a conclusion which is helped by the fact that *Porphyridium*, a genus sometimes placed in one group and sometimes in the other, has no sexual reproduction. So far as pigments are concerned, some of the Rhodophyceae have phycocyan and some of the Cyanophyceae have phycoerythrin.

Although a careful investigation of the whole life history of several members of the Bangiales is desirable, it seems probable that any future study of the group will confirm ISHIKAWA's conclusions.—C. J. CHAMBERLAIN.

Ecology of Urtica dioica.—In an interesting study of the factors which locally limit the distribution of the common nettle, *Urtica dioica*, OLSEN[4]

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[3] ISHIKAWA, M., Cytological studies on *Porphyra tenera*. Bot. Magazine Tokyo **35**: 206-218. 1921.

[4] OLSEN, CARSTEN, The ecology of *Urtica dioica*. Jour. Ecol. **9**: 1–18. *pl. 1*. 1921.

states that it is abundant in Denmark, growing mostly in large close communities. Its gregarious habit is due to the activities of horizontal rhizomes, which also account for the sharpness of the boundaries separating it from other vegetation. It thrives well in full light, and in shade up to 5–10 per cent of open daylight, the greatest vigor being shown in 10–20 per cent of full illumination. A moderate amount of soil moisture also seems necessary to meet its requirements; but while OLSEN has determined the percentage of soil moisture in the habitats under consideration, he has not attempted to determine the growth water available.

By several authors *Urtica dioica* has been placed among nitrogen-requiring plants, and the principal feature of this investigation was the experimental examination of this quality. Microchemical tests showed that the plants themselves always contained considerable nitrates, especially in the stems, rhizomes, and roots. Soil samples were then taken at a depth at which the roots of the plant abound, in twenty localities, some within and others without the nettle communities. The nitrate content of such samples was determined, both at once after they were taken, and again after the soil had been kept moistened in a jar at 18° C. for twenty-five days. This procedure was in order to obtain an expression of the nitrifying power of the soil. These data were tabulated, together with the hydrogen-ion concentration, the percentage of soil moisture, and the light value of the locality. The table makes it evident that the only factor varying directly with the presence of *Urtica dioica* is the nitrate content of the soil.

Experimental cultures of the plant were made, using portions of rhizomes planted in washed sand and watered with nutrient solutions containing varying amounts of nitrates. Growth was in direct proportion to the relative amounts of available nitrogen; hence the conclusion was reached that *Urtica dioica* in nature is able to make sufficiently vigorous growth to enable it to compete successfully with other vegetation only where there is a relatively large amount of nitrogen in available form present in the soil. The experiment also showed that nitrification proceeds in soils showing an acid reaction as high as $P_H = 3.6$, and that ammonium used as a source of nitrogen was toxic to the nettle.—GEO. D. FULLER.

Pink root of onions.—Another of the many diseases directly attributed by farmers to alkali in the soil has recently been shown to be due to a parasite, as a result of investigations carried on in the Texas Experiment Station.[5] Isolation and inoculation studies have definitely connected a species of *Fusarium*, tentatively called *F. malli*, with the pink root disease of onions. Over twenty-five species of fungi were found in diseased plants, including several species of *Fusarium*, and the association of some of these fungi with *F. malli* increased the virulence of the latter. All varieties of onions and garlic

[5] TAUBENHAUS, J. J., and MALLY, W., Pink root disease of onions and its control in Texas. Texas Agric. Exp. Sta. Bull. 273. pp. 42. *figs.* 3. 1921.

inoculated appeared to be more or less susceptible to the pink root organism, but other liliaceous plants, such as *Funkia*, *Tulipa*, *Calla*, *Iris*, and *Lilium* were immune. Pink root of onions has been observed in California, Iowa, Wisconsin, New York, and the Bermuda Islands. In Texas it seriously threatens the industry of growing onions for the early northern markets, which industry has become an important one. Losses vary from $150 to $400 per acre.

Symptoms of pink root include yellowing of the roots, followed by their pink discoloration, drying, and death. The bulb exhausts its energy in producing new roots. Alkali soil, deficiency in nitrogen and humus, excessive temperatures, eel worm and thrips attacks are factors favoring the disease. The seed is not a carrier, but onion "sets," both dry and green, may harbor the causal fungus. Suggested control methods include the use of virgin soil for seed bed and field plantings, steam or formaldehyde disinfection of seed beds known to contain the pink root fungus, rotation of crops, the use of quickly acting fertilizers, careful use of tools, and various cultural practices favoring continued growth of the crop. An attempt to control nematodes by adding cyanimide to the soil failed because the amount required to affect nematodes killed the crop.—J. G. Brown.

Carbon nutrition.—Storage rot fungi of the sweet potato have been investigated by Weimer and Harter,[6] who find that seven of eight species causing rot can utilize glucose as a source of carbon. Five of them are able to increase the acidity of the culture medium, and certain species increased the osmotic concentration of the substratum. The glucose is utilized partly as a source of energy, partly in producing mycelium, and perhaps in still other ways. The respiratory activity of these organisms has been studied by the same authors,[7] who used the amount of CO_2 set free as the measure of the carbohydrate used in this process. *Penicillium* sp., *Botrytis cinerea*, and *Sclerotium bataticola* grew slowly, produced relatively large amounts of dry material, consumed nearly all of the glucose, and produced CO_2 most freely. The other species grew more rapidly, but produced comparatively small amounts of CO_2 and did not consume all the glucose. The economic coefficient was found unusually high in two species. *Fusarium acuminatum* required 17.11 G. and *Mucor racemosus* 22.86 G. glucose for each gram of dry matter grown. The CO_2 set free is not equal to the theoretical amount that could have formed from the sugar consumed. Some of the sugar evidently was not completely respired, as alcohol and acids appeared in some of the culture solutions.—C. A. Shull.

Transmission of potato wilts.—Among the various wilts which are responsible for heavy losses sustained by potato growers are those due to attacks of

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[6] Weimer, J. L., and Harter, L. L., Glucose as a source of carbon for certain sweet potato storage rot fungi. Jour. Agric. Res. **21**:189–210. 1921.

[7] ———, Respiration of sweet potato storage rot fungi when grown on a nutrient solution. Jour. Agric. Res. **21**:211–226. 1921.

Fusarium oxysporium and *Verticillium albo-atrum*. In order to determine the degree of transmission of these wilts through seed tubers, McKay[8] has carried on experiments with numerous varieties of potatoes for four years. *Verticillium albo-atrum* occurs somewhat more extensively in small tubers than in medium-sized ones, 30–50 per cent of the crop grown from infected seed tubers being diseased with *Verticillium* wilt, as shown by cultures. *Fusarium oxysporium* is transmitted in a lesser degree, and it appears to be capable of remaining virulent in the soil for several years after the production of a crop of potatoes. Vascular discoloration is an unreliable index of *Verticillium* infection, since approximately 7–17 per cent of cultured tubers which produced the fungus show no discoloration, and 55 per cent of the tubers which show brown vascular discoloration give no organism parasitic for the potato. Although the discoloration occurs at the stem end of the tuber, stem-end seed pieces give no more disease than eye-end pieces of the same infected tuber. Numerous species of *Fusarium* and other fungi mostly saprophytic in nature appear in cultures of wilt diseased tubers.—J. G. Brown.

Colloidal hydration.—In two recent papers MacDougal[9, 10] discusses the effects of bases, salts, and other substances on the hydration capacity of prepared colloidal bodies and masses of vegetable cells. In a previous paper[11] he had reported that 0.01 N hydroxides retard the hydration of colloids, and suggested that the chief function of the base forming metals required by plants might be the restricting or limiting of the hydration capacity of the living protoplasm. He now finds that when concentrations of 0.001 to 0.0001 M solutions of chlorides and nitrates, and 0.001 to 0.0001 N hydroxides are used, concentrations comparable to those occurring in living cells, the hydration is increased and not restricted. He therefore reinterprets the function of the metallic elements as accelerators of hydration and growth. Correction is also made regarding the effects of HCl. At a P_H value of 4.2 the acid is now shown to cause more swelling than water. Some interesting studies of the hydration of roots of different ecological types, and of roots grown in different types of soil are reported. In general he concludes that all substances which are known to facilitate growth in plants will at appropriate concentrations increase the hydration capacity in some of the colloidal objects tested.—C. A. Shull.

Vertical distribution of Fucus.—*Fucus* has long been regarded as characteristic of the zone of tidal play, largely because of its high light requirement

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[8] McKay, B. M., Transmission of some wilt diseases in seed potatoes. Jour. Agric. Res. **21**:821–847. 1921.

[9] MacDougal, D. T., Water deficit and the action of vitamines, amino-compounds, and salts on hydration. Amer. Jour. Bot. **8**:296–302. 1921.

[10] ———, The action of bases and salts on biocolloids and cell masses. Proc. Amer. Phil. Soc. **60**:15–30. 1921.

[11] ———, Growth in organisms. Science **49**:599–605. 1919.

and its capacity for exposure to the air. GAIL has made some exact studies to determine the controlling factors in distribution.[12] High light requirement is well shown by the fact that the average vertical distance occupied by *Fucus* on south slopes is over 2 m., while on north slopes it is less than one-third of a meter. North exposures with a high shore line have no *Fucus* at all, and there is little or no *Fucus* under overhanging trees. Careful experimental study showed that mature *Fucus* plants are more resistant to low light intensities than are sporelings, that reduced light intensities cause the death of well grown *Fucus* plants 1 m. below the water surface, and that reduced light causes the death of oospores and sporelings when planted more than 3 dm. below the water surface. Well grown *Fucus* plants receiving less than one-fourth total light become darker in color, and decomposition takes place. From these considerations it is properly concluded that light is a controlling factor in determining the lower limit of *Fucus*.—H. C. COWLES.

Vegetation of the Dry Tortugas.—The Tortugas are the westernmost of the Florida keys, and are the seat of a marine laboratory of the Carnegie Institution. While engaged in other work, BOWMAN took occasion to make a detailed study of the distribution and special ecology of the vegetation of the Dry Tortugas.[13] After brief statements on the geology and the climatic conditions, the author presents a general sketch of the vegetation, which speaking broadly belongs entirely to the strand flora. Even *Rhizophora* is lacking in the sense of an association, because of the xerophytism of the conditions. Four communities are recognized, dominated respectively by *Uniola paniculata*, *Suriana maritima*, *Opuntia Dillenii*, and *Chamaesyce buxifolia*. A detailed account then follows of the special vegetation of each of the eight keys that make up the group. Of especial interest is the author's comparison of the vegetation of the islands in 1915 and 1916 with their vegetation in 1904, as reported by LANSING.—H. C. COWLES.

Scrophulariaceae and Orobanchaceae.—BOESHORE[14] has reached the conclusion that the Orobanchaceae represent an extreme offshoot from the Scrophulariaceae. This conclusion is based upon a detailed study of the roots, stems, leaves, flowers, and seeds of both families. From a review of these details, the author concludes that there is ample evidence "to show that direct and distinct continuity can be established from non-parasitic through semi-parasitic Scrophulariaceae to the most degraded parasites of the family, and that these again show direct continuity with the still more degraded

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[12] GAIL, FLOYD W., Some experiments with *Fucus* to determine the factors controlling its vertical distribution. Publ. Puget Sound Biol. Sta. **2**:139–151. 1918.

[13] BOWMAN, H. H. M., Botanical ecology of the Dry Tortugas. Carnegie Inst. Washington Publ. **252**:109–138. *pls. 6. figs. 7.* 1918.

[14] BOESHORE, I., The morphological continuity of Scrophulariaceae and Orobanchaceae. Contrib. Bot. Lab. Univ. Penn. **5**:139–177. *pls. 12–16.* 1920.

and condensedly parasitic types of Orobanchaceae." The author cites the genera that represent the various stages in this series, and also describes the progressive changes in the various structures.—J. M. C.

Permeability.—The permeability of *Laminaria agardhii* as affected by anions from various inorganic and organic salts of sodium has been measured by RABER,[15] who used OSTERHOUT'S electrical conductivity method. All of the anions increase permeability, following in a general way the HOFMEISTER series. The anions arrange themselves by their effects into several groups: thus the monovalent, bivalent, and trivalent groups can be recognized by the quantitative difference in permeability change with members of each group. The tetravalent anion, $Fe(CN)_6$, did not produce a fourth group, but this is explained as due to low concentration of the salt. The author believes that the effects of anions on permeability depend upon the valency of the anion, regardless of whether the salts are organic or inorganic.—C. A. SHULL.

A maritime species.—Following the methods employed by BONNIER of dividing individual plants and growing the resulting halves under different climatic conditions, DANIEL[16] in 1902 separated plants of *Asphodelus luteus* growing at Rennes (France) and planted portions of them in a seaside garden at Erquy. As a result of the maritime climate such striking changes resulted in the general form of the plant, in the branching habit of the inflorescence, and in other structural features that at present the seaside forms are sufficiently distinct to be regarded as a distinct species. This derived species he has named *Asphodelus luteoides*. This he believes to be the first recorded instance of maritime conditions transforming a plant to such an extent that the resulting form is entitled to specific rank.—G. D. FULLER.

Animal burrows an ecological factor.—On some small islands in the outer archipelago of Stockholm possessing a humid oceanic climate ROMELL[17] reports that voles eating the grass roots within their burrows upset the ecological equilibrium and cause strips of *Sphagnum* to replace the turf. The irregular mosaic thus formed, however, is not permanent, as the *Sphagnum* seems unable to resist the invasion of the grass.—GEO. D. FULLER.

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[15] RABER, O. L., A quantitative study of the effect of anions on the permeability of plant cells. I. Jour. Gen. Physiol. **2**:535–539. 1920; II. Amer. Jour. Bot. **8**:366–368. 1921.

[16] DANIEL, LUCIUS, Obtention d'une espèce nouvelle d'Ashphodèle par l'action du climat marin. Rev. Gen. Bot. **33**:225–237, 316–327, 357–371, 420–436. *pls. 3. figs. 12.* 1921.

[17] ROMELL, L. G., Voles as a factor in plant ecology. Svensk Bot. Tidsk. **15**:43–45. 1921.

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CYTOLOGY OF CHLOROPHYLL TYPES OF MAIZE

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(WITH PLATES XI–XVI)

Introduction

During recent years there have been discovered a number of instances of chlorophyll inheritance which are characterized by features due to unusual modes of chlorophyll distribution and behavior. These phenomena are obviously of interest both to the geneticist and the cytologist; to the geneticist because some of them almost certainly represent distinct categories of inheritance, and to the cytologist because of the manner in which they involve the origin and behavior of cell organs other than the nucleus, which latter has been generally held to be mainly responsible for the transmission and development of inherited characters. Much attention has been devoted to this subject by geneticists, whereas the cytological aspects of the problem have not as yet received as much attention. It has been felt that a more adequate knowledge of the behavior of the visible constituents of the cell is of prime importance in the attempt to find a solution of the problem. The present study was undertaken for the purpose of determining whether or not there are in the cells of certain chlorophyll types of maize any visible structural differences which can be shown to be responsible for, or correlated with, the known genetic behavior of these plants.

TYPES OF PLASTID INHERITANCE

There have been described numerous cases of chlorophyll variation which involve an unequal and frequently a very irregular distribution of green color in various regions of the plant. The inheritance of such color patterns has not been found always to conform to the behavior usually ascribed to Mendelian characters. A number of these cases will be reviewed briefly, and for convenience those which have been reported in maize and other known cases will be considered separately.

PLANTS OTHER THAN MAIZE.—A large number of more or less distinct color patterns have been shown to behave as simple Mendelian recessives. Albino seedlings, devoid of chlorophyll and which consequently die in the seedling stage, have been reported in *Antirrhinum latifolium* and *Melandrium album* by BAUR (3), in *Hordeum distichum* by KIESSLING (38), and in *Phaseolus vulgaris* by TJEBBES and KOOIMAN (55). Pale green seedlings have been described in *Urtica pilulifera* by CORRENS (6), and in *Ipomoea hederacea* by MIYAZAWA (49). Yellowish green seedlings have been reported in *Mirabilis jalapa xantha* by CORRENS (7), and in *Nicotiana rustica* by ALLARD (1). Various types of chlorophyll variegation have also been shown to be inherited as simple Mendelian recessives: in *Aquilegia vulgaris* by BAUR (3), in *Pisum arvense* by KAJANUS (37), and in *Capsella bursa-pastoris* and *Arabis albida* by CORRENS (8).

In other cases similar characters do not seem to be inherited in a Mendelian fashion, but are transmitted from one generation to the next through the female parent alone. Since the male parent does not seem to be definitely concerned in the transmission of the character, such cases have been called "maternal inheritance." The first case of this sort was described by CORRENS (6) in *Mirabilis jalapa albomaculata*. Plants of this strain produce branches having green leaves, others having white leaves, and still others with leaves which are partly green and partly white. All types of branches occur on the same plant, and all bear flowers. It was found that flowers from a green branch when self-fertilized produced only green seedlings in the following generation, and bred true thereafter. When crosses were made between flowers of green

and white branches occurring on the same plant, the resulting progeny always resembled the branch which produced the female gamete, regardless of the way in which the cross was made. The results obtained showed clearly that, so far as chlorophyll characters were concerned, the offspring were not affected by the pollen. CORRENS explained these results by assuming that the absence of chlorophyll was due to a cytoplasmic disease, which, although manifesting itself in the plastids, may or may not be limited to these organs. The diseased condition is accordingly transmitted from one generation to the next only through the egg cytoplasm, the male parent not affecting the character of the offspring, since no male cytoplasm is brought into the egg at the time of fertilization.

A situation similar to this was described by BAUR (3) in *Antirrhinum majus albomaculata* and *Aquilegia vulgaris*, and also by GREGORY (23) in *Primula sinensis*. These workers, however, are inclined to the view that two kinds of plastids, diseased and normal ones, become segregated during somatic mitoses to different cells and consequently to different regions of the plant tissue. This results in the variegated appearance common to plants of these strains. The diseased plastids are described by GREGORY (**23**, *pl. 10, fig. 10*) as being pale yellow and smaller than the normal plastids. In young actively growing leaf tissue both kinds are present in the same cell. Another interpretation should be placed on these figures of GREGORY, as will be discussed later.

A somewhat different case is that reported by BAUR (**2**) in *Pelargonium zonale albomarginata*. In this form plants occur which have green branches and entirely white branches. Flowers borne on either green or white branches when self-fertilized produce offspring in succeeding generations which are like the original branch. When, however, crosses are made between green and white branches, mosaic seedlings (green and white) result, regardless of the way in which the cross is made. This case differs from that of *Mirabilis* in that the inheritance is not the maternal type. Here both the male and female gametes must be concerned in the transmission of the character. BAUR is led to assume that plastids rather than the nucleus are directly responsible for this unusual type of inheritance, and that they are brought in by the male

gamete to the cytoplasm of the egg at the time of fertilization. This assumption is directly contradictory to that of CORRENS. BAUR further assumes that there are present in these plants two kinds of plastids, green and colorless ones, which are permanent cell organs and are sorted out and unequally distributed to daughter cells during somatic mitoses. Thus there results a segregation of green and colorless plastids in different parts of the plant, and this is held to account for the absence of chlorophyll in certain regions of the plant. BAUR (4) later reported similar cases in a strain of *Antirrhinum majus albomaculatum* and in *Aquilegia vulgaris*.

Additional evidence has been furnished by IKENO (36), who has worked with variegated races of *Capsicum annuum* and obtained results similar to those of BAUR in *Pelargonium*. Such strains of *Capsicum*, however, differ from *Pelargonium zonale albomarginata* in that all the plants produced show some degree of variegation, although green branches may occur. Furthermore, when pollen is taken from flowers on either variegated or green branches of a variegated strain and used to pollinate flowers on normal green plants, the resulting progeny are always variegated, although to a less degree than in the variegated parents. IKENO believes that this character is not controlled by the nucleus, but by plastids (diseased or normal) which are transmitted from one generation to the next by both parents. An apparently significant fact in this connection is that the two types of hereditary transmission, maternal and biparental, have not been found to occur in the same species. It is possible that this may mean that male cytoplasm regularly enters the egg in some species and not in others; but adequate cytological evidence for this is lacking.

MAIZE.—Numerous cases of chlorophyll inheritance have been reported in maize. Many distinct types have been described which differ greatly, not only in the mode of their inheritance, but also in the amount of chlorophyll present and the distribution and appearance of the pigment during the growth period. EMERSON (20) described several chlorophyll types, and presented evidence to show that albino seedlings conform to a Mendelian type of inheritance, and that the factor concerned is a simple Mendelian recessive. GERNERT (22) presented similar evidence. MILES (47)

furnished additional data on the inheritance of certain types of albinism, and as a result of a cytological investigation of the albino seedlings concluded that plastids are entirely absent in plants of that type. LINDSTROM (43), in a comprehensive study of chlorophyll inheritance in maize, described the behavior of eight distinct types, including those already reported on. In every case the inheritance was shown to be Mendelian. Two of these types, albino and yellow, are seedling characters, the plants failing to mature because of the absence of a sufficient amount of chlorophyll. In a third type, known as "virescent," the seedlings at first are yellowish white, but later become green, and in the mature condition cannot be distinguished from normal green plants. The five other characters described by LINDSTROM as "golden," "greenish-striped," "japonica white striped," "japonica yellow striped," and "fine striped," are manifested only in the mature plants; the young seedlings have a wholly normal appearance. From these studies LINDSTROM concluded that plastid inheritance in maize is typically Mendelian.

At the present time, however, cases are known in which the inheritance of certain aberrant chlorophyll types is not Mendelian. ANDERSON, in genetic studies as yet unpublished, has found that in a certain strain of maize there are produced some plants which are uniformly green, others uniformly yellowish green without sufficient chlorophyll to reach maturity, and still others with distinct yellowish green and green stripes.[1] Breeding experiments so far carried out have failed to show any inheritance of the unusual character through the male parent. The plants which are yellowish green lack a sufficient amount of chlorophyll to reach maturity, and no offspring have been obtained from them. The striped plants, either when self-fertilized or when pollen is used from a green plant of an unaffected strain, produce some green plants, some yellowish green plants, and some striped plants in varying proportions, which seemingly depend upon the amount of yellowish green tissue in the region of the plant producing the ear. Ears have been obtained which have produced only yellowish green

[1] Described in a paper read by E. G. ANDERSON before the Society of American Naturalists at Chicago, December, 1920. A published account is to appear shortly.

plants. The evidence indicates that this is a case of maternal inheritance. In no case has pollen from the affected plants of this strain produced any visible effect when used in crosses on green plants, either in the first or succeeding generations.

It is clearly evident from these cases that the inheritance of chlorophyll variations can hardly be accounted for on the basis of a single explanation. There are widely different categories which at present seem to be entirely distinct. In the case of the Mendelian behavior, the explanations offered are the same as for any other character behaving in a similar manner, although other cytoplasmic organs having a certain degree of individuality are apparently more directly involved in bringing the characters to expression. In this category characters expressed by intracellular organs are apparently under the control of nuclear factors, just as are other Mendelian characters expressed by tissues and multicellular organs. The hypotheses advanced to account for the non-Mendelian behavior of similar characters assume that the plastids themselves are permanent cell organs capable of transmitting certain characteristics, and are not controlled by nuclear factors; and furthermore, that in certain cases plastids are transmitted to succeeding generations by both parents, and in other cases only by the female parent. It must be admitted that such explanations are highly speculative, in view of the number of cytological observations which have so far been made.

METHODS AND TECHNIQUE

The general procedure followed has been to examine the material in the living condition, the observations thus made being supplemented by a study of fixed and stained preparations. It was found that meristematic tissue, including apical meristems and young leaf tips of germinating seeds, could readily be studied in this way. It was necessary in the case of older tissues to remove first the epidermal layer of cells before examining the mesophyll cells beneath. A solution of cane sugar of 7.5–10 per cent concentration was found to be a favorable medium in which to examine fresh material. Cells which are protected by one or two cell layers or by epidermal cells will remain living for a considerable period of

time, even in a water mount. Thus it was found that bits of embryonic (meristematic) tissue will remain living and apparently in an entirely normal condition for 24–36 hours in an isotonic solution of cane sugar. In most cases, however, observations were made immediately after making the mounts in order to avoid the possibilty of any modification of the cell contents. Intra-vitam stains, such as neutral red, Cresyl blue, Janus green B, etc., have been used, but unexpectedly proved to be of relatively little value. Various methods of fixation and staining were used, including those of BENDA, REGAUD, CHAMPY, LAGUESSE, BENSLEY, and other special methods which have been reported as being useful in such studies.

Description

The chlorophyll types here described do not include all the known cases of chlorophyll abnormality in maize, but have been selected as representative of certain distinct categories, illustrating markedly different kinds of behavior, both in development and in inheritance. These types are discussed in genetical literature as "normal green," "Mendelian white," and "Mendelian virescent." The "maternal inheritance strain," discovered by ANDERSON, has not yet been described in the literature.

The development of plastids may readily be followed in the meristematic and mesophyll tissues of young rapidly growing seedlings. In the growth stages immediately following the germination of the seeds, leaf tissue is being formed through the activity of the apical stem meristem, and the embryonic leaves thus formed are increasing rapidly in size through an active division of the cells making up the leaf meristem and through a division and growth of the cells throughout the leaf tissue. The meristematic tissues during these and later stages furnish very favorable material for a study of the cytoplasmic inclusions of the living cells. Since it has been found that the cytoplasmic inclusions of the epidermal cells, as well as those of the vascular system, differ markedly from those of the mesophyll cells in mature leaf tissue, the study of plastid development has been limited to the regions from which mesophyll cells are derived.

NORMAL GREEN STRAIN

Detailed attention will be given to the development of plastids in a normal green plant, and in the consideration of the other types emphasis will be placed on the points in which such types differ from the normal plants.

APPEARANCE IN EMBRYONIC CELLS.—The youngest stages examined were those found in the cells of the promeristematic region of seedlings 36–48 hours after germination. This region occupies the apex of the stem, and is composed of undifferentiated parenchymatous cells which are actively undergoing division in all planes. In undifferentiated tissue in all cases the observations were made on subepidermal cells. In such cells the cytoplasm contains minute granules which may clearly be seen without the aid of special staining methods (fig. 1). These granules appear in the living cell as refringent globules, which are constantly changing their position in the cell as a result of cytoplasmic streaming; this movement seems to be a constant and characteristic feature of actively dividing embryonic cells. The size of these bodies is variable. In the promeristematic region they are rarely more than 1 μ in diameter, and careful observation reveals a closely intergrading series between these and other smaller ones lying just within the lower limit of visibility. Whatever their size, these bodies always appear sharply distinct from the homogeneous cytoplasm in which they are imbedded, and in this material cannot be interpreted as granular constituents of the cytoplasm itself. At this early stage the cytoplasm appears to be entirely free from other accumulations which might be confused with these small bodies.

Appearances which might be interpreted as division stages are frequently seen, both in the living cells and in fixed and stained preparations. In the living cells a condition which strongly suggests division by a simple constriction and bipartition of these bodies is frequently noted, but their extremely minute size and their constant movement make it impossible to follow a single granule for a sufficient length of time to obtain direct evidence for its division. Moreover, chance association and separation are of frequent occurrence, especially in cells displaying active cyto-

plasmic streaming. Association may be but momentary, or granules which have been seen to come together may remain attached for a considerable period of time. Thus when a separation is seen to occur, it is not possible to tell whether one is observing an actual division of a single granule, or a reseparation of two granules formerly distinct. The fact that a paired arrangement occurs much more frequently than do groups of three or groups of four might be considered as suggestive, but in view of the observed behavior described, this can scarcely be considered as convincing proof of division. This point is of interest in view of the undoubted division of similar bodies which have become larger in older cells. The marked variation in the size of these bodies and the fact that evidence for their division in these early stages is uncertain raise the question of their nature, a question which will be taken up in the discussion.

Observations on the living cells afford no evidence for the existence of more than one kind of body in the class of cell elements under discussion. An appearance suggesting the presence of two kinds of bodies, lighter and darker ones, is seen when the cells are examined with lenses of high magnification. This, however, is due to their relative position in the cytoplasm. In the living cells these minute bodies are continually in motion, and as they pass into the plane of focus first appear faintly colored, but when sharply in focus they are much darker in color, then become lighter again as they pass out of focus. Thus a single granule may appear light or dark depending on its position in the cytoplasm. The use of intra-vitam stains, such as are commonly employed in studies of cytoplasmic organization, and the fixing and staining methods which have been shown by recent workers to preserve the cytoplasm faithfully with its various inclusions, have also failed to differentiate distinct kinds. Treatment with osmic acid does not appreciably alter the appearance of these bodies (fig. 52). When treated with BENDA'S fixation and stained with haematoxylin, the cytoplasmic inclusions are well preserved, and their appearance is similar to that of living cells (fig. 53). Since these bodies have been found to occur as a constant feature of the cytoplasm of meristematic cells in maize, and inasmuch as they have been found to be definitely

concerned with the formation of chloroplasts, the term "proplastid" will be used for such bodies. Other cytoplasmic granules and transitory accumulations not concerned with the development of plastids may be present later. Oil globules which can be distinguished from the proplastids by their characteristic highly refractive appearance and microchemical reaction, and other metaplasmic masses in the vacuole (fig. 5) are to be found at certain stages. The chemical nature and significance of these bodies are not known, and a detailed consideration of this phase of the problem is hardly within the scope of the present work.

The position and behavior of the proplastids during cell division have been observed in the living cells. There is apparent no definite sorting out of equal numbers to daughter cells, or more active division of individuals during this period. The proplastids are grouped at opposite ends of the cell during mitosis, and their passive distribution to the daughter cells seems to depend wholly upon their chance positions in the cytoplasm at the time of cell-plate formation.

PLASTID DEVELOPMENT.—The formation of mature functional plastids from minute granular proplastids may readily be followed in the subepidermal cells of the tips of leaf buds forming from the apical meristem. The earlier transitional stages are present in the meristematic cells near the apices of successively older seedling leaves which are growing rapidly, and which are not yet exposed to sunlight. Later stages are found in the leaves which are about to emerge from the surrounding sheath. In passing from the tip of such a leaf toward a point somewhat below the tip a series of stages may be observed. Numerous well developed plastids are present in the mesophyll cells of fully exposed seedling leaves.

In leaf tissue recently formed from the apical meristem the cells contain some proplastids which have increased noticeably in size, and others similar in size and appearance to those found in younger cells (fig. 2). Vacuoles are present in these cells, and the cytoplasm becomes more or less limited to the region surrounding the nucleus and to the periphery of the cell, with connecting strands between. Cytoplasmic streaming, which is commonly active at these stages, carries the proplastids about through the cell, even the largest ones being translocated in this manner.

In somewhat older stages (figs. 3, 4) the largest bodies remain stationary or nearly so, and are not affected by the cytoplasmic streaming. A grouping of proplastids of various sizes is often seen in certain parts of the cell. This most frequently occurs in the parietal portion of the cytoplasm, whereas in certain other cells there may be a grouping of almost all of the proplastids about the nucleus (fig. 51). In the present study these and other modes of grouping have not been correlated with any other phenomenon of cell activity.

Evidence for the division of the developing plastids is not difficult to obtain in the later stages (fig. 5). Cytoplasmic streaming is not so active in such cells, and the proplastids which have reached a size of 2 μ or over may frequently be seen in the process of division.

A variation in the size of the proplastids which are present in a single cell is markedly characteristic of the tissues studied. In such a cell as that of fig. 6 there is a close intergrading series, from proplastids which are so small as to be barely visible, up to others which have a diameter of 4.5–5 μ. The largest proplastids of this cell appear faintly green. The presence of these transitional stages within a single cell furnishes convincing proof of the origin of chloroplasts from bodies which at first are scarcely visible, and which one is led to believe may even arise *de novo* from the cytoplasm. In no case has the presence of green color been found to be associated with the proplastids until they have reached approximately one-half of the size and shape of mature functional plastids. The appearance of starch in the developing proplastids, which sometimes occurs, is perhaps associated in some way with chlorophyll formation.

The final stages in the maturing of chloroplasts may be observed in the cells near the tip of a seedling leaf which is about to emerge from the enveloping sheath (figs. 7, 8). Subepidermal cells near the apex contain a small number of partially developed plastids in which chlorophyll is present, and many smaller proplastids of varying size (fig. 7). Farther back from the tip the mesophyll cells contain an increasingly large number of nearly mature plastids, the majority of which are markedly green, and a diminishing number of proplastids (fig. 8). Finally, in the cells of

mesophyll tissue of well developed seedling leaves which are exposed to sunlight the enlarging plastids have attained a diameter of 7–8 μ. In such cells the plastids are closely packed in the parietal layer of cytoplasm, and are intensely green (fig. 9). In these cells, in addition to the larger plastids there are present a certain number of smaller bodies of varying size which cannot be distinguished from the proplastids found in the earlier stages. This fact seems to indicate that such structures represent partially developed plastids and not bodies of a separate category, as some observers have believed. Thus not only meristematic cells, but cells of mature mesophyll tissue as well, contain proplastids in various stages of enlargement, as well as plastids which have attained the maximum size and color.

The condition found in the mesophyll cells of the leaf tissue of a mature plant is very similar to that just described for fully green seedling leaves. In the former the plastids are slightly larger in the exposed portion of the leaf blade, and the number of proplastids is somewhat smaller than in the latter. In portions of mature leaves, however, which are protected from sunlight by other enveloping leaves or leaf sheaths, the development of the proplastids has not progressed as far as in the exposed portion of the leaf, regardless of the fact that the tissue in question is structurally mature. Chlorophyll is almost or entirely absent in such cells. In passing outward toward the green portion of the leaf, the proplastids become progressively larger, and there is a relatively smaller number of more minute primordia. Chlorophyll makes its appearance with the development of the plastids. Figs. 42–46 illustrate the transitional stages found in such a leaf, and the amount of shading used in representing the proplastids indicates the relative intensity of green color to be found in these stages.

There appears to be a close correlation between the development of plastids and the appearance of chlorophyll in the leaf tissue of normal green plants, whether in seedling stages or in the mature plant tissue. Maturing plastids 4–5 μ in diameter are very faintly green, and as they increase in size the intensity of the green color also increases, until the plastids have attained their maximum dimensions, when they are bright green.

EPIDERMAL CELL INCLUSIONS.—In maize the epidermal cells of leaves rarely contain plastids; if present at all they are few in number. Certain very characteristic structures are present, however, which will be discussed only briefly, inasmuch as they seem to have no direct bearing on the problem of chlorophyll development.

The epidermal cells of the meristematic region may be studied without removing them from the remainder of the leaf tissue. Their walls are not heavily cutinized during their early development, and the cells beneath are sufficiently transparent to permit a careful examination of cytoplasmic structures. The cytoplasm is limited to the parietal region of the cell, except for strands which pass through the large central vacuole to the region occupied by the nucleus near the inner wall. The cytoplasm of cells near the tip of embryonic leaves contains minute granules and short rods (fig. 47). These bodies are very numerous, and are carried rapidly about by the flowing cytoplasm, both rods and granules continually changing their positions. In older cells, that is, those farther removed from the tip, the rods are longer and more numerous (fig. 48). In still older cells many elongate filaments are to be found, as well as numerous granules and short rods (fig. 49). Cells have been observed in which even the elongate filaments are actively translocated by the cytoplasm; a single one may thus be observed for some time. This phenomenon furnishes definite proof that the filaments are structurally distinct elements, and not merely lines of flow, or other artifacts due to fixation. The fact that the filaments are longer and more numerous in the older cells than in meristematic cells suggests that they have resulted from an elongation of shorter ones, and that these in turn may have originated from the granular bodies. Convincing proof of their division has not been obtained. A small number of colorless bodies which resemble the partly developed plastids of mesophyll cells are also found in these cells, but mature green plastids are rarely present. In addition to these structures, oil globules are present which may be distinguished by their greater refringence in living cells and by their characteristic reaction to osmic acid. The relation of these structures found in epidermal cells to the proplastids of mesophyll cells will be discussed later.

MENDELIAN WHITE STRAIN

In the "Mendelian white" strain studied there are completely green and completely white plants. Albinism is here inherited as a simple Mendelian recessive. The green plants are entirely like green plants of normal strains, so far as any visible structures are concerned. Microscopic examination of the cells reveals a series of stages in plastid development which is indistinguishable from the series observed in normal green plants already described. In the white (albino) seedlings proplastids are present in the early embryonic stages just as in the green plants (figs. 10–12). In older tissues (figs. 13–16), however, corresponding to similar regions in a green plant (figs. 7–9), there is a striking difference in the behavior of the proplastids. They increase in size very slowly and irregularly, and although the leaf tissue itself continues to grow and differentiate, doubtless at the expense of reserve food stored in the seed, the proplastids do not develop as rapidly, or in as large numbers, as in the tissues in the same stages of differentiation in a green plant. Granules measuring 0.8–1.2 μ in diameter are found just as in normal green tissue, but development beyond this stage occurs only in a few scattered instances. The few proplastids which do become larger frequently present an abnormal appearance (figs. 14–16). In the living cells they are colorless or nearly so, and may sometimes have a darker irregular mass near the periphery (fig. 15). When fixed according to BENDA'S method and stained with haematoxylin, they bear a striking resemblance to small nuclei, and the irregular mass within these bodies bears a striking resemblance to densely staining nucleoli. When the tissues have reached a stage comparable with that at which chlorophyll appears in a normal green plant, there are very few plastids as large as those in which chlorophyll first develops in the green plant, the cell being characterized rather by a large number of proplastids, a condition suggesting retarded development (fig. 16). There also occur irregularly shaped masses, giving the appearance of degenerating plastids which had become partly mature. Figs. 14 and 15 represent the appearance of the cytoplasmic bodies in mesophyll cells occurring between the vascular bundles of the leaf. Fig. 16 shows the condition which is found in a cell nearer the bundle.

Here the proplastids in general are larger and more numerous, and often faintly yellowish green. This appears to indicate that the first steps of chlorophyll elaboration are initiated in these cells.

Although not any of the plastids are normally green in most of the tissue of the leaf blade, there has been found at the extreme tip of the albino seedling leaf a limited region in which the cells contain green chloroplasts (figs. 17–19). In such a region, even within a single cell, are found transitional stages between colorless partially mature plastids and those which are fully green (fig. 18). Chlorophyll, therefore, is not entirely absent from the albino seedlings of the "Mendelian white" strain. In these albino seedlings, however, there is never developed a sufficient amount of chlorophyll to enable the plant to live beyond the seedling stage. Microscopic examination shows clearly that the initial structural basis for plastid development is present, chloroplasts which are normal in appearance being actually found in some cases. This latter condition never becomes general throughout the plant. It therefore seems clear that in this case failure of the plant to become green is not to be explained as the result of an absence of plastids or plastid primordia.

Mendelian virescent

A third category is "Mendelian virescent." In this strain the affected plants in the young seedling stage resemble those of the "Mendelian white" strain. The seedling leaf is at first white, but later nearly the entire leaf becomes green, the color deepening rapidly. Later formed seedling leaves are somewhat greener in the early stages, while leaves formed toward maturity are entirely green from the start.

In the virescent plants the early stages in the development of the plastids are similar in all respects to the corresponding stages in a normal green plant. So far as it is possible to tell, the number, size, and development of the proplastids is identical in the two cases (figs. 20–23). In the tips of the young leaves, as they increase in size and become exposed to sunlight, the growth of the proplastids and the development of chlorophyll proceed as in green plants (figs. 24–26, 32). In the main portion of the leaf blade, on the

other hand, the mesophyll tissue fails to become green as rapidly as the tissue near the tip, and the cells between the bundles are found to contain only partly developed proplastids (fig. 27). Cells nearer the vascular bundles contain larger plastids with more chlorophyll (fig. 28), while in cells adjoining the bundles the plastids have reached nearly the maximum size and color (fig. 29). In somewhat older leaf tissue which is gradually becoming green there is a corresponding increase in the size of the proplastids (figs. 30–32), and the resulting plastids eventually become fully green (fig. 32). Here, then, is a case in which the usual formation of chloroplasts with their pigment seems to be merely delayed, but, unlike the "Mendelian white" seedlings, development continues until the whole plant becomes fully green.

Maternal inheritance strain

In this strain there are plants of three kinds. Some are entirely green and others are uniformly yellowish green; a third class is made up of plants which show longitudinal stripes of the two colors. The number and character of such stripes are extremely variable. Some plants are distinguishable from normal green plants only by the presence of one or two stripes, which may be of any width, and which may be of a color only slightly lighter than normal green. On the other hand, the appearance of some plants is rendered most striking by a large number of stripes, which, although their yellowish green color varies in depth to a certain degree, are never white. Frequently a plant may be divided sharply and almost equally throughout its entire length into green and yellowish green halves. Again, plants are observed which are completely yellowish green, except for one or two fully green stripes. The light stripes, whatever the intensity of their color, are not always continuous from the base to the tip of the leaf. In such cases they may end more or less abruptly at any point in the leaf, or may begin at any point above the base and terminate anywhere between this point and the tip.

Cytological features.—With regard to the green plants, careful cytological examination has failed to reveal any differences between their cells and those of green plants of unaffected strains.

The cells of the yellowish green plants show a series of stages in the development of chloroplasts from minute proplastids which are similar in all respects to the stages in the green plants, with the exception of the maximum size and depth of color attained. The proplastids increase in size until many of them have become well differentiated plastids with a diameter of 4.0–5.5 μ (figs. 33–41), whereas the plastids in normal green plants have a diameter of 7–8 μ; very exceptionally the latter size may be attained (fig. 41). The plastids in the pale areas of the seedling leaves are fewer in number, and the proplastids are more numerous than in the corresponding cells of a green plant (fig. 39). A study of the living cells shows a variable behavior as regards the elaboration of chlorophyll in the young seedling leaves. In the mesophyll cells most of the plastids become faintly green as soon as they become partially mature (fig. 39), while in cells near the vascular bundles plastids are often observed in which the color has become nearly as deep as in a normal mature chloroplast (fig. 41). In cells somewhat removed from the bundles all gradations between the lighter and darker shades are seen (fig. 40). In addition to the uniformly colored plastids, there are others which show an uneven outline and an irregular distribution of the pigment within them. For example, the color is often confined to one or two regions within the plastid, the limits of these regions in some cases being clearly marked and in others very vague (fig. 39). Such appearances strongly suggest degeneration or other disturbances of a serious nature. Not only young cells, but cells which are completely matured, contain proplastids in various stages of enlargement.

The plastids are much more fully developed in the yellowish green plants than in the albino seedlings of the "Mendelian white" strain, but a sufficient amount of chlorophyll for continued growth fails to be developed, and the plants die in the seedling stage. They may become slightly larger, however, than the white seedlings of the Mendelian strain. In the yellowish green plants there is little correlation between the degree of development reached by the plastids and the amount of chlorophyll elaborated. There are abundant well differentiated plastids present but only a rela-

tively small amount of chlorophyll. The failure of the plants to become green cannot be ascribed to a failure of the plastids to develop, but rather to an absence of a sufficient amount of chlorophyll.

The striped plants of this strain possess varying amounts of green and yellowish green tissue, as already pointed out. To the naked eye the boundary between the green and yellowish green areas appears to be very sharp, but when viewed with the microscope there is seen a region of transition one to several cells in width, in which are found plastids showing many intermediate sizes and depths of color, even within a single cell. Although the transition regions may vary in width, careful search has so far never failed to reveal cells which are in some degree intermediate in character. In some cases there is a single transitional cell which may contain plastids of many shades of green, whereas in other cases there may be a series of several transitional cells in each of which all or nearly all of the plastids are of one intermediate shade. Fig. 50 illustrates the condition which is most frequently found to occur in transitional regions. The cells on the left are characteristic of yellowish green tissue, those on the right are typical of green tissue, the ones between contain plastids which vary greatly, both in size and in intensity of green pigment. In the light of such facts the inapplicability of hypotheses involving a simple sorting out of plastids of two completely distinct types by successive cell divisions is clearly evident, so far as the color types in the strain of maize under consideration are concerned. To this point we shall return.

Intra-vitam stains and the use of fixing and staining reactions commonly employed have as yet given no evidence that the class of cell elements described comprise bodies of more than one kind. It is hoped that further studies may contribute something to this phase of the problem.

Summarizing, all of the plant types examined have been found to be the same as regards the cytoplasmic inclusions of their meristematic cells. Minute proplastids of the same size, shape, and general appearance have been observed in the living cells of all the chlorophyll types of maize studied. Intra-vitam stains and the fixing and staining reactions which have been used by other

workers for the study of cytoplasmic structures have given no evidence for the assumption that the group of cell elements under investigation consists of bodies belonging to more than one class. The observed differences between the various types of plants lie in the subsequent behavior of the proplastids with reference to the development of chloroplasts. From a structural standpoint the cells of all the plant types appear to be initially alike. The differences which later appear seem rather to be dependent upon the relative amount of chlorophyll developed in organs, the primordia of which are present in all cells. It is probable, therefore, that the ultimate explanation of unusual types of behavior may involve functional rather than structural differences.

Discussion

BEARING OF RESULTS ON PLASTID INHERITANCE.—The hypotheses which have been advanced to explain plastid inheritance have been based chiefly on the breeding behavior of the plants. The question remains as to how far these hypotheses will be substantiated by cytological observations. For the most part such hypotheses involve the assumption that plastids are permanent cell organs, arising only by a division of others of their kind, either while they are in the form of minute primordia or after they have reached the mature stage. The fact that proplastid-like bodies are present in the early embryonic stages, and the well substantiated evidence that mature plastids arise from bodies which cannot be distinguished from these elements, however, do not seem to constitute sufficient proof of the theory that plastids are cell organs having an unbroken continuity through all stages of the life cycle. Observations suggest that plastids may also differentiate anew at certain times in the life of the cell, which is obviously of the highest importance in connection with the problem in hand, and will be fully discussed later.

The frequent occurrence of chlorophyll abnormalities has furnished abundant material for a study of plastid inheritance. Many cases have been reported, and the breeding behavior carefully studied. The inheritance of some of the known cases is clearly Mendelian, but other types are clearly non-Mendelian. Thus the

known cases of plastid inheritance do not appear to be in the same category, and as yet have not been explained by a common well substantiated hypothesis.

Examples of chlorophyll characters which are inherited as Mendelian recessives have been described by numerous workers. Among the researches dealing with such characters may be mentioned those of EMERSON and LINDSTROM in maize, and those of BAUR, KIESSLING, TJEBBES and KOOIMAN, MIYAZAWA, CORRENS, and ALLARD on a number of other plants. The hypothesis held to account for the transmission of these characters is the same as that upon which the behavior of other Mendelian characters has been explained. Although the character in question is expressed in the plastids, the cell activities which result in the appearance of the character seem to be under the control of nuclear factors. If the character is thus under the control of a nuclear factor, it is nevertheless of importance to determine whether plastids are present in colorless cells as well as in normal ones; and also to determine to what extent the plastid is a permanent cell organ multiplying by division, especially if it is desired to gain insight into the process through which the character is brought to expression.

MILES (47), in a cytological investigation of albino seedlings in maize, reported that in the pure white plants no plastids could be differentiated. This does not at all correspond with the findings of the writer. Partially matured plastids are present in all of the mesophyll tissue of the plant, and in certain regions a limited number of mature functional plastids are present in some cells. Plastids, therefore, are not entirely absent, but their development is permanently retarded in the white seedlings. In the virescent plants the normal development of the plastids is interfered with only in the seedling stage.

Other chlorophyll types in maize which are Mendelian appear to be normal in the seedling stage, but the character appears in the plant as it approaches maturity. In fact there are known in maize markedly different chlorophyll types, and it seems certain that additional ones will be described. These cases are of interest cytologically in that apparently there is a wide variation in the condition of the plastids in the affected plants.

The cases in which the inheritance of plastid characters is non-Mendelian present a very complex problem. Added significance is here attached to the plastids, and workers are led to assume that the plastids themselves in the main are responsible for the inheritance of the characters which are manifested in them. As in the case of the Mendelian plastid characters, this assumption involves the question of the origin and permanence of the plastids, to be discussed later. The cases of non-Mendelian inheritance of chlorophyll variation have been classified as (1) maternal and (2) biparental.

Attention has already been called in the introduction to the well known cases of maternal inheritance reported by BAUR, CORRENS, and GREGORY, as well as to that found in maize by ANDERSON, whose results have not yet been published. CORRENS assumes that in *Mirabilis* the absence of chlorophyll is due to a cytoplasmic disease which in some way affects the plastids. Two kinds of plastids are said to be present, green and colorless ones, whose segregation during the divisions of the somatic cells is assumed to explain the presence of "checkered" leaves, as well as the complete absence of color in entire leaves and branches. BAUR offers a somewhat different explanation for similar cases. He believes that there are two kinds of plastids, diseased and normal ones, present in the mature leaf tissue, both being permanent cell organs with a definite individuality. The primordia of the two kinds of plastids are supposed to be transmitted from one generation to the next through the cytoplasm of the egg. A somatic segregation of these primordia to different cells during the growth of the plant accounts for the green and white areas of the mature plant. The hypothetical nature of this explanation is to be admitted, inasmuch as different kinds of primordia have not been demonstrated in these plants, from which it follows that the postulated segregation has not been observed. An examination of the mesophyll cells of plants produced by the maternal inheritance strain in maize fails to lend support to either of these theories. The development of the plastids from granular proplastids has been traced carefully in the living cells, and the condition of the plastids in the leaf tissue of the seedlings and mature plants has been

studied thoroughly. A variation, not only in the size of the plastids, but in the amount of color pigment as well, is characteristic of all the plant types.

Two distinct kinds of plastids, normal (green) and chlorophyll-less ones do not occur in the strains of maize studied; the green and colorless plastids observed in these strains do not represent distinct categories at all. The yellowish green plants of the maternal inheritance strain produce some plastids which have a diameter equal to that of the plastids present in normal green plants, and the intensity of the green color may equal that of green plants. In general, however, the plastids are slightly smaller and paler in color. The striped yellowish green and green plants show no sharp segregation of two distinct kinds of plastids. Cells on the border line between the two regions contain plastids of varying sizes and intensities of color. A single cell in such a region often contains plastids showing all degrees of variation found in either yellowish green or green areas. Such intermediate conditions are prevalent in the transition region between the two kinds of tissue, and at no time can there be found two distinct kinds of plastids, either in different cells or in the same cell. GREGORY made a study of the breeding behavior of a strain of *Primula sinensis* which produced chlorotic plants. The experimental results showed the character to be maternal in its inheritance. In chlorotic cells the plastids were shown to be pale yellow and smaller than in cells of the green tissue, but in any individual cell of mature tissue the plastids were found to be all alike. In young actively growing cells, however, different kinds of plastids occur in the same cell, which are similar to the two kinds found in the green and chlorotic cells of the mature leaf. GREGORY uses this as evidence that both kinds occur together in embryonic tissue, and later become segregated to different regions of the plant, and concludes that the abnormality is localized in the plastids. There is, however, another interpretation to be placed on the variation in the size and color of the plastids present in the growing cells of the leaf. In maize there can be no doubt that this condition is due to the presence of different stages in the development of plastids of one kind. The association of plastids showing a great variation in

size and color in the same cell is characteristic of embryonic cells in normal green plants. Since it was in such cells that GREGORY reported the presence of the two supposedly different kinds of plastids, this interpretation seems to be a more plausible one for his observations. Convincing cytological evidence of the occurrence of two distinct kinds of plastids and their segregation to different regions of the plant is at present lacking. In view of the condition observed in maize, it is clearly evident that visible structural differences in meristematic cells cannot be held to account for the inheritance and development of chlorophyll patterns.

All of the plant types, both the Mendelian and maternal, have the same initial cell structure, so far as observations have gone. It has not been possible to distinguish different kinds of proplastids in the youngest cells examined, that is, those of the promeristematic tissue of very young stems and the meristematic regions of embryonic leaves. The actually observed differences lie in the subsequent behavior of the proplastids with reference to the evolution of plastids.

Whether the proplastids arise by division or *de novo*, they are found to be present in the meristems of all of the chlorophyll types. The fertilized eggs of the different strains have not been examined, but if they differ as regards their visible cytoplasmic inclusions, corresponding differences would certainly be expected in the undifferentiated embryonic tissues of these strains; but careful examination of such tissues has failed to reveal any differences. As already stated, therefore, the differences in such types seem to be due to different modes of subsequent behavior on the part of proplastids in different plants and in different regions of the same plant, rather than to initial proplastid differences in the fertilized eggs of the different types. This view is supported by the occurrence of striped plants in the maternal inheritance strain, and also in the Mendelian strains which are not here reported.

It is necessary to account for different kinds of behavior on the part of proplastids which are, so far as microscopic examination shows, initially alike. Since visible bodies of more than one kind cannot be seen segregating, it might be suggested that there is an invisible structural difference in the cytoplasm of the different

plant types, or even in different parts of the same plant (striped). Thus the difference between the green and white plants in both the maternal and Mendelian categories depends upon an invisible differentiation process in the cytoplasm of the different cells, which influences the course which the proplastids take in their development, and the degree of development reached. One is then led to inquire at what stage in the life cycle this differentiation, which may be regarded as a physiological process, occurs. In the case of the maternal types, the irregular distribution of the green and white plant-producing seeds on the ear suggests that it may occur in the formation of the egg, or even earlier in the development of the ear, because of the frequent presence of irregular patches of affected seeds. The occurrence of the differentiation at some stage in embryogeny would account for the striped plants, in early division stages for the plants with large areas of the pale green tissue, and in the later stages for the plants having smaller amounts of such tissue. In the Mendelian types differentiation which is probably of a different kind must occur at sporogenesis, like any other Mendelian character. The two types of behavior, the Mendelian and maternal, are not in the same category, and as yet cannot be explained by a common well substantiated hypothesis. It is hoped that further studies of cytoplasmic structure will furnish evidence of value in the solution of the problem, and show more clearly what phases of the problem are to be definitely assigned to the physiologist.

RELATION OF PROPLASTID TO CHONDRIOSOMES.—One of the most outstanding questions arising from the present study is that regarding the relation which may exist between the proplastid of the foregoing description and the minute cell elements which other investigators have described under the name of chondriosomes, mitochondria, etc. There is great confusion at the present time concerning the nature and significance of these elements. It is plain from a survey of the very extensive literature dealing with the cell elements of this general class that the opinions of the most competent observers are in conflict on many important points. It is probable that cell inclusions of many different kinds may have been designated by the term chondriosomes, and whether

any particular body falls under this class depends upon the definition employed.

The present study has shown beyond a doubt that plastids develop from minute primordia in the different types of maize under consideration. What has so far been determined regarding the nature of these primordia and the relation which they may bear to chondriosomes must be regarded as insufficient to warrant an extended critical review of this question. It is planned to continue the investigation of this point. Nevertheless a brief comparison with the observations of other investigators who have devoted special attention to the chondriosomes may contribute something toward a solution of this puzzling problem.

The development of plastids from granular primordia has been observed by many workers, but there is a wide diversity of opinion as to the relation of the primordia to other cytoplasmic inclusions. One group of workers maintains that plastids arise from chondriosomes (mitochondria) which are permanent cell organs, and which correspond to similar bodies described in animal cells (MAXIMOW, PENSA, LEWITSKI (**41**), GUILLIERMOND, EMBERGER). GUILLIERMOND (**24–34**), as a result of many researches on plant cells, believes that plastids arise from rodlike mitochondria (chondriokonts) and differ from the latter only in size and to a certain extent in chemical constitution. TWISS (**56**) reports that in fixed and stained root tip cells of *Zea Mays* an unbroken series from globular, ellipsoid, or short rod-shaped mitochondria to mature plastids can be traced from the embryonic region backward, and concludes that mitochondria are normal constituents of the cytoplasm. He believes, however, that the evidence for the division of the mitochondria, as well as that for their function in heredity, is inadequate. EMBERGER (**18**) reported that there are two kinds of bodies (young plastids and mitochondria) present in the cells of certain plants, which differ slightly in size and staining intensity; both are to be classed under the general term mitochondria. Other workers hold that the plastids do not come from chondriosomes, but from bodies distinct from them (**48, 50, 52, 53, 54**). A third group of workers (TISCHLER, VON DERSCHAU, etc.) contend that the plastids are ultimately of nuclear origin, since they arise from chromatic

bodies extruded from the nucleus. Still others question the relationship between plastids and chondriosomes. HARPER (35) states as follows:

> None of the evidence so far adduced as to the specific genetic relationship between chondriosomes and plant plastids is in any way adequate . . . . that in certain cells the plastids can be recognized as very small cytoplasmic bodies with no starch in them was adequately established by SCHIMPER, but that the plastid bodies necessarily and regularly arise from the chondriosomes it seems to me is by no means proved by such crude and diagrammatic figures and seriations as those so far presented.

Views similar to this have been held by many other workers (LUNDEGARDH **46**, LÖWSCHIN **44**, **45**, etc.).

From this brief review it is evident that there are, at present, many conflicting views in regard to the class of cell inclusions known as chondriosomes, and the relationship which exists between plastids in their initial stages and these bodies. It is furthermore apparent that this diversity of opinion is in some measure a consequence of the lack of agreement as to what constitutes permanent cytoplasmic organs, and as to what bodies should be included in the class "chondriosomes." The problem has been complicated further by the fact that certain observers have used a great variety of fixatives, which in some cases appear to have preserved the bodies in question with relatively little alteration, while in other cases artifacts have been produced which have led to various misconceptions.

No reader of chondriosome literature can fail to recognize the fact that much of the obscurity which surrounds the subject is due to the lack of uniformity in the application by various workers of the term chondriosome. By some the term has been applied very widely to a considerable variety of minute cell inclusions, some of which are seen to develop into plastids. Others are inclined to make a distinction between chondriosomes on the one hand and the primordia of plastids on the other. Still others further restrict the application of the term chondriosome, using it only with reference to a concrete class of cell inclusions which can be shown to have a particular histochemical constitution. Thus COWDRY (**10**) defines chondriosomes primarily as "substances which occur in the form of granules, rods, or filaments in almost all

living cells, which react positively to Janus green, and which, by their solubilities and staining reactions, resemble phospholipins and, to a lesser extent, albumins."

Noteworthy in this connection are the statements made by MOTTIER (**50**) as a result of his studies on the origin of plastids. In his first contribution on this subject leucoplasts and chloroplasts were shown to be derived from minute "plastid primordia." Other minute bodies present in all cells but not developing into plastids were referred to as chondriosomes. In a more recent paper (**51**), in which the development of protein bodies (aleurone grains) from minute primordia is reported in the endosperm cells of *Zea Mays* and other plants, emphasis is no longer placed upon this distinction between primordia and chondriosomes. It is stated that the latter, because of their obscure rôle, were previously called chondriosomes mainly for convenience. In view of the fact that the terms mitochondria and chondriosome have been so loosely applied to plastid primordia and many other cytoplasmic inclusions as well, MOTTIER does not use these terms in his most recent description, and even suggests that much ambiguity might be avoided by dropping the terms entirely.

The present writer has also felt that much confusion might result from the use of the term chondriosome in his description. In the material studied there appears to be but one class of cytoplasmic granules, as already stated, these granules representing the early stages in the development of plastids. In order to avoid any implication as to the ultimate nature of these bodies and their relation to other cytoplasmic inclusions described by various workers, they have been designated in the present paper by the term proplastid. Whether or not the proplastid is a chondriosome is a question which can be answered only when the histochemical nature and developmental history of cytoplasmic inclusions have become better known, and when a definite and uniform terminology has been settled upon. The proplastids can be readily observed in the living cells. They are clearly visible in the cells of the promeristematic tissue of young stems and embryonic leaves, and their activities in these cells and their subsequent behavior in older cells have been closely followed through all stages of their develop-

ment. Although they can be so studied in the living cells, it is nevertheless necessary to employ methods which will not only permit one to view the living cell for a moment in the "living" condition, but which will allow continued observation of the actively functioning cell over a considerable period of time, thus avoiding alteration in the cytoplasm which may be brought about very quickly when the living tissue is improperly handled.

The proplastids appear in their initial stages as refringent globules of varying size, always sharply distinct from the ground substance of the cytoplasm. They are constantly changing their position in the embryonic cells, being often carried about rapidly by the streaming cytoplasm. This phenomenon makes it appear certain that even the most minute of the proplastids are special structures distinct from the principal mass of cytoplasm in which they lie. Particular attention has been devoted to these minute proplastids which lie just within the lower limit of the range of visibility. They have been examined with lenses of very high resolving power (Zeiss apochromatic objective, 2 mm. N.A. 1.40 and compensating ocular 6), and their activity has been repeatedly watched very closely. Very important questions concerning the ultimate nature and significance of proplastids are here involved, and will be discussed in another connection later. The subsequent enlargement of the proplastids to form chloroplasts can be traced step by step throughout their course of development, abundant material being readily obtainable from young seedlings.

Although comparatively few investigators have based their interpretations mainly on the study of living cells, they agree with the present writer in reporting the presence of such minute bodies in the living meristematic cell. In older cells, however, many phenomena have been described which the present writer has not observed, although this may be due in part to the fact that this study has been limited largely to mesophyll cells. The few observations made on the cells of other tissues indicate that additional types of cell elements and modes of behavior may be present there. For example, conditions similar to those figured by GUILLIERMOND in epidermal cells of flower petals and other floral organs have been seen in the epidermal cells of maize, but not in the mesophyll cells.

Intra-vitam stains, a number of which have been employed, have unexpectedly failed to give a definite reaction to the proplastids. Such stains as Janus green B, violet of Dahlia, neutral red, etc., have given uncertain results. In some cases the proplastids have appeared to be faintly stained by Janus green B, but in no case has a strong staining reaction been observed, neither has it been found possible to differentiate different kinds of minute bodies in the cytoplasm of the cell. COWDRY (11) reported that plant mitochondria are stained with Janus green B, but that it is much more difficult to obtain a good stain in plant cells than in animal cells because of their well developed cellulose walls. A similar conclusion has been reached by other plant workers.

Chondriosomes in both plant and animal cells were first observed in fixed and stained preparations, and the lack of uniformity in the results of early workers may have been largely due to the use of different fixatives and alterations resulting from poor fixation, as has been pointed out above. The special methods of fixation and staining commonly used in a study of the cytoplasm have been fully described in recent literature (COWDRY 11, GUILLIERMOND 31, KINGSBURY 39). In general chondriosomes have been found to be preserved by potassium bichromate, chromic acid, neutral formalin, and osmic acid, and these are the chief ingredients of the fixatives used for their study. Such substances as alcohol, ether, chloroform, and acetic acid dissolve the chondriosomes or produce profound changes in their shape, etc.

The methods of fixation ordinarily employed often appear to preserve quite faithfully the structures in question, but direct observation on the living cell is unquestionably of far greater value, since it obviates the possibility of misinterpretation to a very large extent. Furthermore, in tissues which are favorable for a study of the living cells the cytoplasmic inclusions are sharply delimited, and actually are more easily observed than in fixed and stained cells. Conflicting results have been obtained by those who have attempted to demonstrate the existence of different kinds of cytoplasmic bodies by the use of various fixing and staining methods. The use of such methods in this study has failed to furnish convincing evidence for the presence of more than one type of proplastid in the initial stages.

Chondriosomes of widely different shapes have been observed, the commonest forms being the granular mitochondria and the rod or thread-shaped chondriokonts. These and other types are not looked upon as belonging to distinct classes by plant cytologists, but rather as transitional stages between granular elements and structures such as chloroplasts, chromoplasts, elaioplasts, etc., concerned in the elaborative functions of the cell. In animal cells the chondriosomes have been seen to undergo marked changes in shape (LEWIS and LEWIS 40), but it may be that such changes are more gradual in plants. The proplastids in maize are of a single uniform shape in the mesophyll cells, being granular in the early stages and spherical or ovoid in later ones. Rodlike elements are present in the epidermal cells, but not in the cells associated with chloroplast formation. This variation in the size, and the different reactions to certain methods of fixation and staining have been used by workers as evidence for the existence of different kinds of elements which can be distinguished in embryonic cells and which perform certain definite functions. Prominent workers maintain that plastid primordia can be distinguished from mitochondria at all stages in their development (RUDOLPH, MOTTIER, DANGEARD). This conclusion is based chiefly on size differences and on slight variations in fixing and staining reactions. This contention has been supported by the statement that unaltered mitochondria are present with the chloroplasts in mature cells, the inference being that all the plastid primordia have developed into plastids, while the mitochondria remained unchanged. The statements of MOTTIER already cited are noteworthy in this connection. The present study has shown that in maize these small bodies in the mature cells are without doubt to be regarded as plastids which are for the most part in a very retarded state of development. A sufficient number of intermediate stages between the smallest ones and mature plastids are present to make it appear certain that these bodies do not represent a distinct category unlike the proplastids of meristematic cells. This question of the possible existence of more than one type of initial granule has received critical attention throughout the present study, and inasmuch as the origin of the chloroplasts can be traced backward through

successively smaller stages to proplastids which lie just within the lower limit of visibility, there appears to be no justification for the view that more than one kind of initial granule is present in the meristematic cell. It is only through a study of these minute initial stages that many of the principal questions involving chondriosomes and plastids can be answered. These earliest stages seem to have received but scant attention from previous workers. It is to be emphasized that the evidence at hand leads directly to the conclusion that the various structures observed in later stages (green and white plastids) have arisen by a process of differentiation from bodies of one kind, rather than from bodies initially unlike. The cause of such a difference in behavior on the part of the primordia can only be conjectured.

INDIVIDUALITY OF PLASTIDS.—The proplastids have been found to be a constant feature of cytoplasmic organization in maize, and the question arises as to the origin of these bodies. The definiteness of their behavior in relation to the evolution of chloroplasts indicates that they are at least concerned in the elaboration of certain products of cell activity. Whether or not they may also be found to be associated with other vital functions remains as a problem for further research.

Plastid primordia have been considered by previous workers as essential constituents of the cytoplasm which retain their individuality and persist throughout the life cycle (FORENBACHER 21, PENSA, CAVERS 5, GUILLIERMOND, MOTTIER, EMBERGER). The evidence from primitive plants (ALLEN, SAPEHIN, SCHERRER), in which well developed plastids are present throughout the life cycle, may be considered as evidence favoring this view. The occurrence of division stages in partially developed chloroplasts suggests also that the smaller bodies may divide, but my observations indicate rather that such an assumption is hardly warranted. Appearances have frequently been found which suggest division stages, but definite proof of division is very difficult to obtain. The fact that a frequent association and subsequent separation of the proplastids occur in embryonic cells, as herein described, renders it unsafe to draw conclusions on this point. Even if it were shown that these minute bodies multiply by

division only, it would still remain to be shown whether or not the same would hold true for stages before the bodies have become large enough to be seen. The writer has repeatedly satisfied himself that the series of stages observed actually grades off to the lower limit of visibility.

In view of the uncertainty regarding the mode of plastid origin in young meristematic cells, it seems probable that the question of the genetic continuity of plastids would not be answered with finality by a study of the gametes by methods at our command. In the present research the gametes have not been observed. If it were found that the fertilized egg contains no visible proplastids, the presumption would be strong in favor of the *de novo* origin of these bodies in embryonic cells. It is conceivable, however, that proplastids too minute to be observed might nevertheless be present and be multiplying only by division. On the other hand, if the fertilized eggs were found to contain proplastids, the mode of their origin and multiplication would probably be no easier to determine than in older meristematic cells which have been studied and described. It therefore seems that the impossibility of determining the mode of proplastid origin in meristematic cells requires that the question of the continuity of plastids shall remain an open one, regardless of any condition which one might expect to observe in the gametes. In view of these facts, explanation of plastid behavior which are based on the assumption of a complete individuality on the part of these organs are unsound. The only other alternative is that of *de novo* origin. The conception of the cytoplasm as a substance in which certain processes become localized, with the accompanying new differentiation of regions which are cell organs, has been furnished with a clear statement by HARPER (**35**), as follows:

> What seems to me the most important advance in our knowledge of cell architecture has been in the direction of the recognition of localized spatially differentiated regions of the cell body in which certain processes occur . . . . the plastid is to be regarded as a region of the protoplasmic complex rather than a differentiated and definitely delimited body . . . . cytologically the chloroplast is perhaps little more than an area of the cytoplasm impregnated or infiltrated with chlorophyll.

The possibility of the *de novo* origin of cell organs is one which should be more generally recognized. With regard to the plastids it must be admitted that the evidence so far obtained by cytologists does not permit a definite decision in favor of either the *de novo* or the individuality theory. The exact manner in which plastids originate in the cell is obviously of the greatest importance to those who are searching for the explanation of the behavior of inherited characters which manifest themselves in these organs. If plastids are not passed on as permanent individuals, some other explanation must be offered for their repeated appearance and regular behavior in successive generations.

Summary

1. All the chlorophyll types examined were found to contain the same initial cell structure, minute "proplastids" of the same size and general appearance being present in every type.

2. In normal green plants the proplastid first appears in the cell as a minute granule at the limit of visibility, gradually enlarging and developing chlorophyll until it becomes a mature chloroplast. In plants of the other chlorophyll types studied (Mendelian white, Mendelian virescent, and the maternal inheritance strain) the unusual characters of the plants are due to the failure of the proplastids initially present to develop into plastids with the normal size, or color, or both.

3. The green and colorless plastids found in different plants or in different portions of the same plant do not represent two fundamentally distinct types, but are rather to be regarded as the end members of a continuous series which comprises also all intermediate conditions. No cytological evidence was found favoring the view that the primordia from which the variously developed plastids arise are of more than one kind.

4. Partially developed and fully matured plastids may be seen multiplying by division, but when first visible the proplastid is so minute that it is impossible to determine the mode of its origin. The division of partially mature and mature plastids emphasizes the fact that they have a distinct individuality at such stages;

but in view of the obscurity which surrounds the origin of the minute primordia from which the plastids first appearing in the embryonic cells arise, the question regarding the extent to which the plastids are to be considered permanent cell organs with an unbroken genetic continuity throughout the life cycle must remain an open one.

5. In the case of those strains in which the inherited characters are transmitted according to Mendelian rules, it is inferred that the behavior of the proplastid is at least in part under the control of the nuclear mechanism. In those strains in which the inheritance of the unusual characters is non-Mendelian (maternal strains), it is very probable that an explanation of another kind will be found necessary.

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EXPLANATION OF PLATES XI–XVI

All figures were drawn at the level of the table with the aid of an Abbé camera lucida under a Spencer objective, 1.8 mm., N.A. 1.25, with compensating ocular 18. They have been reduced approximately one-third and show a magnification of about 2250 diameters. Observations of critical stages were made with a Zeiss apochromatic objective, 2 mm., N.A. 1.40, with compensating ocular 6.

PLATE XI

Normal green plant

Except where noted, drawings are of living cells of subepidermal tissue from mesophyll region of leaf, or of meristematic cells which will later become part of mesophyll tissue.

FIG. 1.—From promeristematic region of germinating seed.

FIG. 2.—From tip of first leaf bud formed from apex of stem; median section.

FIGS. 3–6.—Developmental stages from meristematic tips of successively older embryonic leaves; figs. 3–5, median sections; fig. 6, section through cytoplasmic layer just beneath cell wall; largest proplastids in fig. 6 faintly green.

FIGS. 7, 8.—From 16 mm. embryonic leaf just before it emerges from sheath, showing cytoplasmic layer beneath cell wall in face view.

FIG. 7.—Cell near tip, green color appearing in proplastids.

FIG. 8.—Cell somewhat farther back from tip, proplastids more fully developed.

FIG. 9.—From mesophyll tissue of fully developed seedling leaf, plastids bright green.

PLATE XII

Mendelian white seedling

Amount of green color present in chloroplasts after they have reached a size of 2.5 μ indicated by depth of shading; stages represented in this series taken from a 3-inch seedling about six days after germination.

FIGS. 10–12.—Successively older stages in development of proplastids in undifferentiated meristematic tissue; cells in median section; fig. 10, promeristematic cell from stem tip; fig. 11, from tip of leaf bud recently formed from apex of stem; fig. 12, from tip of 18 mm. embryonic leaf.

FIGS. 13–16.—From different regions of 35 mm. leaf just before emerging from enveloping leaves; fig. 13, undifferentiated cell near tip (note abnormal appearance of some of proplastids); irregular shape and presence of darker regions within proplastids characteristic of certain ones; figs. 14, 15, cells 3–5 mm. from tip; fig. 14, green color entirely absent in all proplastids; fig. 15, faint greenish color in one proplastid; others show conditions suggesting degeneration; fig. 16, typical mesophyll cell of seedling leaf; slight trace of yellowish green color in some of proplastids; others almost colorless or containing irregular opaque masses.

FIGS. 17–19.—Cells from tip of unfolding seedling leaf.

FIG. 17.—Undifferentiated cell at tip of leaf; largest proplastids faintly green.

FIG. 18.—Cell 3 mm. from tip; larger proplastids (plastids) clearly green.

FIG. 19.—Mesophyll cell 4.5 mm. from tip; plastids bright green.

PLATE XIII

Mendelian virescent

Depth of shading indicates intensity of green color in later stages of plastid development; material of same age as in the case of Mendelian white.

FIGS. 20–23.—From promeristematic and embryonic leaf tissue corresponding to similar early stages in green plant.

FIGS. 24–26, 32.—From meristematic undifferentiated regions of successively older leaf tips; figs. 24–26, before exposure to sunlight; fig. 32, after exposure to sunlight.

FIGS. 27–29.—Cells from pale green area of seedling leaf.

FIG. 27.—Mesophyll cell between vascular bundles.

FIG. 28.—Mesophyll cell nearer vascular bundle.

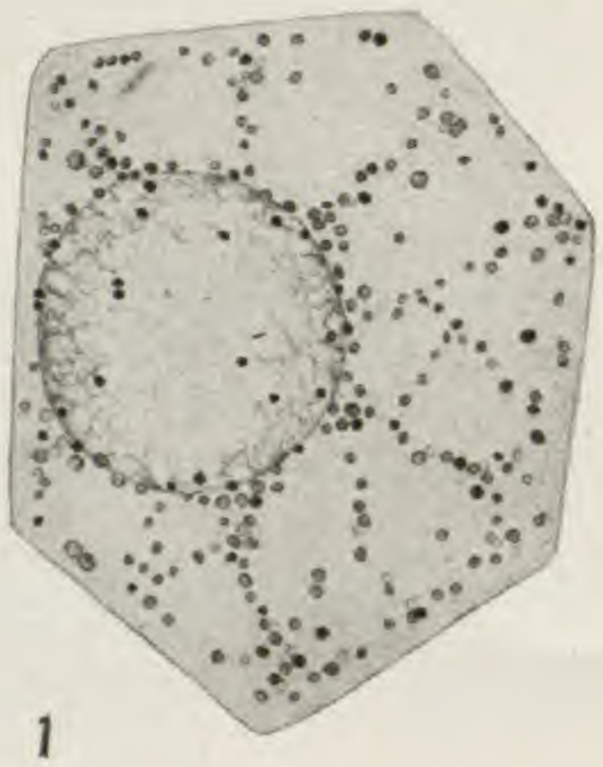
FIG. 29.—Cell lying next to vascular bundle.

FIGS. 30–32.—From transition region between pale green and green areas of seedling leaf.

FIG. 30.—Mesophyll cell from slightly green region.

FIG. 31.—From deeper green region.

FIG. 32.—Cell from apex of green leaf.



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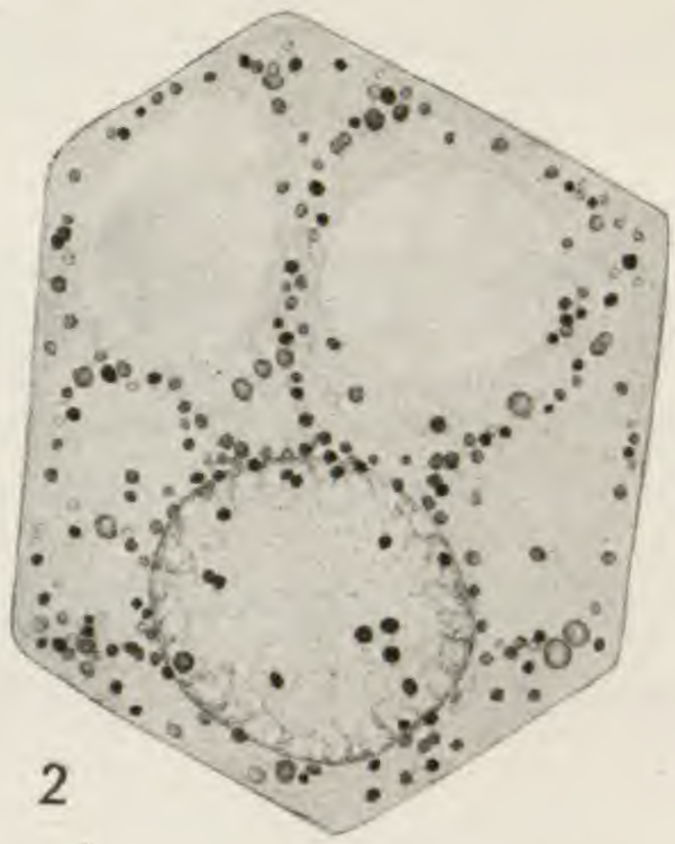

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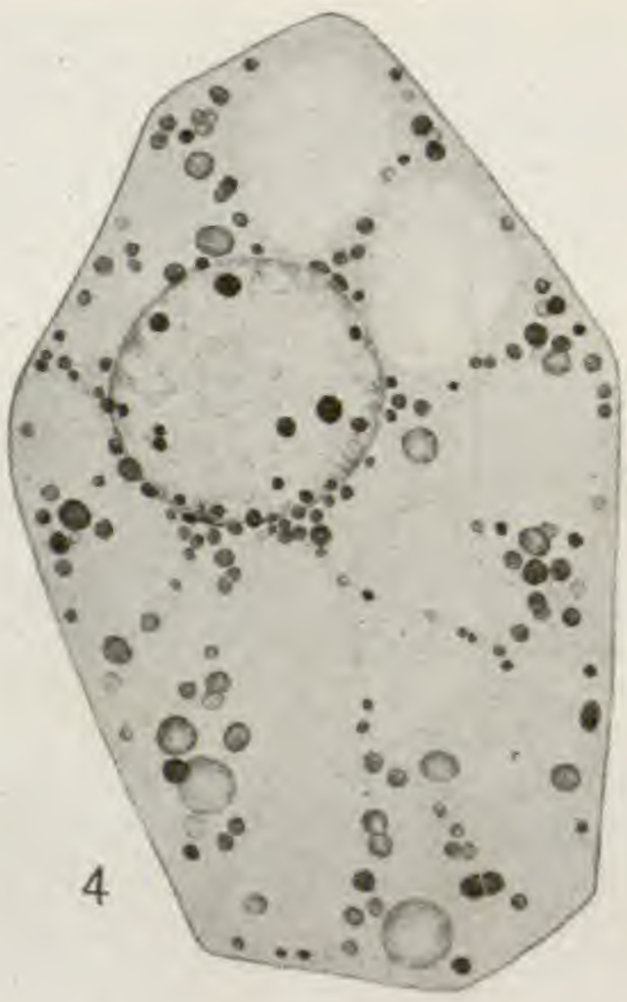

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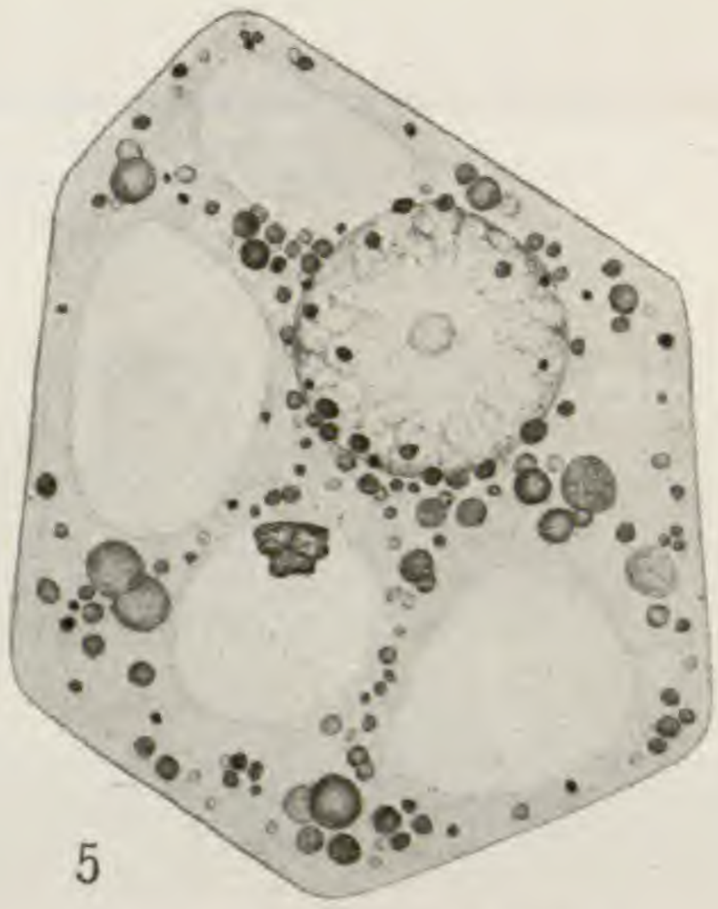

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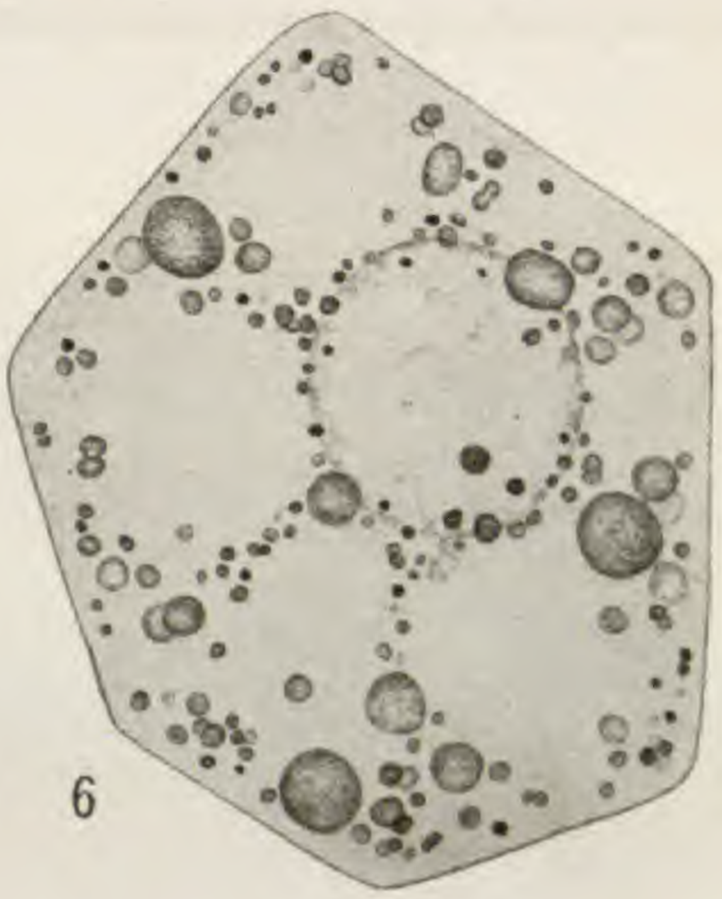

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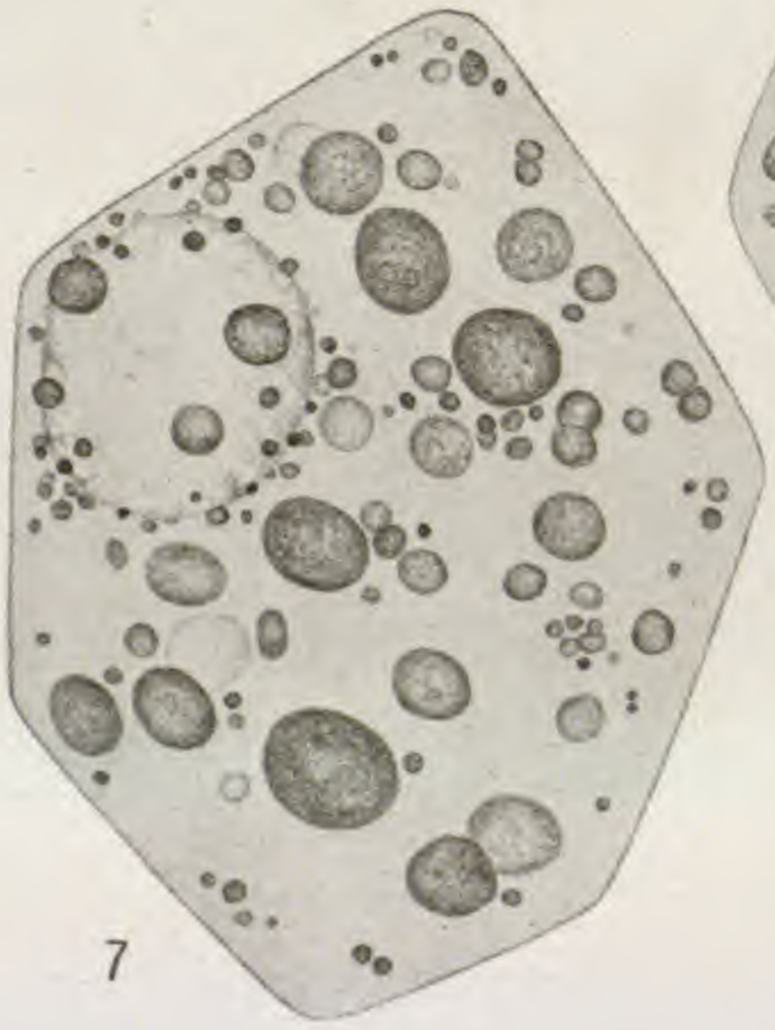

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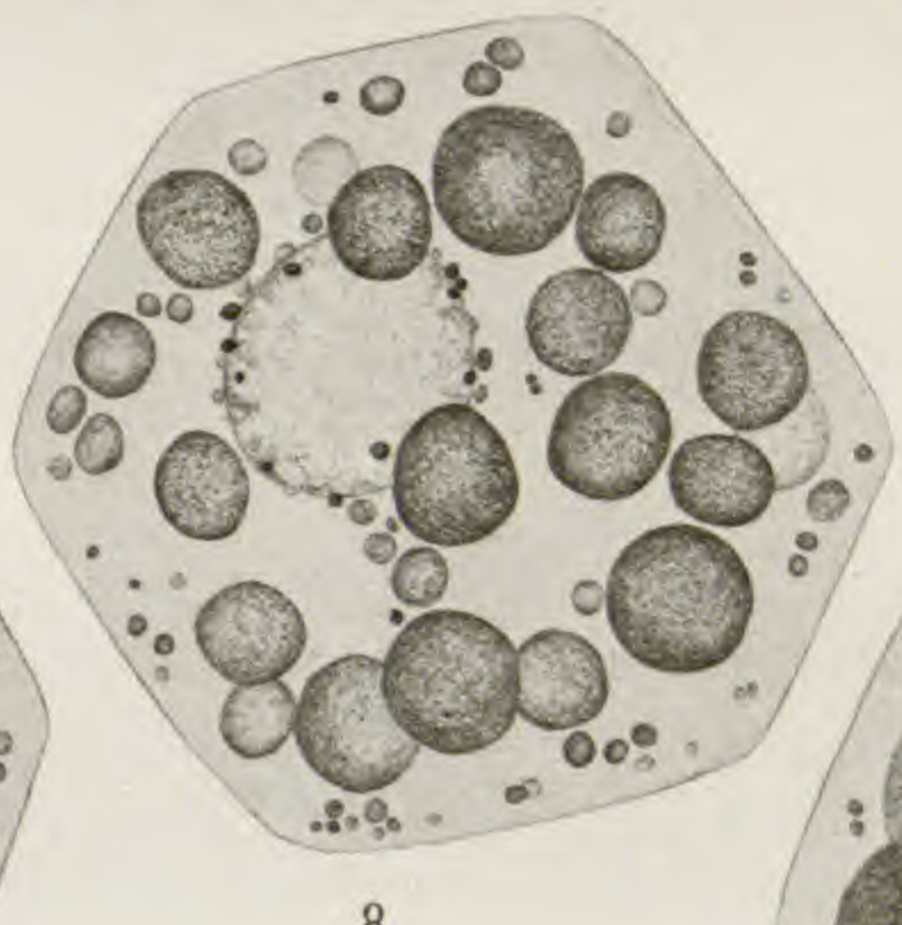

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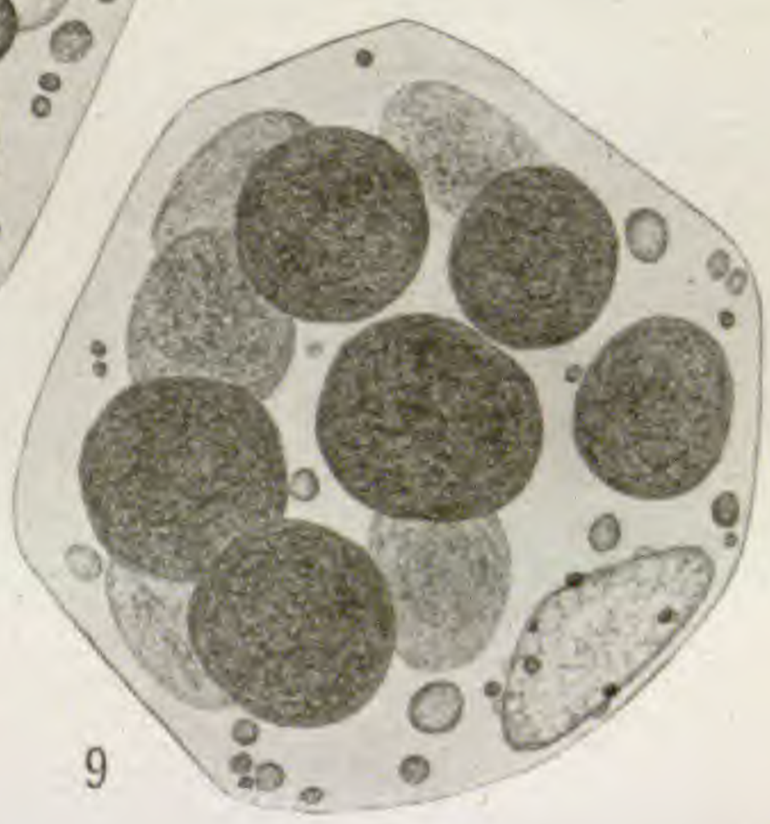

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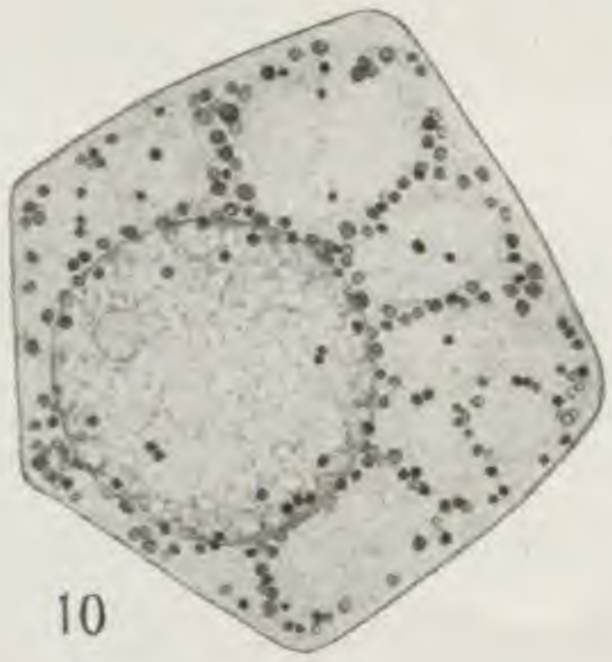

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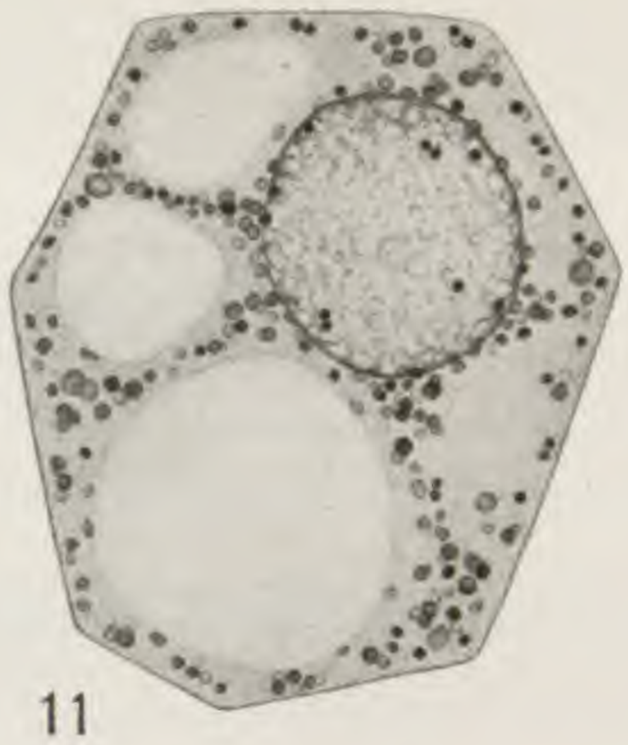

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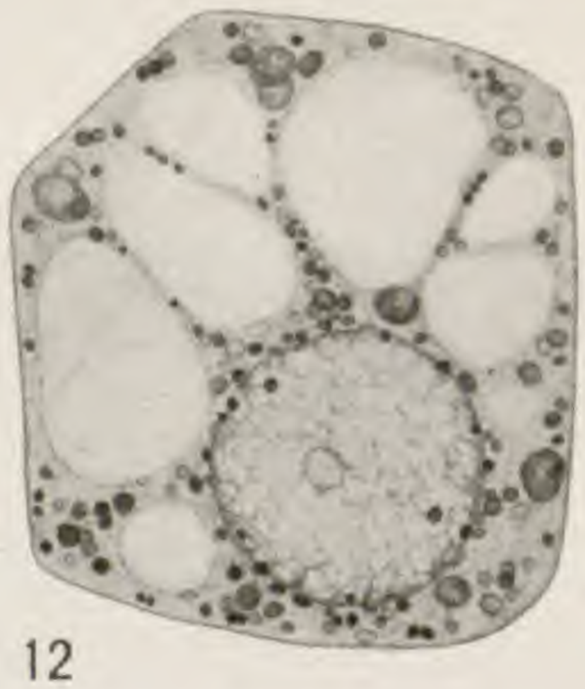

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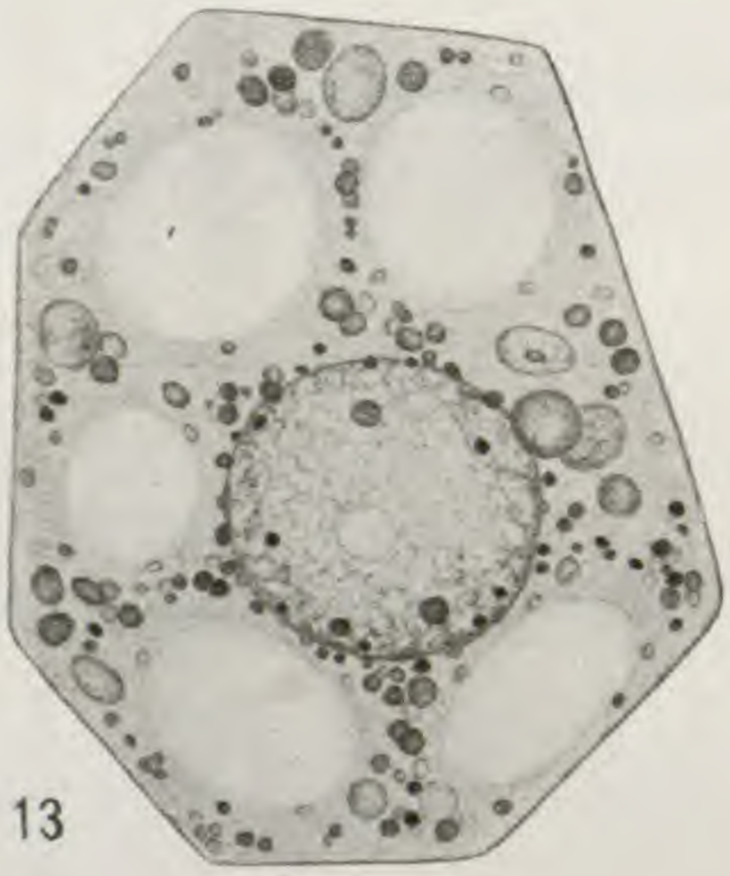

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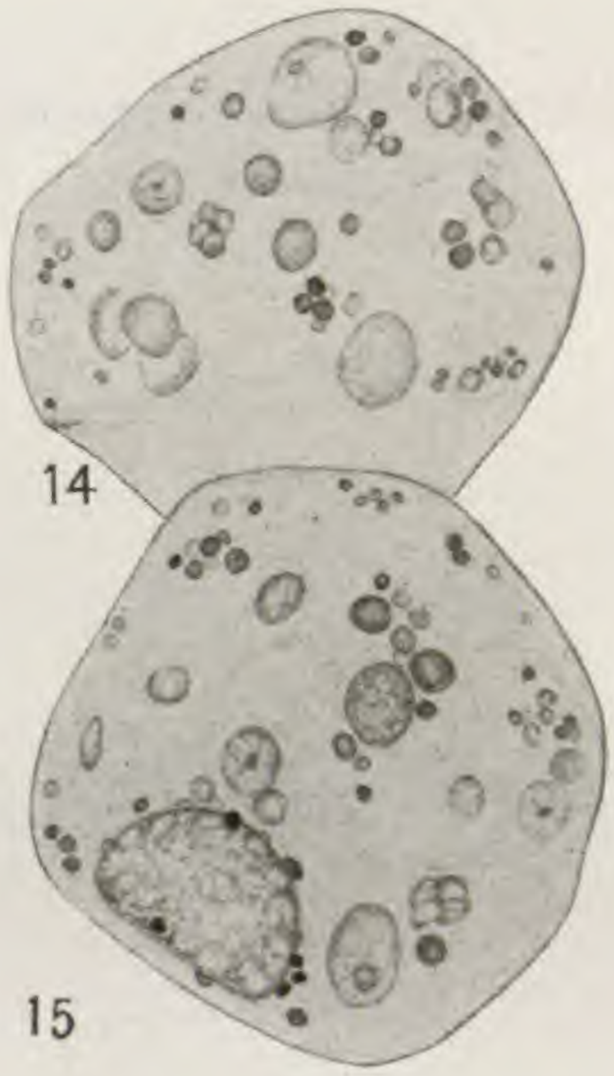

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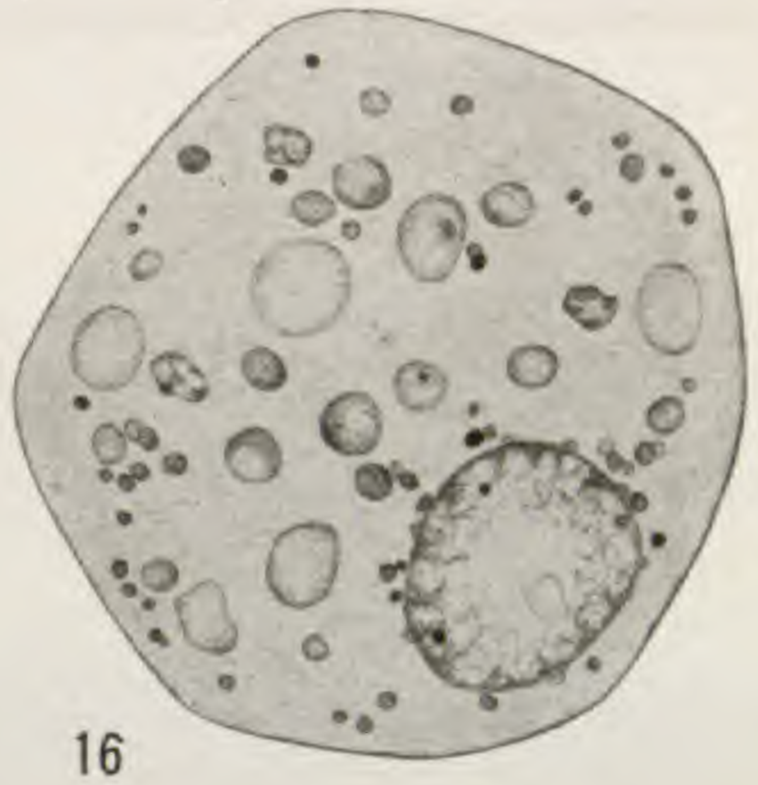

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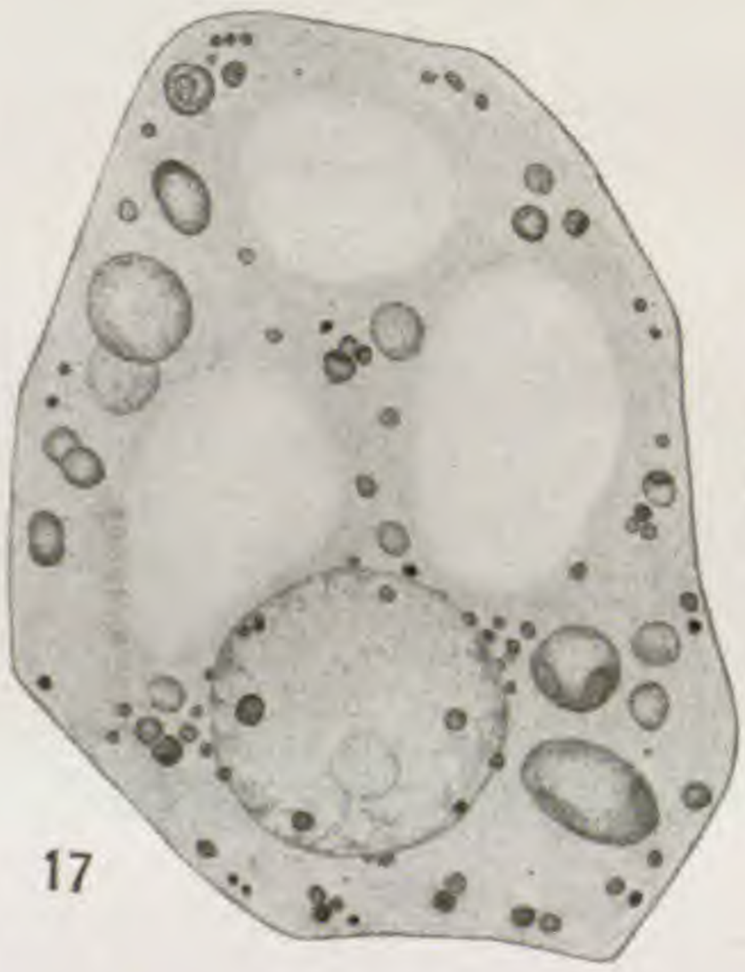

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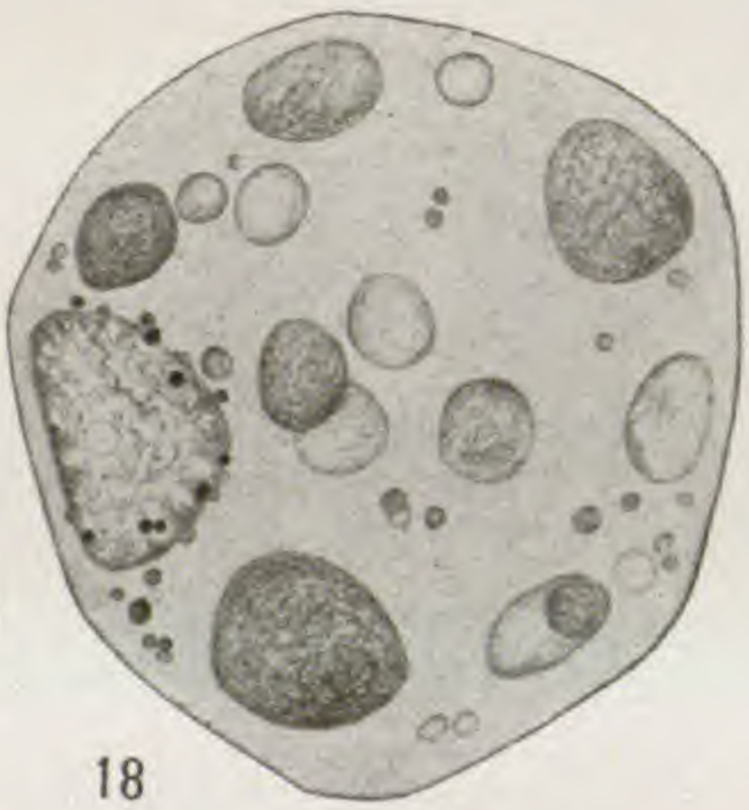

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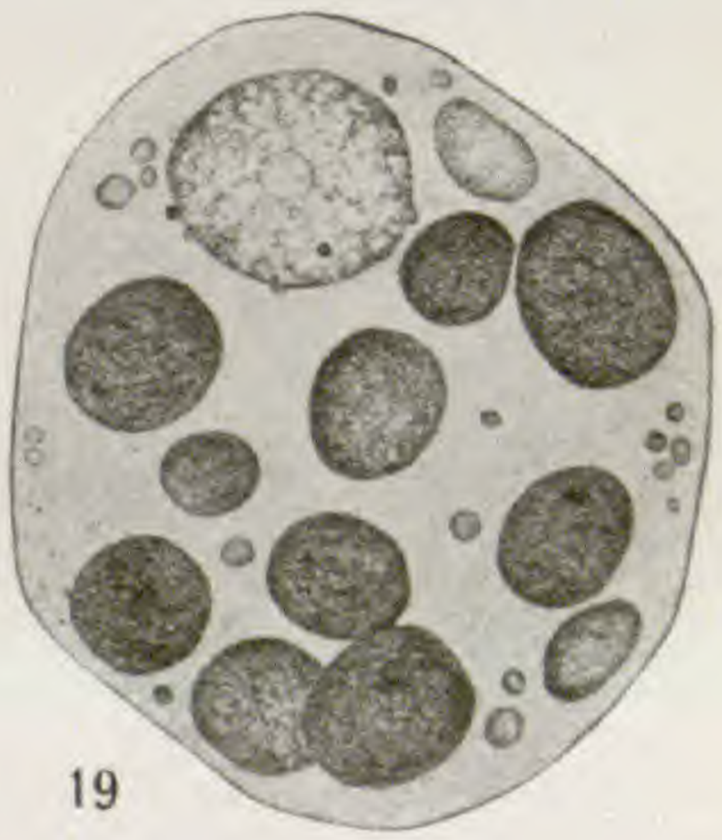

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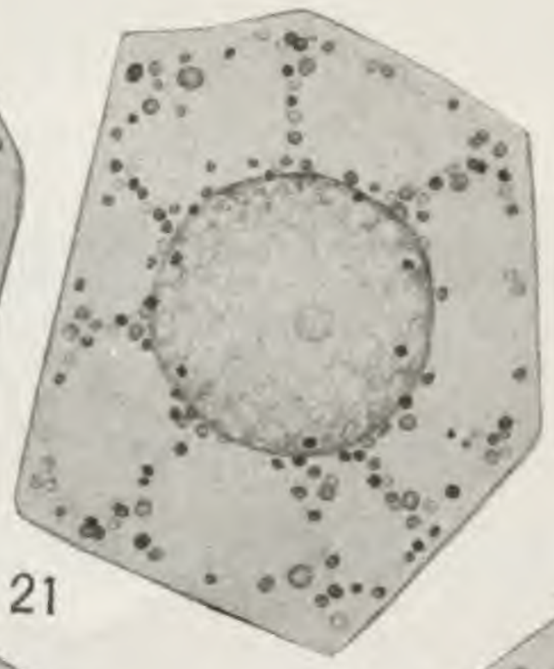
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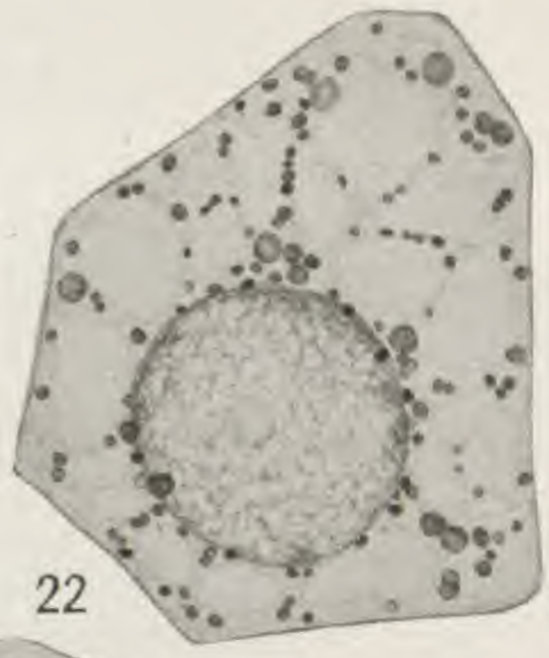
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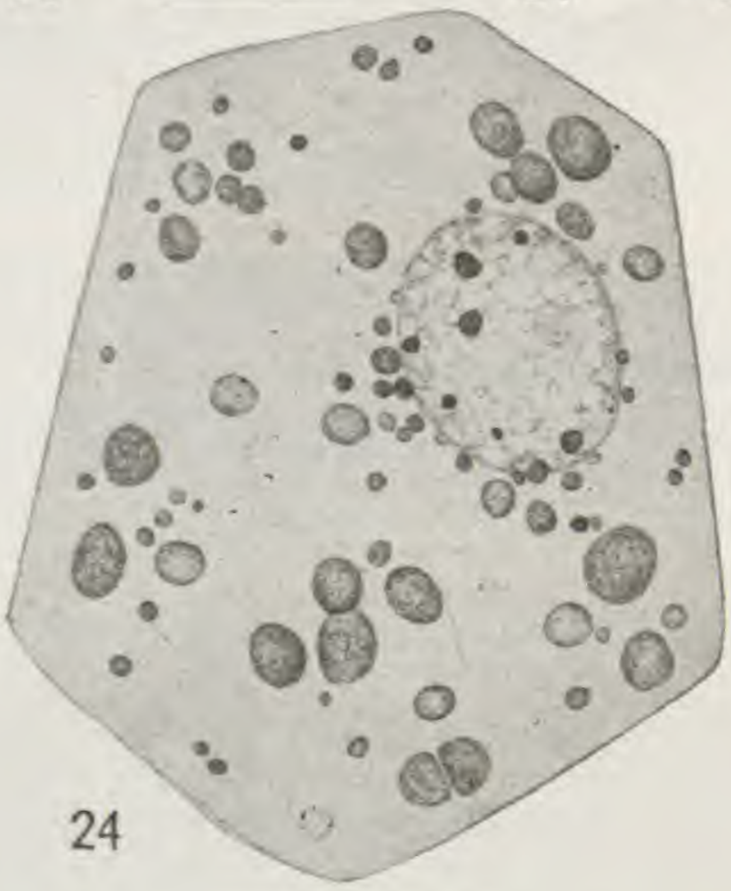
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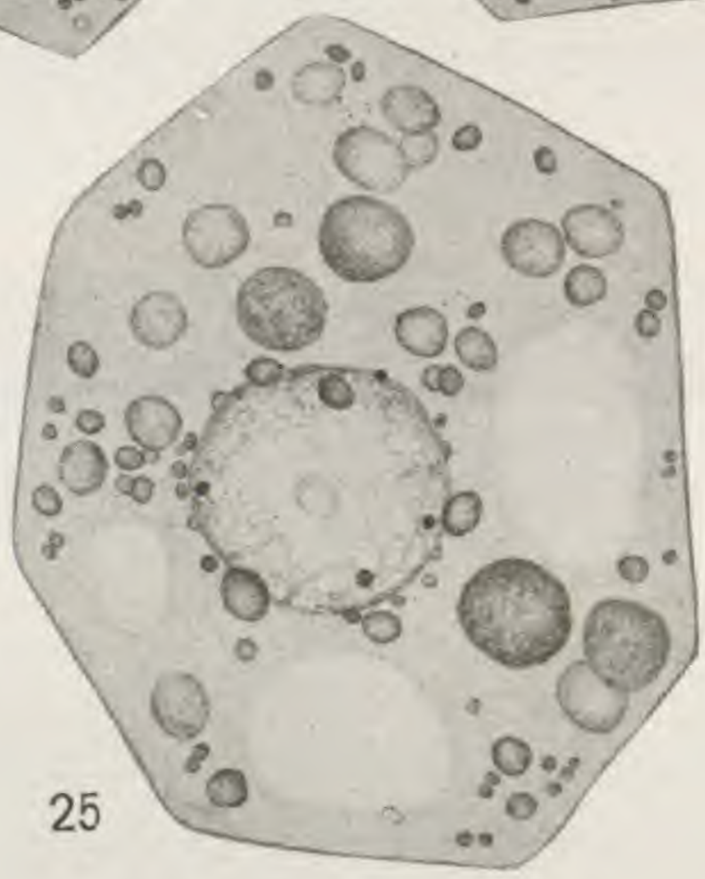
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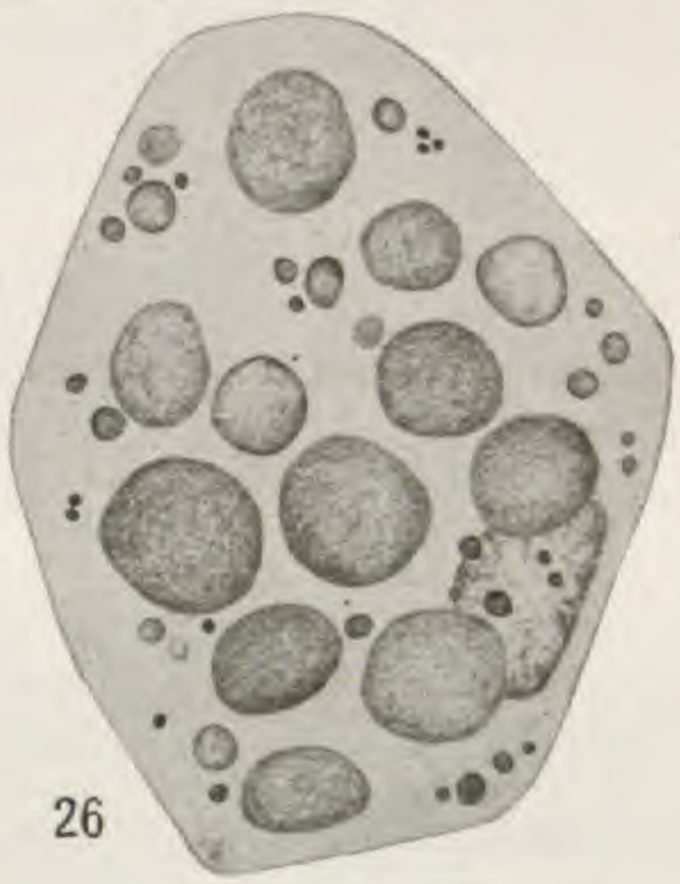
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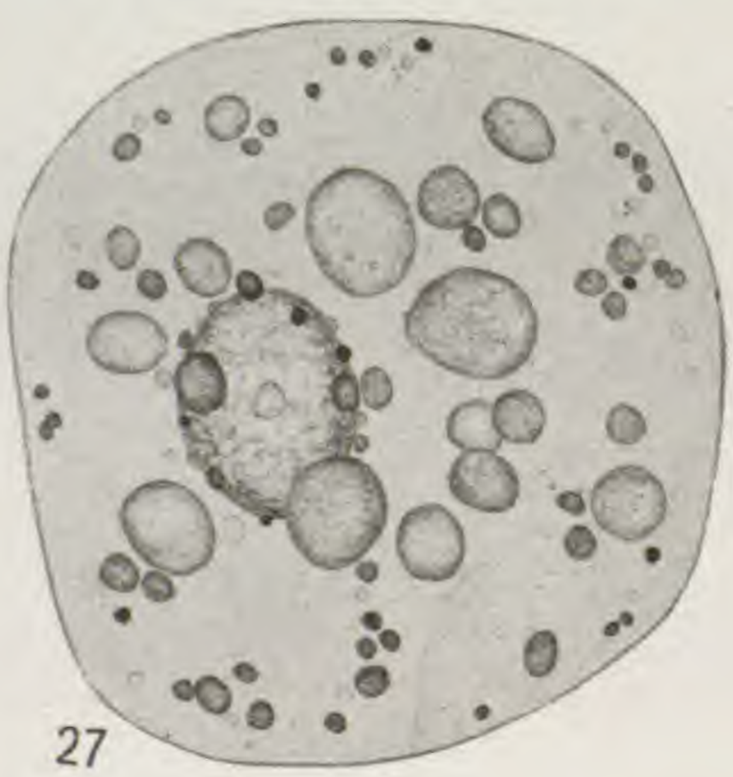
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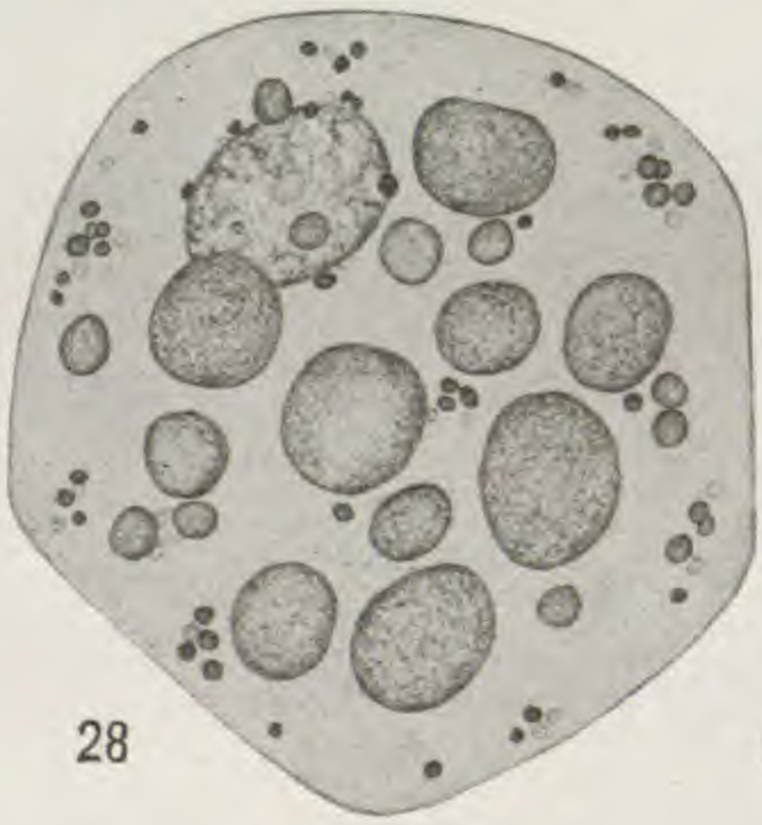
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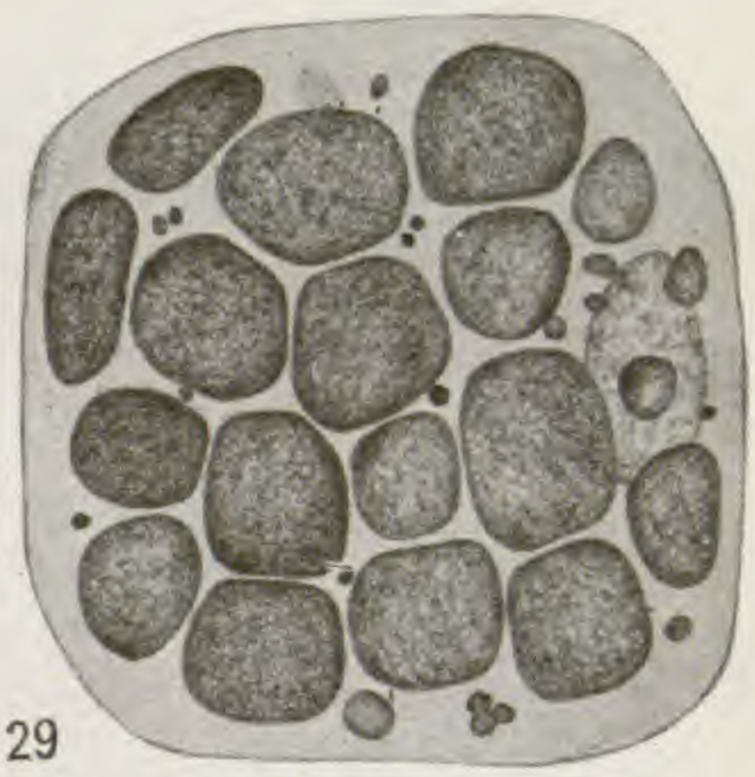
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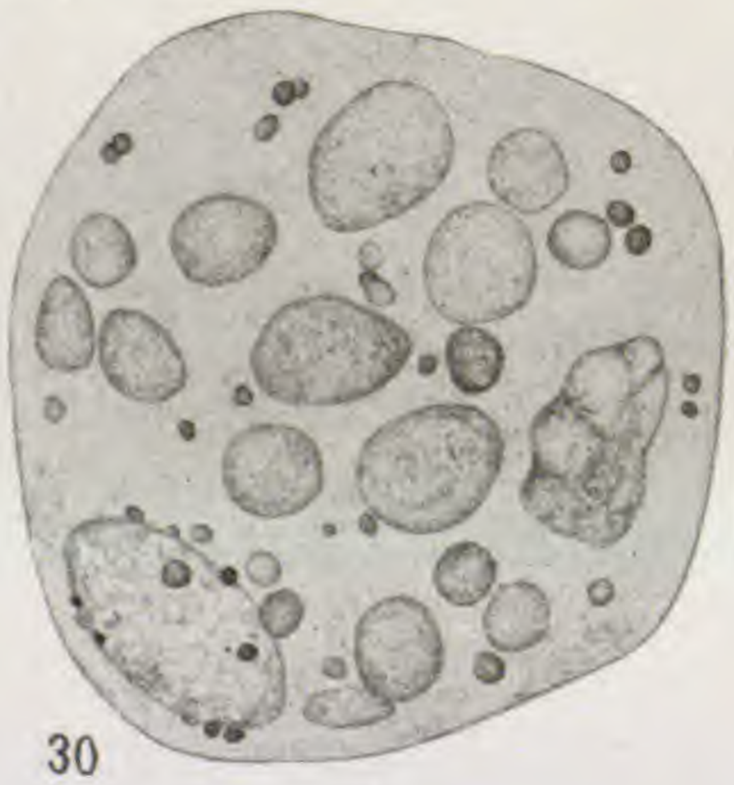
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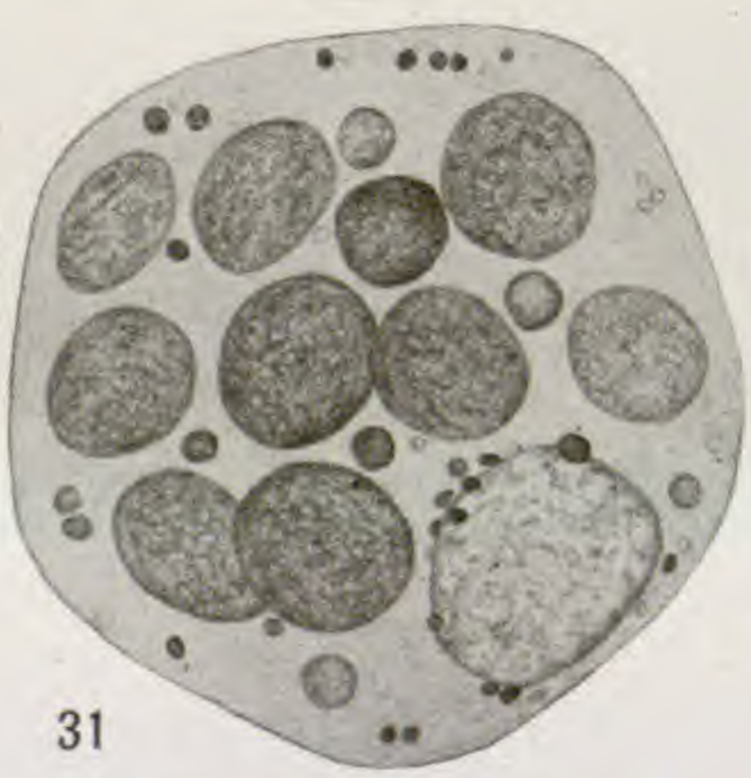
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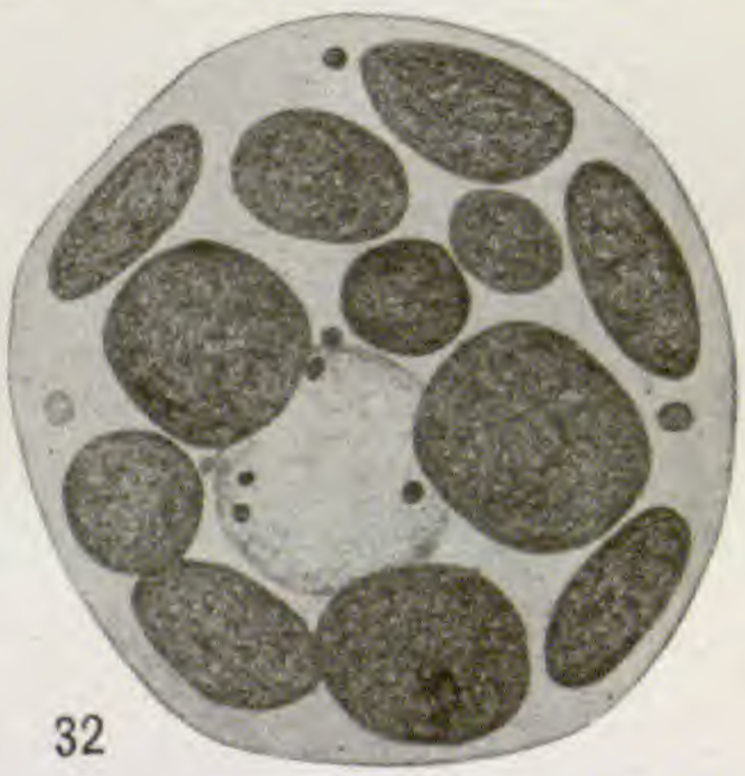
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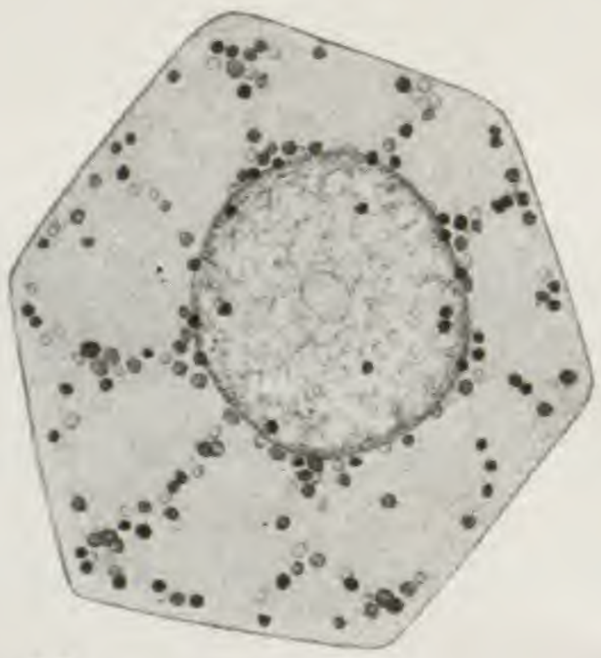

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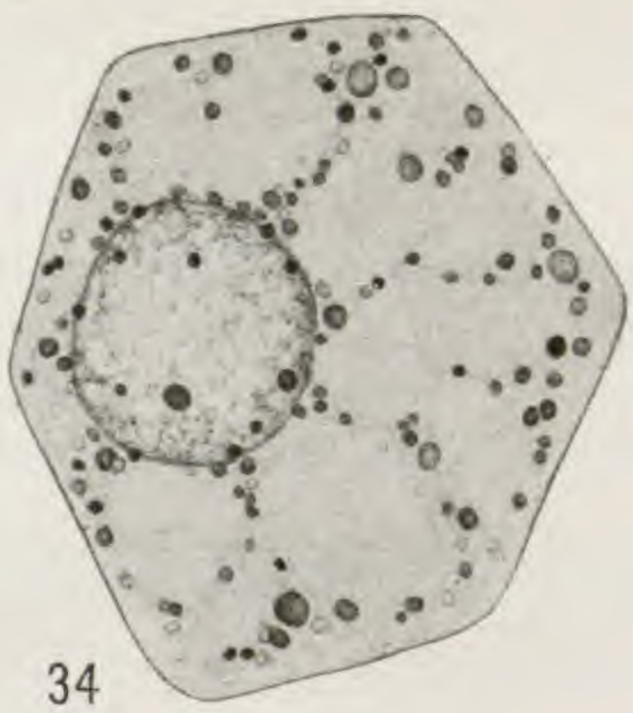

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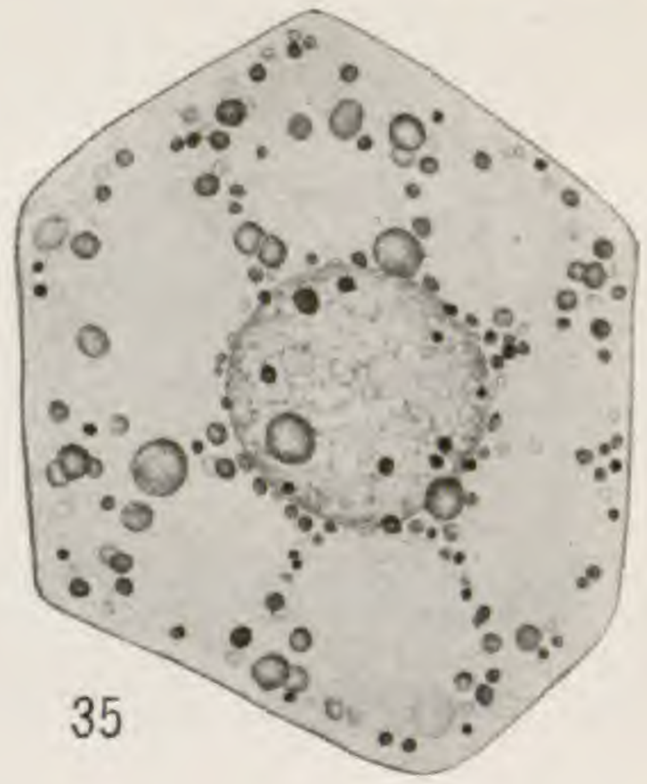

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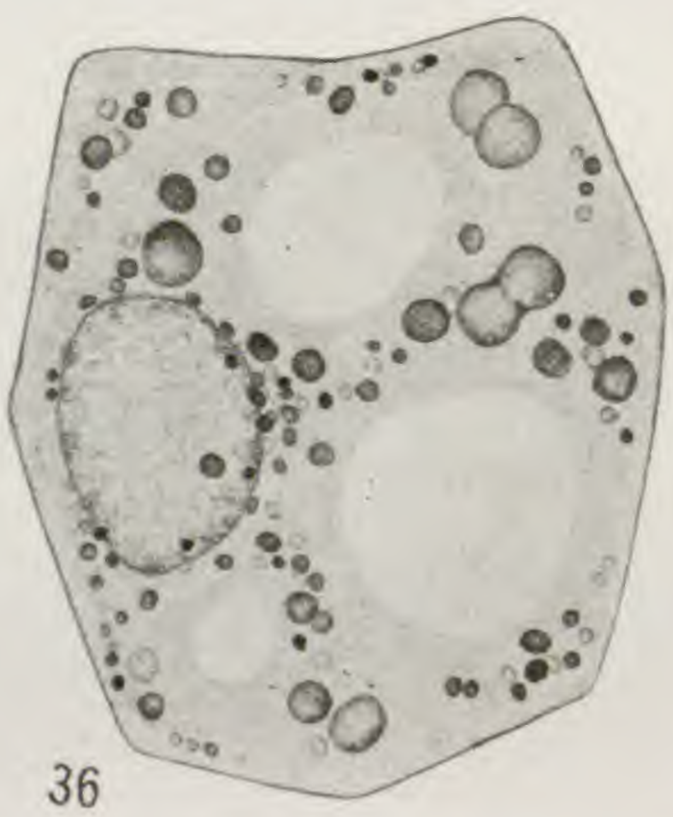

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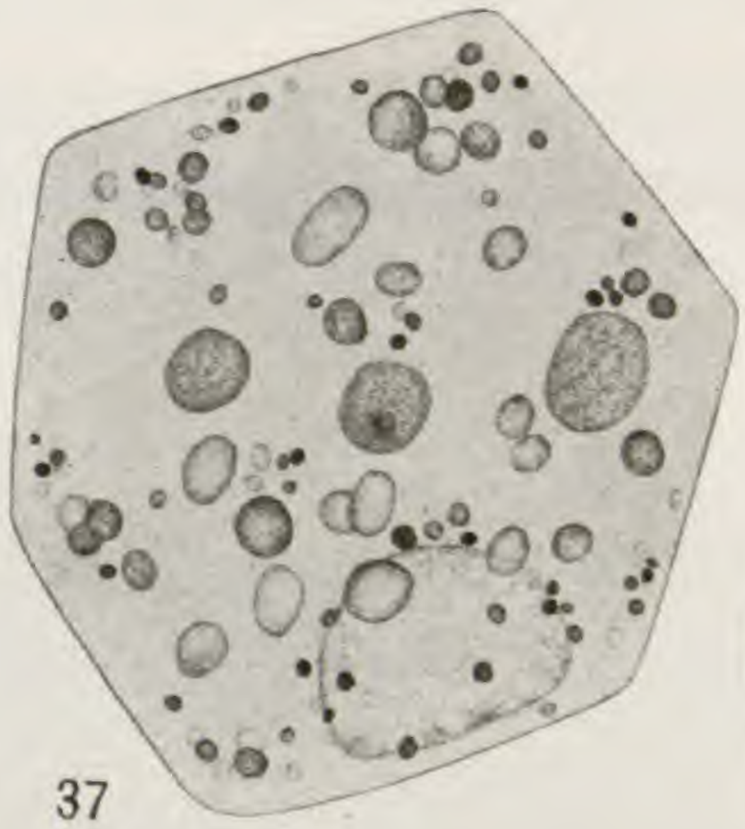

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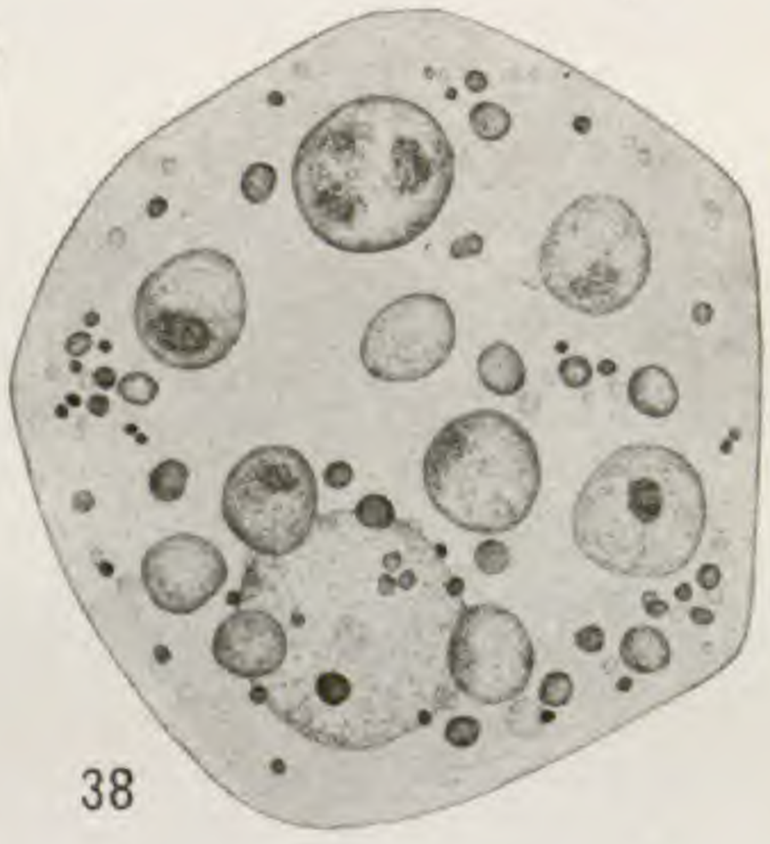

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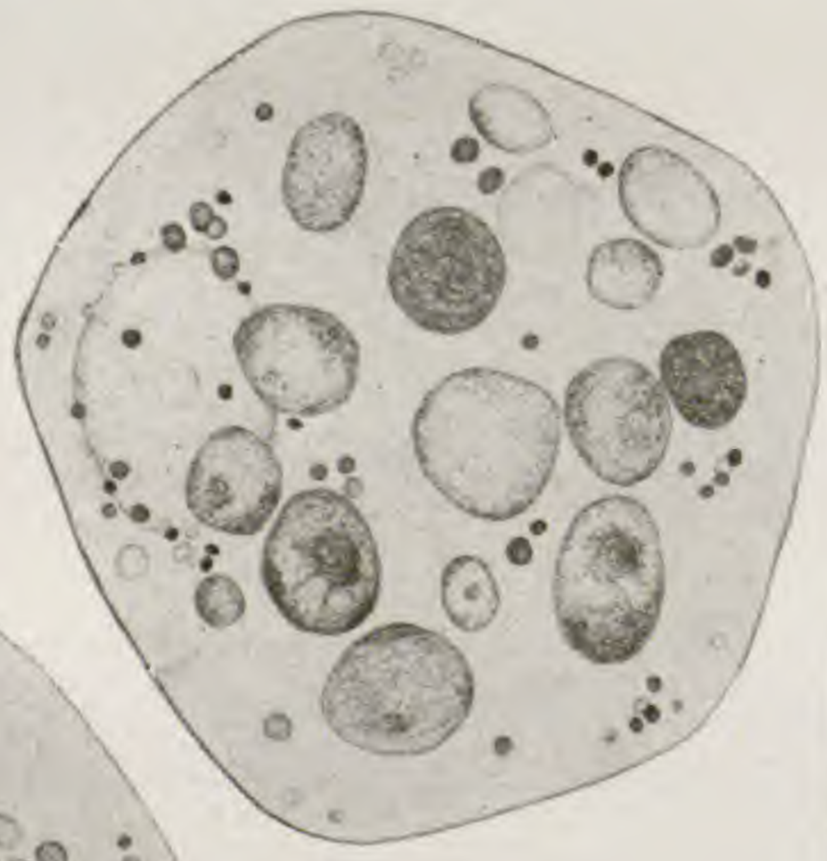

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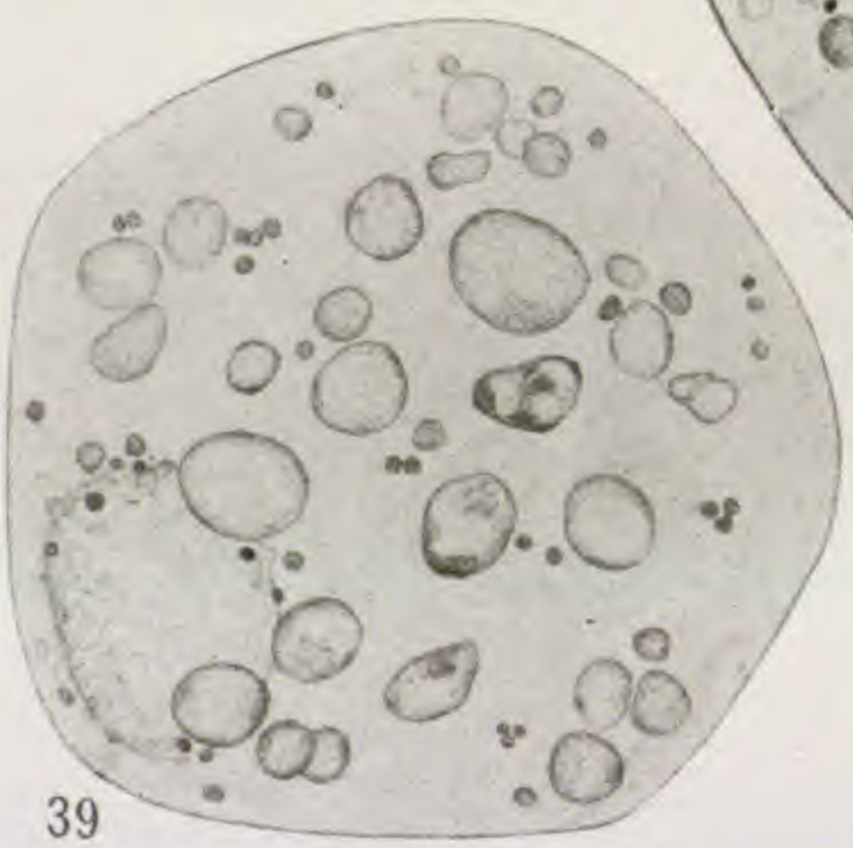

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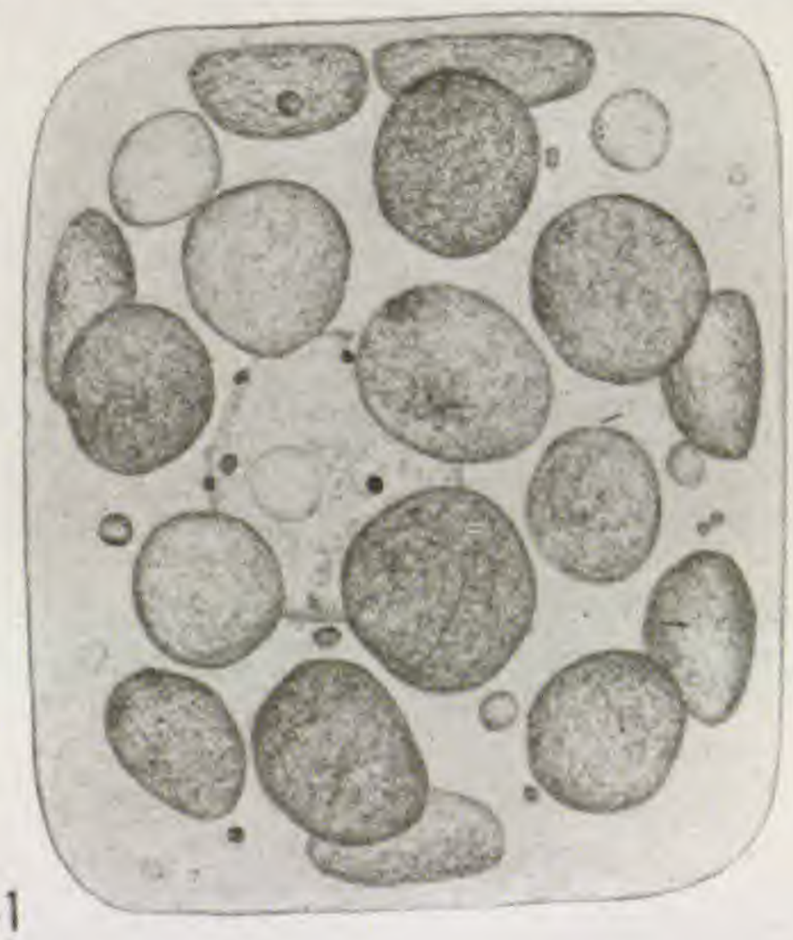

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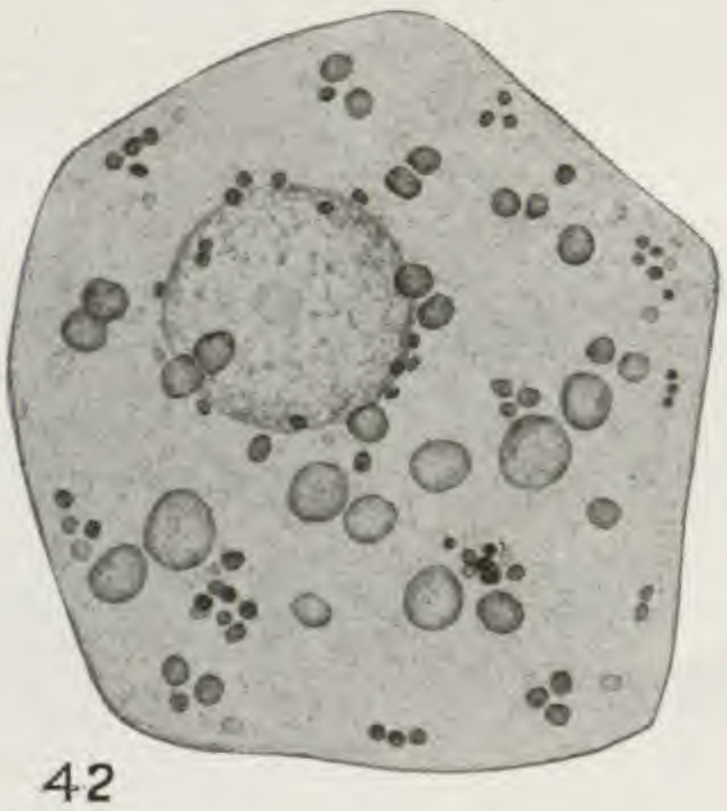
RANDOLPH on MAIZE



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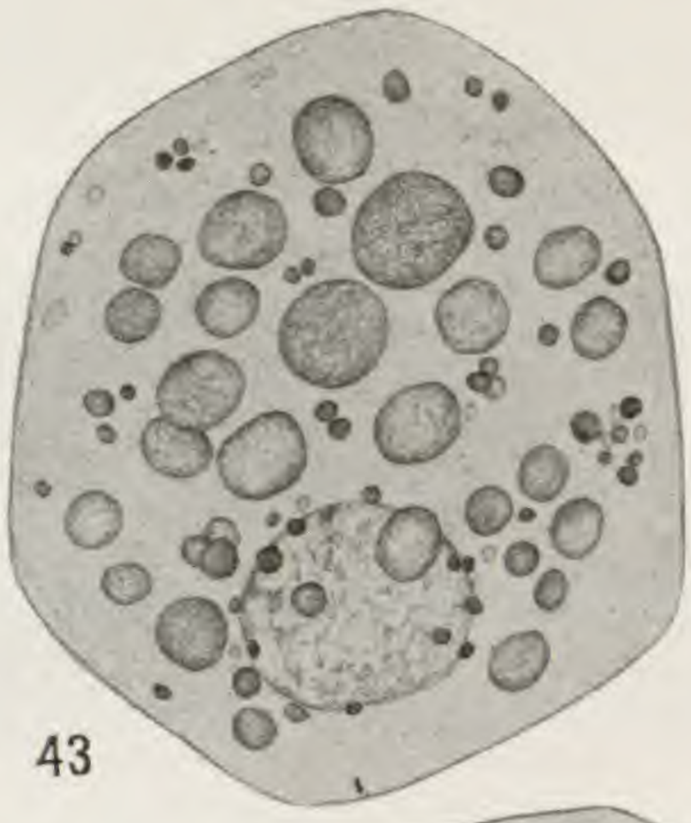

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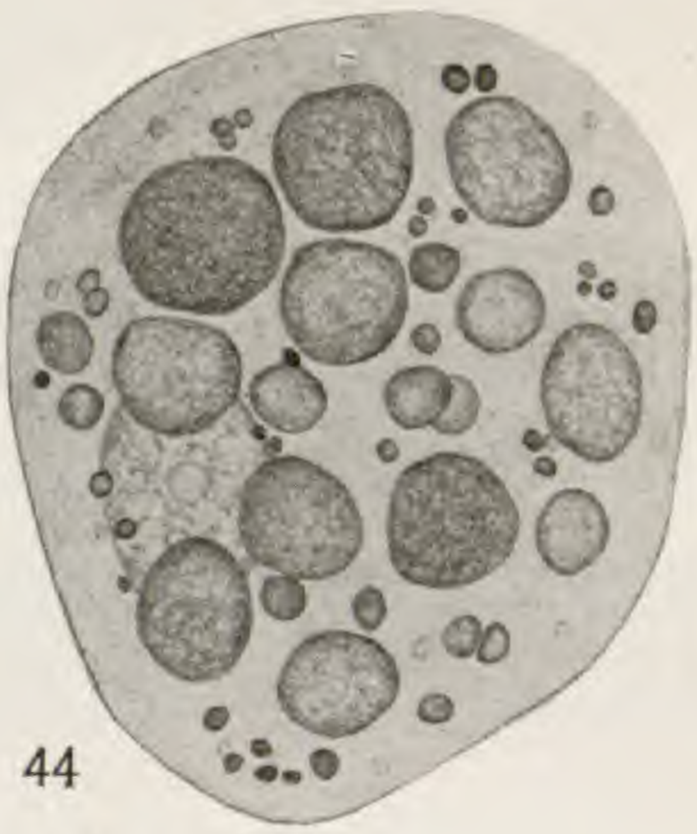

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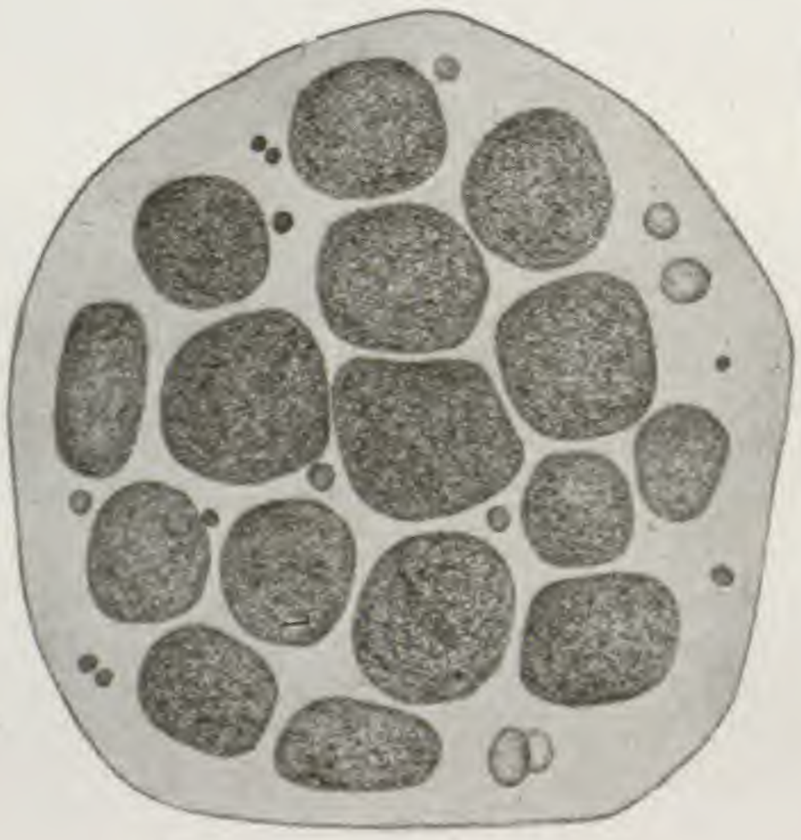

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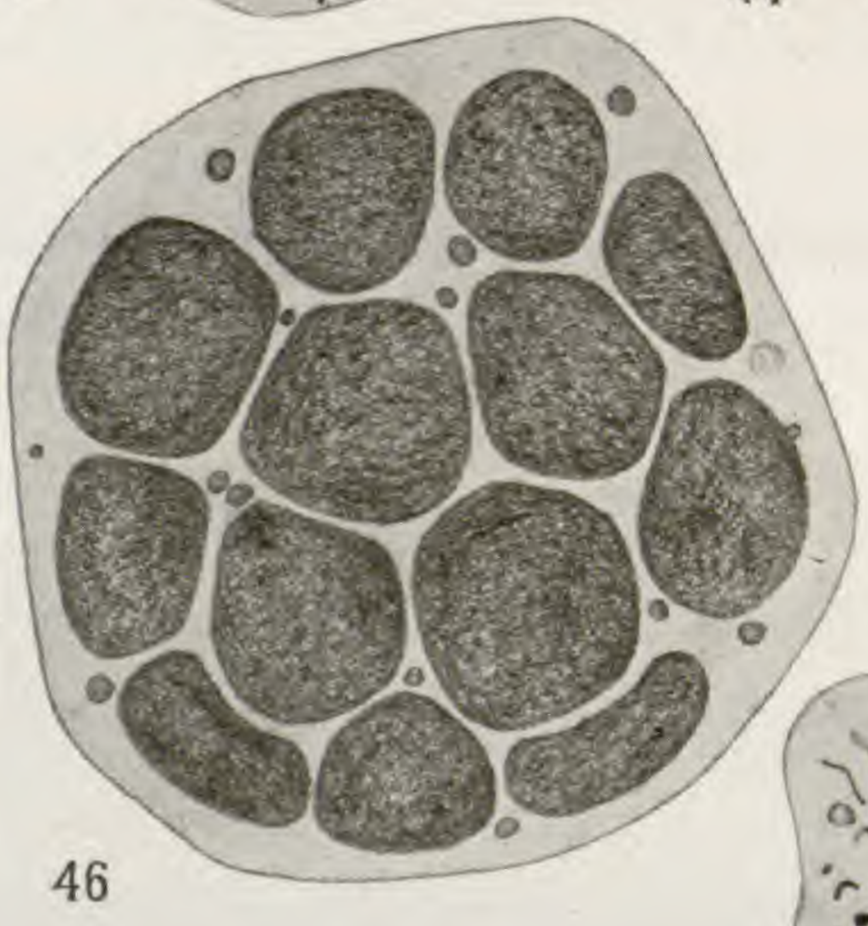

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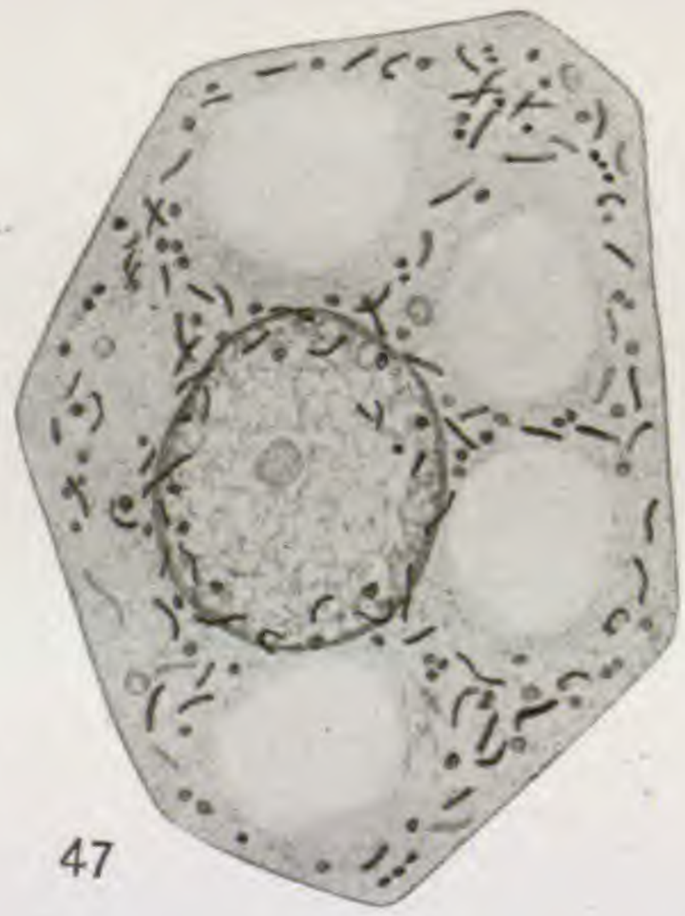

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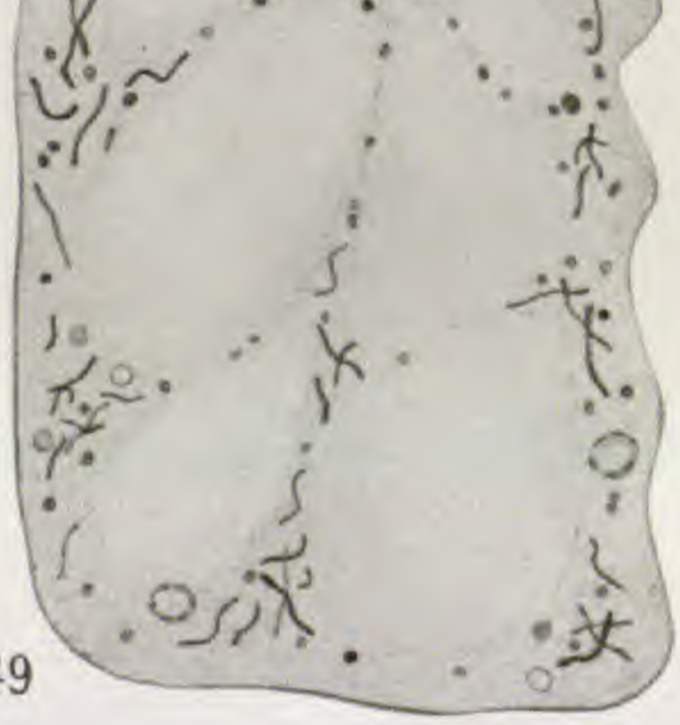

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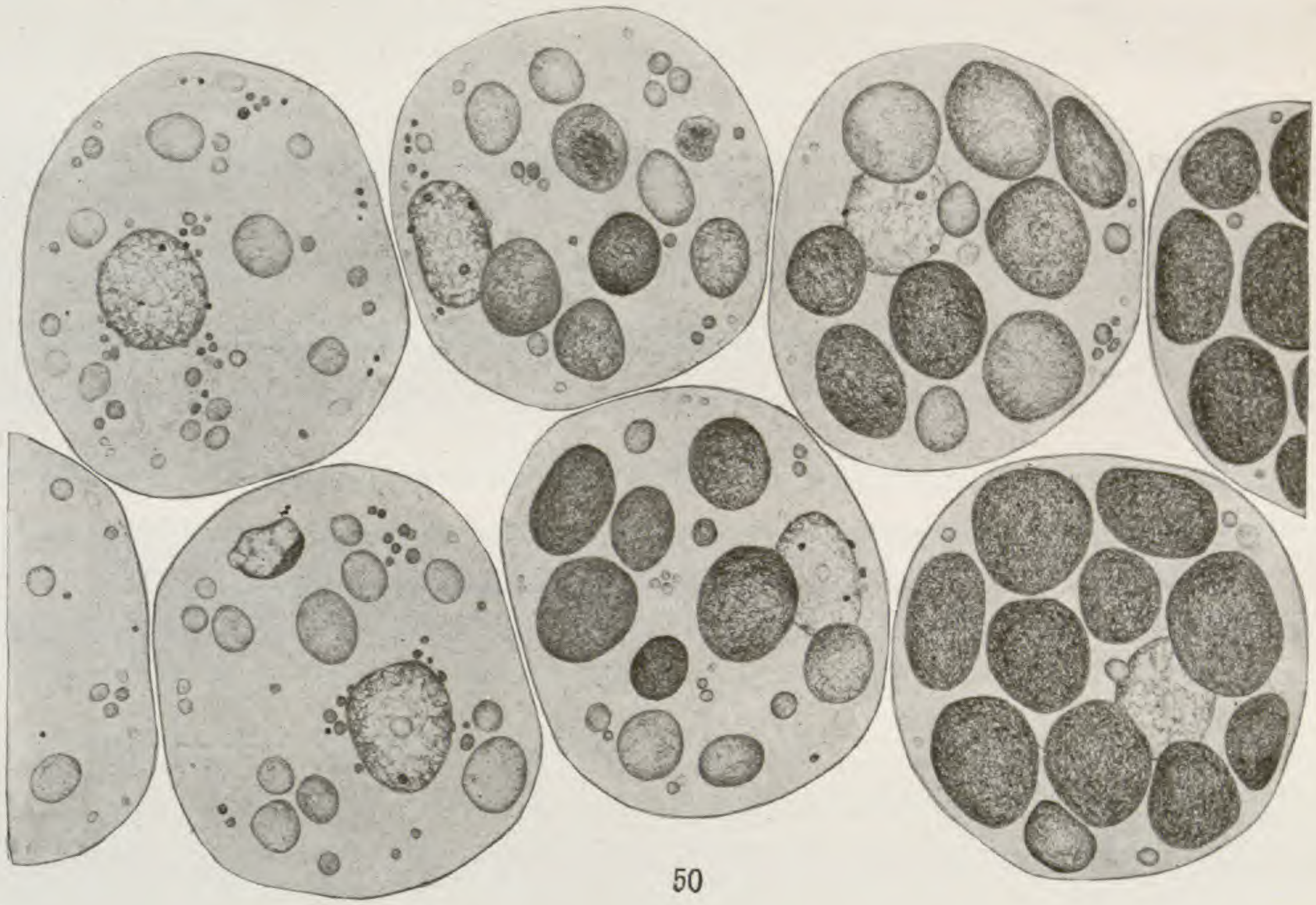
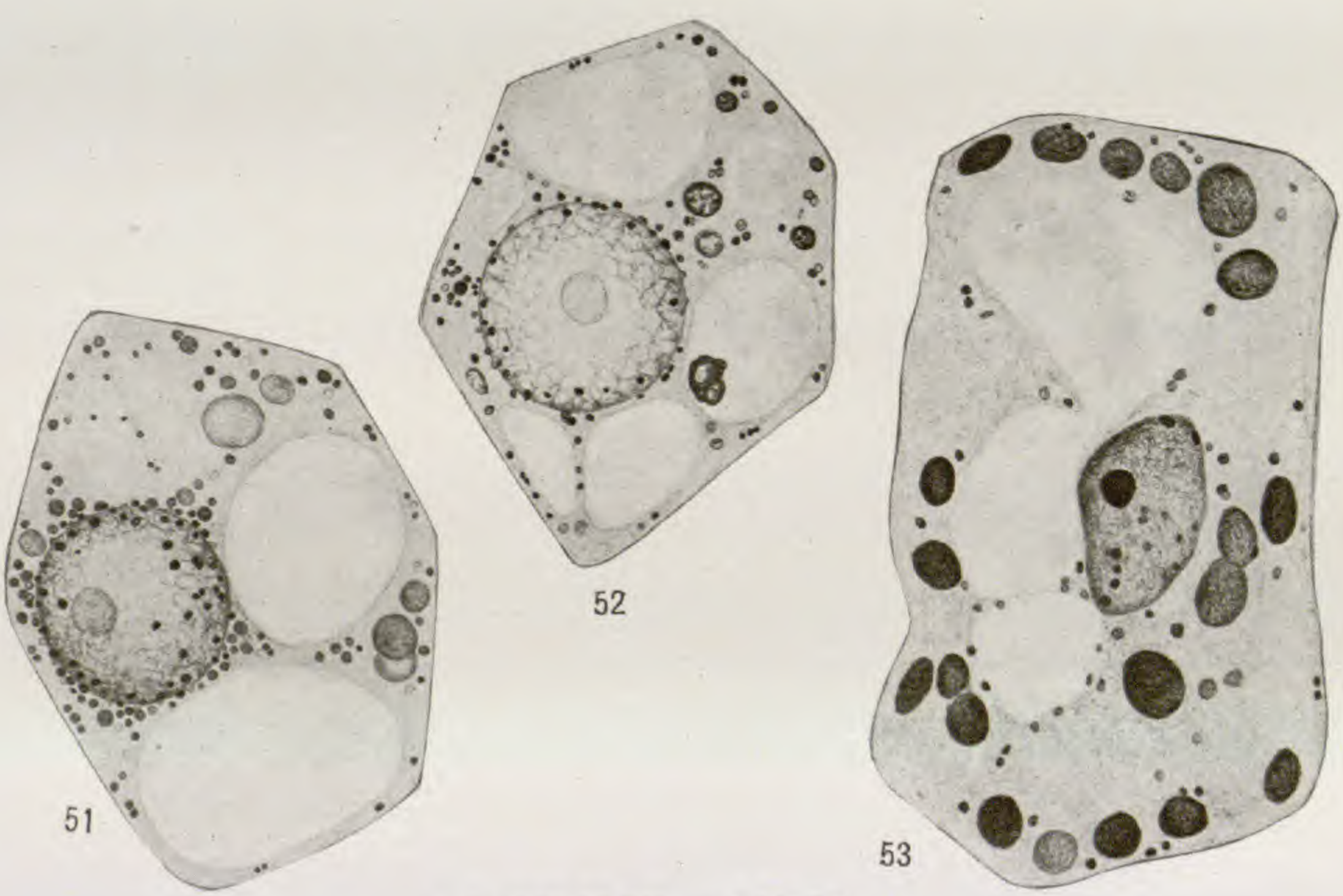
RANDOLPH on MAIZE





RANDOLPH on MAIZE

PLATE XIV

Maternal inheritance strain

Material from uniformly yellowish green 3-inch seedling; amount of green color indicated by depth of shading.

FIGS. 33–38.—Showing development of plastids in undifferentiated apical regions of successively older seedling leaves.

FIG. 33.—Promeristematic region.

FIG. 34.—From first leaf bud.

FIG. 35.—From leaf bud 4 mm. long.

FIG. 36.—From tip of embryonic leaf 20 mm. long.

FIG. 37.—From tip of embryonic leaf 40 mm. long.

FIG. 38.—From tip of leaf about to emerge from sheath.

FIGS. 39–41.—From mesophyll tissue of 3-inch seedling leaf; fig. 39, cell between vascular bundles; fig. 40, cell nearer vascular bundle; fig. 41, cell adjacent to vascular bundle.

PLATE XV

FIGS. 42–46.—From fully developed mesophyll leaf tissue of mature normal green plant; amount of green color indicated by depth of shading.

FIG. 42.—Cell from region of leaf blade inclosed in enveloping leaves.

FIGS. 43–45.—Stages occurring in mesophyll tissue between unexposed and exposed regions of same leaf.

FIG. 46.—Mesophyll cell from exposed region of fully developed leaf.

FIGS. 47–49.—Epidermal cells of seedling leaf tissue of 3-inch seedling: fig. 47, cell near apex of 40 mm. embryonic leaf; fig. 48, cell from same leaf, but slightly farther from tip; fig. 49, cell of 40 mm. embryonic leaf blade.

PLATE XVI

FIG. 50.—From striped plant of maternal inheritance strain, showing cells from transitional region between yellowish green stripe and green stripe; depth of shading indicates amount of green color present.

FIG. 51.—From 35 mm. embryonic leaf of a normal green 3-inch seedling, showing grouping of many proplastids about nucleus.

FIG. 52.—From same tissue as fig. 51, showing effect of osmic acid (1 per cent aqueous solution) fixation on proplastids.

FIG. 53.—From young leaf tissue after Benda's fixation and iron-haematoxylin staining.

CULTIVATION OF EXCISED ROOT TIPS AND STEM TIPS UNDER STERILE CONDITIONS[1]

WILLIAM J. ROBBINS

(WITH FOUR FIGURES)

The growth of higher plants under sterile conditions is a necessary procedure in the investigation of problems involving the direct use by higher plants of organic or inorganic substances which may be altered by bacterial action. Failure to observe sterile conditions throws doubt on the conclusions drawn from the results secured in any experiment where the direct use by plants of an organic compound and some inorganic substances such as ammonium salts or nitrates is investigated.

As indicated by WILSON (15), the methods which have been used to grow plants under sterile conditions have either attempted to grow the entire plant under sterile conditions, or to keep that part of the plant which is of importance in the special investigation in a sterile environment. LUTZ, LAURENT, LEFEVRE, MOLLIARD, GRAFE, RAVIN, KNUDSON (11), BRANNON (4), and others have described methods of cultivating entire plants under sterile conditions. MAZÉ and PERRIER (12), SHULOW, HUTCHINSON and MILLER (9), and WILSON (15) have described in some detail methods by which higher plants may be grown with their tops exposed to the normal aerial environment. MAZÉ and WILSON grew corn plants to maturity with the root systems in sterile water cultures. Isolated and mature plant embryos have been cultivated under non-sterile conditions for longer or shorter periods by BROWN and MORRIS, ANDRONESCU (2), URBAIN (16), and others. KNUDSON cultivated corn embryos, and BUCKNER and KASTLE (3) bean embryos for short periods under sterile conditions. Isolated and immature embryos of species of *Rhapanus* and of *Cochlearia danica* have been grown with some success under sterile and non-sterile conditions by HANNIG (8). HABERLANDT (5) attempted to grow

[1] Published with the permission of the Director of the Agricultural Experiment Station, University of Missouri.

the isolated cells of the leaves of higher plants, and succeeded (**6, 7**) in obtaining some cell division under certain conditions in isolated pieces of the tissue from tubers, stems, and leaves of higher plants. To the writer's knowledge, however, the cultivation in sterile and artificial media of a portion of the meristematic tissue of higher plants has not been accomplished.

In the present paper a method is described by which isolated meristematic tissue (root tips and stem tips) of higher plants may be grown with some success under sterile controlled conditions. There are presented also the results of some preliminary experiments in which the method has been used, and which indicate the possibilities and limitations in growing excised root tips and stem tips under these conditions.

The experiments described were performed for the most part in 1917 at the Alabama Polytechnic Institute and Experiment Station, as the first step in an investigation to define the classes of materials required by a plant shoot or root for continued growth. In order to eliminate the influence of the shoot and its products on the root, or of the root on the shoot, it was considered necessary to grow the root tips isolated from the tops, and the shoot tips isolated from the roots, in artificial media under sterile conditions. It was thought that if the isolated meristematic tissue of a higher plant (such as the shoot tip or the root tip) could be cultivated successfully, such questions as the synthesis of elaborated nitrogen by the non-green parts of higher plants and the relation of chlorophyll and light to it, the necessity of accessory food substances for the growth of green plants, in short, the complete nutrient requirements of the shoot and of the roots of higher plants, and possibly of individual cells of the plant, could be investigated directly.

Method

The method followed in securing sterile root or shoot tips and cultivating the excised tissue was as follows. Seeds were sterilized by WILSON'S (**14**) calcium hypochlorite method, and transferred without washing to sterile Petri dishes containing a thin layer of 0.8 or 1 per cent plain agar. In transferring, a metal spoon made of aluminum with a wooden handle was used, and this spoon was

dipped in alcohol and flamed in order to sterilize it previous to use. When the seeds had germinated and the roots had reached a length of a centimeter or more, a centimeter or thereabouts of the root tip was cut off in the dish with a sterile scalpel, measured, and transferred with a sterile platinum loop to the described culture medium. The growing tip of the shoot was similarly treated. By this method a part of the plant, including the meristematic region, was excised and placed under sterile controlled conditions.

Growth of root tips in sterile nutrient solutions

Using the method described, the root tips of peas, cotton, and corn were placed in (1) a modified Pfeffer's solution,[2] (2) the same solution containing 2 per cent glucose, and (3) the modified Pfeffer's solution containing 2 per cent levulose. Each root tip was transferred to an Erlenmeyer flask of 125 cc. capacity, containing 50 cc. of solution. The flask was set in the dark and allowed to stand at room temperature during the period of the experiment. It was found that in such solution cultures the root tips of peas, cotton, and corn would develop into a considerable root system in the mineral nutrient solution containing carbohydrates, but that little growth occurred in the culture solution to which no carbohydrate was added. For all three plants the greatest growth occurred in the glucose solution. The method of culture and the appearance of root tips of corn in a 2 per cent glucose solution and in a solution lacking carbohydrates at the end of twenty-four days is shown in fig. 1. The detailed results of these early experiments were as follows:

PEAS.—In the experiment with peas, the variety Extra Early was used, and the period of growth was twenty-nine days. Fifteen root tips were used with each sugar solution and thirteen in the mineral solution without sugar. One contamination developed. All of the roots in the levulose solution were darkish brown, particularly at the cut end. Those in Pfeffer's solution were pure white. All were turgid, but the specific gravity of those in the Pfeffer's solution was less than that of those grown in the sugar solution.

[2] The composition of this solution was as follows:
$Ca(NO_3)_2$, 2 gm.; KH_2PO_4, 0.5 gm.; KNO_3, 0.5 gm.; KCl, 0.25 gm.; $MgSO_4$, 0.5 gm.; $FeCl_3$, 0.005 gm.; distilled water, 6000 cc.

The former floated on water, the latter sank. The average growth in length of the roots is given in table I, from which it can be noted

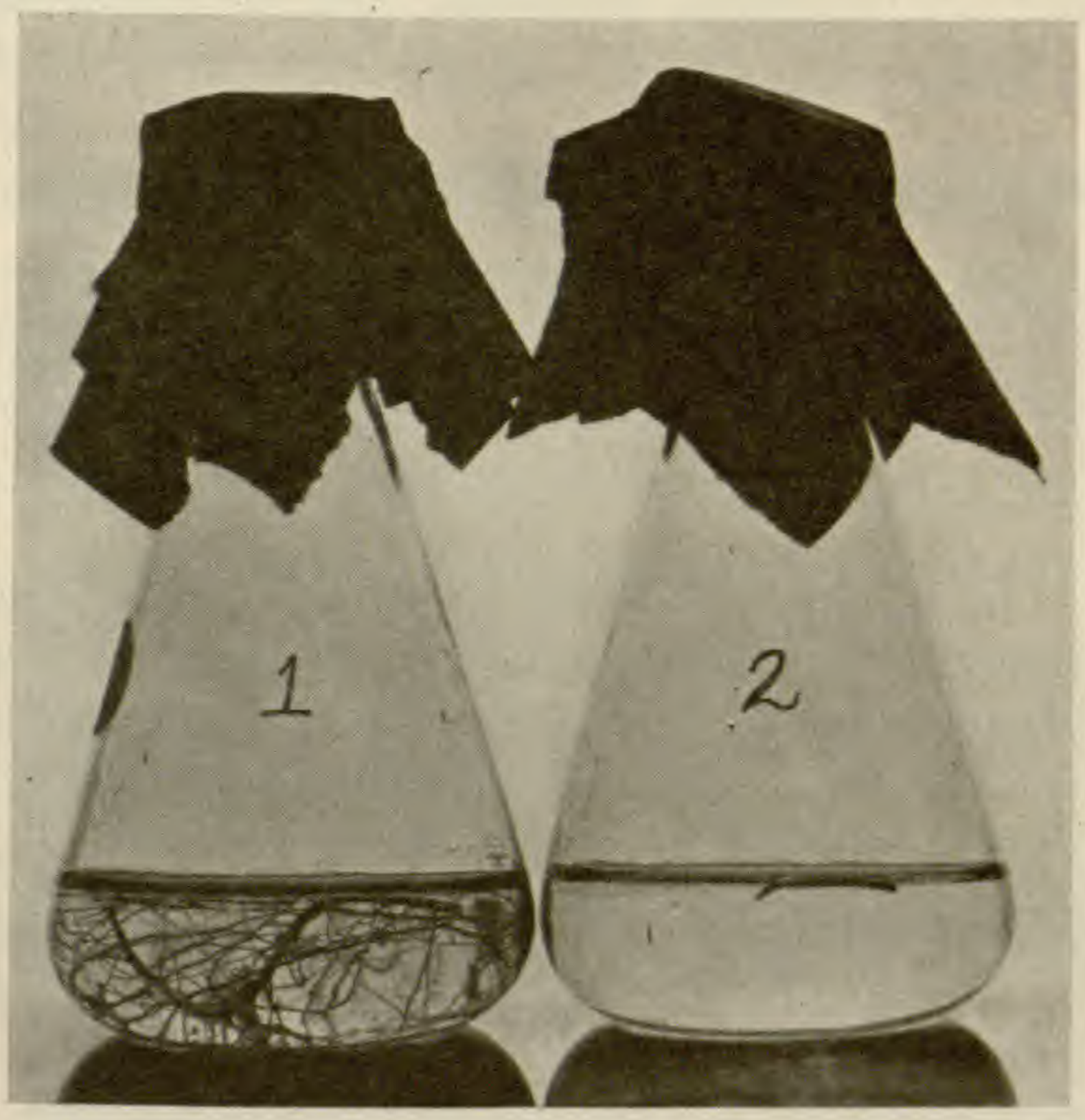


FIG. 1.—Appearance of root tips of corn in Pfeffer's solution at end of twenty-four days; flask no. 1 contains 2 per cent glucose, flask no. 2 contains no carbohydrates; root tip in no. 1 appeared as root tip does in no. 2 at beginning of experiment.

that in the mineral nutrient solution alone the average gain in length was 0.84 cm., and no secondary roots were produced. In levulose the average gain was 2.04 cm. with 2.3 secondary roots;

TABLE I

ROOT TIPS OF PEA GROWN TWENTY-NINE DAYS IN DARK

Solution	Number root tips used	Average original length (cm.)	Gain in length 29 days (cm.)	Side roots, average number per root
Pfeffer's solution.....	13	1.58	0.84	0
Pfeffer's plus glucose.....	14	1.49	4.27	3.5
Pfeffer's plus levulose.....	15	1.54	2.04	2.3

in glucose the average gain was 4.27 cm. with 3.5 secondary roots. Considerable variation in growth was evident. Some of the roots

in the sugar solution made little or no growth, others grew well (fig. 2). These roots which showed little growth lowered the average for the lengths in the sugar solutions. The maximum growth in glucose of a root tip originally 1.5 cm. long was 13.5 cm. with

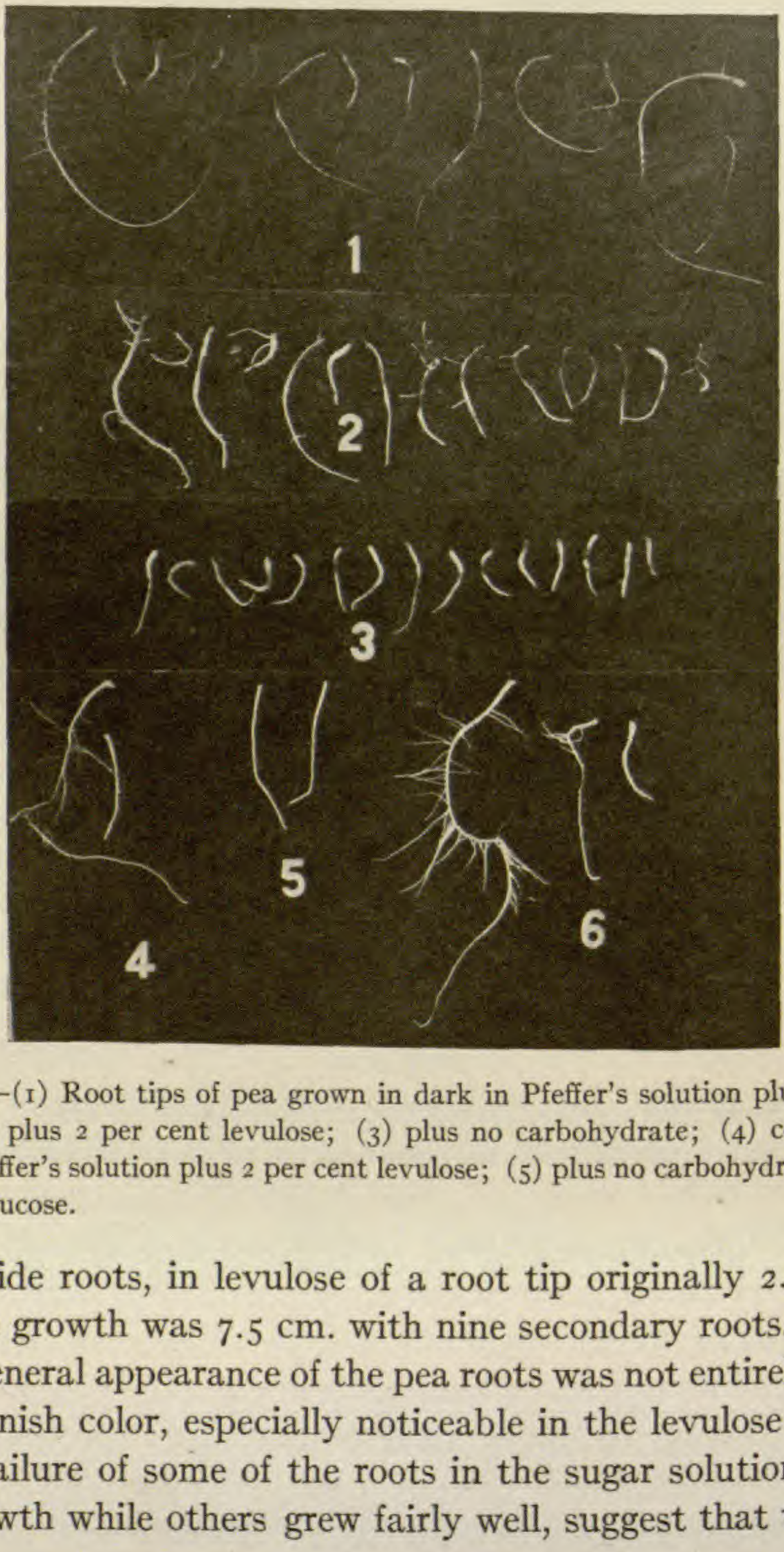


FIG. 2.—(1) Root tips of pea grown in dark in Pfeffer's solution plus 2 per cent glucose; (2) plus 2 per cent levulose; (3) plus no carbohydrate; (4) corn root tips grown in Pfeffer's solution plus 2 per cent levulose; (5) plus no carbohydrate; (6) plus 2 per cent glucose.

thirteen side roots, in levulose of a root tip originally 2.6 cm. the maximum growth was 7.5 cm. with nine secondary roots.

The general appearance of the pea roots was not entirely normal. The brownish color, especially noticeable in the levulose solutions, and the failure of some of the roots in the sugar solution to make much growth while others grew fairly well, suggest that the sterili-

zation of the seeds, the method of handling the roots, or the culture solution was injurious or at least unfavorable. It is of interest to note that when the root curved in its growth the lateral roots were produced on the convex side of the root. This was found to be true also in the roots of all three kinds of plants, and it can be noted in the case of corn and pea roots in fig. 2. JOST (10) states that by bending the main root, development of lateral roots may be prevented from the concave side, and cites the lupine as an example after NOLL.

CORN.—Using the same methods, corn roots were investigated. The period of growth was eleven days in the dark, and the number of roots used in this preliminary experiment was three in the glucose solution, two in the levulose solution, and two in the mineral nutrient solution without sugar. The corn root tips made a much greater growth than the peas, and did not show the browning so evident in the pea roots in the sugar solution, but were white in both the sugar solution and the mineral nutrient solution at the end of the experiment. The data in table II show that the average

TABLE II

ROOT TIPS OF CORN GROWN ELEVEN DAYS IN DARK

Solution	Number roots used	Average original length (cm.)	Gain in length 11 days (cm.)	Average number secondary roots
Pfeffer's plus 2% glucose...	3	2.3	8.33	22
Pfeffer's plus 2 % levulose..	2	3.75	5.95	20
Pfeffer's solution.....	2	4.15	1.60	0

increase in length in the Pfeffer's solution was 1.6 cm. with no secondary roots, the average increase in length in the levulose was 5.95 cm. with twenty secondary roots, and in glucose 8.33 cm. with twenty-two secondary roots. The appearance of the corn roots at the end of the experiment is shown in fig. 2.

This preliminary experiment does not indicate, however, the maximum amount of growth which corn roots may make under the conditions described. Since the experiment was performed, several hundred corn roots have been grown in sterile nutrient solutions. Corn roots with a length of 14–17 cm. and with 80–125 secondary

roots have frequently been grown in two weeks in the dark in Pfeffer's solution containing 2 per cent glucose. The maximum length has been that of a tip originally 2.0 cm. long which attained a length of 32.5 cm. and 131 secondary roots, in forty-three days.

COTTON.—Root tips of cotton were grown seventeen days in the three solutions, and with them even more striking results were secured than with the corn. Tips of ten roots were used in glucose, ten in levulose, and eleven in Pfeffer's solution without sugar. The root tips in glucose lengthened very rapidly, and at the end of seventeen days, as indicated in table III, had attained an average

TABLE III

GROWTH OF ROOT TIPS OF COTTON IN STERILE NUTRIENT SOLUTIONS

Solution	Number of roots	Average original length (cm.)	Gain in length 17 days (cm.)	Average number secondary roots per root	Dry weight per root (gm.)
Pfeffer's plus 2% glucose.....	10	3.06	31.76	61	1224
Pfeffer's plus 2% levulose.....	10	3.11	2.34	29	0.0728
Pfeffer's solution.....	11	2.99	1.92	0.1	0.1240

length of 34.8 cm., while originally they were but 3.06 cm. long. The cut end of the roots in glucose was very dark brown, almost black for 1–1.5 cm., and brownish for 3–4 cm. The root cap was black. In levulose the increase was much less, only 2.34 cm., and the whole root was dark brown. The increase in length in the mineral solution alone was still less, being only 1.9 cm. The roots were pure white. The maximum growth in the glucose solution was that of a root tip 3.2 cm. long which attained a length of 42.2 cm. with seventy secondary roots. In levulose the maximum growth was that of a root tip 2.1 cm. long which grew to 13.4 cm. with thirteen secondary roots.

Growth of root tips in agar

The excised root tips of corn have also been grown in 1 per cent agar. Fig. 3 shows the growth of an excised root tip of corn at the end of two weeks in Pfeffer's solution containing 1 per cent

agar, and in Pfeffer's solution plus 2 per cent glucose containing 1 per cent agar. It can be noted from the figure that the root tip not supplied with glucose has made little growth, while the root tip supplied with glucose has made considerable growth, has responded normally to gravity, and has produced a considerable number of secondary roots.

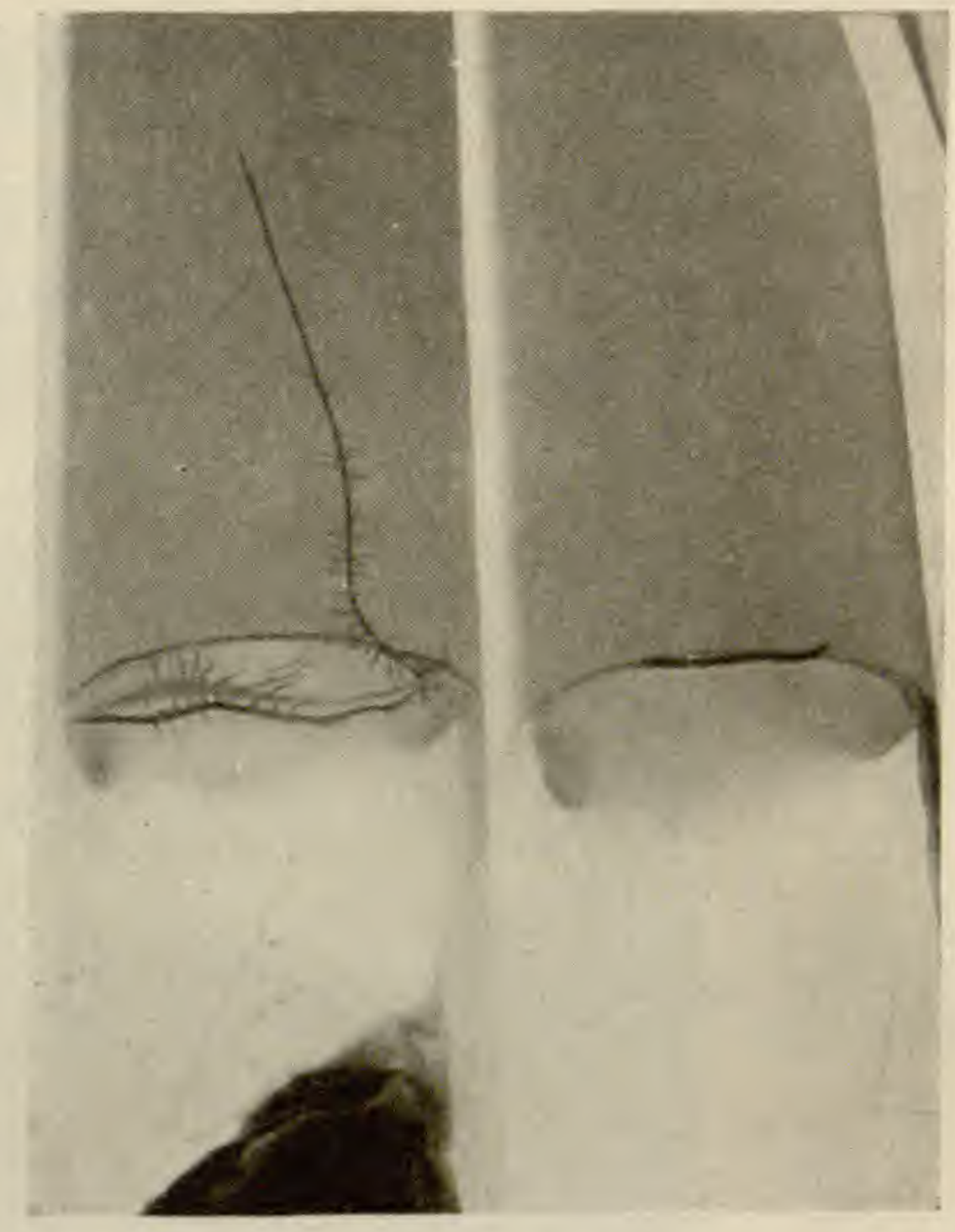


FIG. 3.—Growth of root tips in Pfeffer's solution plus 1.0 per cent agar; tube on left no glucose, tube on right 2 per cent glucose.

Growth of shoot tips in sterile nutrient solutions

The shoot tips of pea, corn, and cotton were placed in the three solutions and grown in the dark. While growth was secured in the carbohydrate solution with cotton, the development was abnormal and measurements were not made. The shoots of peas and corn developed more normally in the carbohydrate solution, and in many cases produced roots. The plants, however, were chlorotic and showed the elongation and small leaf development typical of plants grown in the dark (fig. 4). In both cases, as indicated in table IV, the greatest increase in length occurred in the glucose solution and least in the Pfeffer's solution without sugar. The relative growth of the shoot tips in the three solutions was therefore the same as that of the root tips. Starch was found in the tissue grown in the glucose and levulose solutions, and in the guard cells of the shoots in the Pfeffer's solution.

From these experiments it is evident that in the dark, in solution cultures containing the mineral salts commonly accepted as essential for the growth of green plants and a soluble carbohydrate, the root tips of corn, cotton, and peas, and the stem tips of corn, cotton, and peas make considerable growth. In the same solutions lacking

carbohydrates, only a slight increase in length occurs, probably at the expense of carbohydrate originally present in the root tip.

Glucose is apparently a better source of carbon than levulose for all three plants and for both tops and roots. This is particularly

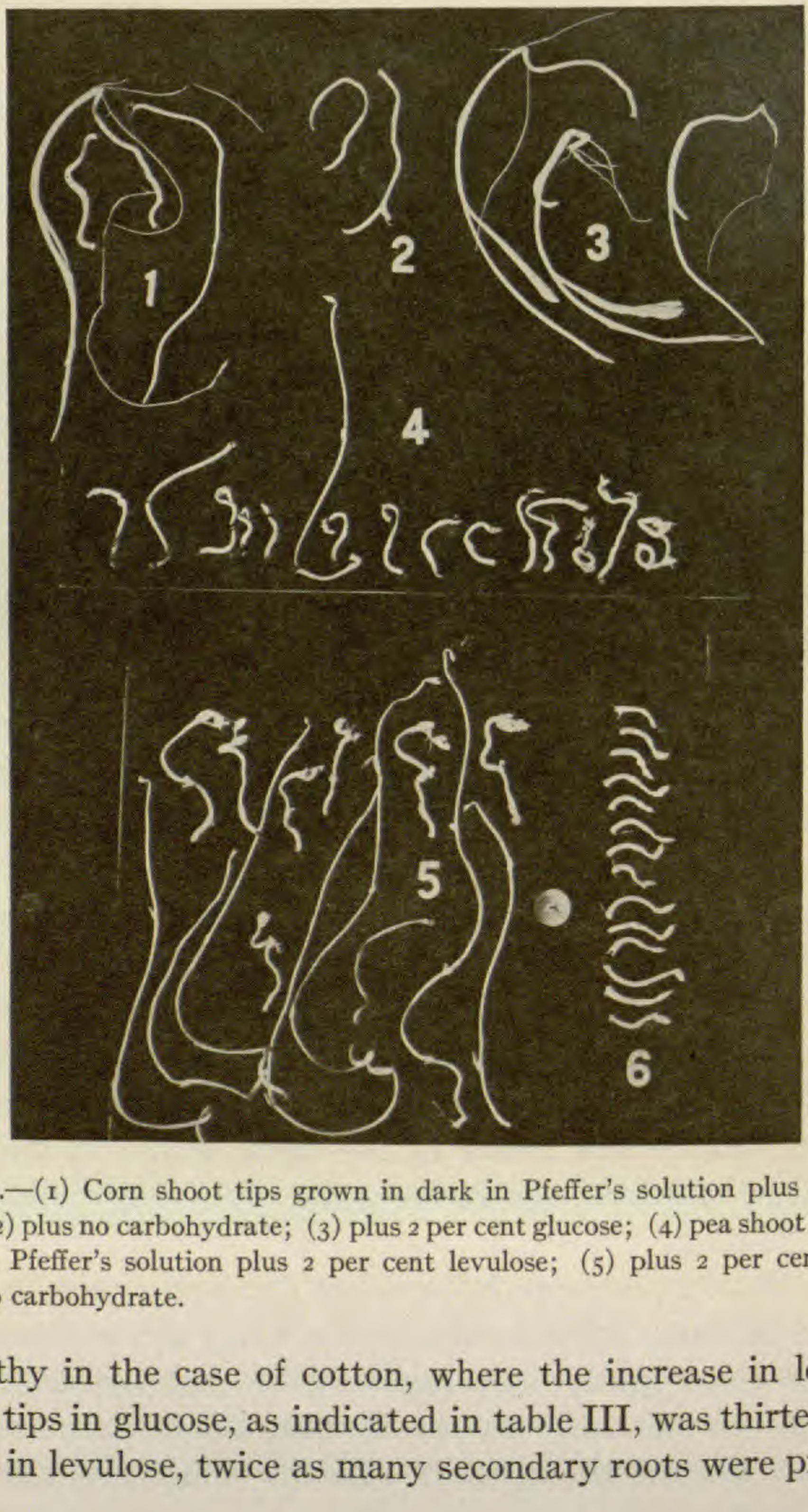


FIG. 4.—(1) Corn shoot tips grown in dark in Pfeffer's solution plus 2 per cent levulose; (2) plus no carbohydrate; (3) plus 2 per cent glucose; (4) pea shoot tips grown in dark in Pfeffer's solution plus 2 per cent levulose; (5) plus 2 per cent glucose; (6) plus no carbohydrate.

noteworthy in the case of cotton, where the increase in length of the root tips in glucose, as indicated in table III, was thirteen times the gain in levulose, twice as many secondary roots were produced,

and the gain in dry weight compared with the check was twice as great. Although the number of corn root and stem tips used in this experiment was small, the roots grown in glucose were better than those in levulose. No comparison between the two sugars could be made in the case of cotton stem tips. The contrast between the relative effect of glucose and levulose on the amount of growth made by the tissue of these seed plants and their effect upon the

TABLE IV

GROWTH OF SHOOT TIPS OF PEA AND CORN IN DARK, TWENTY-NINE AND ELEVEN DAYS RESPECTIVELY, IN STERILE NUTRIENT SOLUTIONS

Additions to modified Pfeffer's solution	Number shoot tips used	Average original length (cm.)	Gain in length (cm.)	Total number roots
	Peas			
Glucose (2%)	15	1.75	12.77	7
Levulose (2%)	15	1.72	3.58	3
None	14	1.75	0.88	14
	Corn			
Glucose (2%)	3	2.16	17.5	13
Levulose (2%)	3	2.90	12.43	8
None	2	3.75	4.50	1

growth made by *Ceratodon purpureus* should be noted. In the case of the latter plant, as reported earlier (13), the amount of dry matter produced with levulose as the carbon source was 2–7 times as great as the amount produced with glucose. The fact that the stem tips in the dark, even in the presence of 2 per cent glucose, are morphologically like etiolated shoots, would also indicate that it is not an absence of available carbohydrate which causes the stem elongation and small leaf development of plants grown in the dark.

Continued growth of root tips in culture solutions

The fact that the root tips in these experiments grew in solutions containing carbohydrates, and made very little growth in the same solutions lacking sugar, would suggest that the complete requirements for the growth of roots are water, mineral salts, carbohydrates, and free oxygen. If such were the case we should expect

that, furnished with sufficient quantities of these materials in the proper proportions, a root would continue to grow indefinitely. The excised roots of corn, however, will not grow indefinitely under the conditions described. Their development is rather definitely limited in the dark in the culture solutions used, as can be noted from the following experiment.

Root tips of corn were grown in the dark in the Pfeffer's solution containing 2 per cent glucose. At the end of eight days the tips of the roots were cut off and transferred to a fresh solution of the same composition, where they were allowed to grow for ten days. At the end of that time the tip was again cut off and transferred to a new solution. Growth took place there for ten days. If the nutrient solution were complete and no inhibiting or injurious factors active, by continued transfers we should be able to keep a root tip growing indefinitely in the same way as cultures of bacteria, molds, or yeasts are kept growing indefinitely by continued transfers to fresh media.

In this experiment the growth during the first period was excellent, and the number of secondary roots large (table V). During

TABLE V

GROWTH OF ROOT TIPS OF CORN IN NUTRIENT SOLUTIONS: ROOT TIPS IN GLUCOSE SOLUTION CUT OFF AND TRANSFERRED AS INDICATED

Period of growth	Number roots used	Average original length (cm.)	Gain in length (cm.)	Number secondary roots per root
	Pfeffer's solution plus 2 per cent glucose			
June 30–July 7	16	3.43	8.98	64.8
July 7–July 17	16	3.48	2.04	20.8
July 17–July 28	13	1.86	0.14	00.0
	Pfeffer's solution			
June 30–July 28	10	3.65	0.90	00.0

the second period the increase in length was one-fourth that of the first period, and the number of secondary roots one-third. In the third period hardly any increase in length was found, and no secondary roots were produced.

The maximum gain in the first period was that of a root tip originally 4.3 cm., which increased to 18.0 cm. and produced ninety-seven secondary roots. The maximum gain in the second period was that of a root tip 2.7 cm. long originally, which increased to 7.4 cm. and produced twenty-one secondary roots. In the third period only three out of thirteen roots showed any increase in length, the maximum being 0.9 cm. This experiment has been repeated many times, and many root tips of corn have been carried through the three periods of culture given. Five varieties of corn have been used: a dent variety from Alabama; Longfellow flint from New York (kindly furnished by Dr. J. K. WILSON); Boone County White and Reed's Yellow dent from Missouri; and Funk's Yellow dent from Illinois. The stoppage of growth in the third period in Pfeffer's solution plus 2 per cent glucose has occurred in every case. No single root tip thus far has made more than a slight amount of growth in the dark in the third period.

Not only does a diminution of growth rate, and a reduction in the production of secondary roots ending in a cessation of growth in the third period take place in the course of these transfers, but the diameter of the root tip continually decreases, until in the third period its diameter is one-fourth or less that of the original root tip. When stoppage of growth takes place, however, the root tips may apparently be normal in macroscopic and microscopic appearance, showing vascular bundles and root hair development.

The failure of an excised root to continue growth when repeated transfers of the root tip are made, at once suggests that the seedling root contains some material derived from the seed other than glucose, the mineral salts of Pfeffer's solution, water, and free oxygen which are necessary for continued growth and which the root cannot synthesize in the dark in solution cultures from the material supplied. Such material or materials would be fractionated by the continued transfers of the root tips.

Other explanations may be suggested. The stoppage of growth may be due to an unbalanced condition of the nutrient solution in which the roots are grown. The root tips, however, at the time of growth stoppage may show no macroscopic evidence of injury, and the fact that root hairs may be present on the root

tip in the third period also would indicate that the stoppage of growth is not due to the toxicity of the solution. The dextrose may penetrate too slowly to furnish sufficient carbohydrate to the root cells for continued growth. This would not appear to be a feasible explanation, however, because the early growth of the root tip is rapid, and the decrease in growth appears progressively greater. The early growth must occur at the expense of glucose which penetrates the root cells, because the root tips furnished with no glucose in the nutrient solution grew very little. It would seem that if the rate of penetration of the glucose determines the stoppage of growth one would have to assume a continuously increasing difficulty of penetration, terminating in entire impermeability of the root cells to glucose. The limited oxygen supply in the solution cultures may account for the growth stoppage. Here again, however, no better supply of oxygen is supplied in the first period than in the later periods, and yet the growth in the first period is the most rapid. If the limited oxygen supply is the factor which eventually causes the stoppage of growth, it must be a cumulative effect, due either to the development of deleterious materials or failure to synthesize some necessary material. It should also be noted that, although the aeration of water cultures of entire corn plants favors root and top development (ANDREWS and BEALS 1), the roots of an entire plant in unaerated water cultures do not show the stoppage of growth evident with the excised roots in solution cultures. It would seem reasonable to assume, therefore, as a working hypothesis from the experiments described, that oxygen, the mineral salts of Pfeffer's solution, glucose, and water are insufficient for the continued growth of excised corn roots.

Summary

1. A simple method of growing the isolated meristematic tissue of higher plants, excised root tips and stem tips, under sterile conditions is described.

2. The excised root tips of peas, corn, and cotton make considerable growth in the dark in solution cultures containing mineral salts and glucose or levulose.

3. The excised root tips of peas, corn, and cotton make little growth in the dark in solution cultures containing mineral salts and lacking carbohydrate.

4. The growth of the isolated root tips of peas, corn, and cotton is markedly greater in solution cultures containing glucose than in those containing levulose.

5. The excised roots of corn respond normally to gravity when grown on agar containing mineral salts and glucose.

6. The isolated shoot tips of peas and corn make considerable growth in the dark in sterile solution cultures containing mineral salts and glucose or levulose, but little in the absence of carbohydrates.

7. The excised shoot tips of corn and peas grown in sugar solutions remain chlorotic, and those of peas show the stem elongation and small leaf development characteristic of plants grown in the dark.

8. When the excised root tips of corn are grown for ten days or two weeks in the dark in a solution culture containing glucose and mineral salts, and the tip is then cut off and transferred to a fresh solution of the same type, the amount of growth in the second period is less than that in the first, and ceases in the third period.

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INFLUENCE OF WHEAT SEEDLINGS UPON THE HYDROGEN ION CONCENTRATION OF NUTRIENT SOLUTIONS[1]

LINUS H. JONES AND JOHN W. SHIVE

Introduction

That the reaction of nutrient culture media bears a very important relation to their biological properties is a fact that is well recognized. Consideration of H ion concentration is of vital importance in connection with plant culture studies, not only because of the profound influence which this factor exerts upon the manner in which plants respond toward certain nutrient elements in the media, but also because of its intimate relation to plant growth in general.

The H ion concentrations of some nutrient solutions commonly used for plant physiological studies undergo rapid and pronounced changes in contact with the roots of growing plants, while the reaction of other nutrient solutions changes only slightly or not at all under similar conditions, owing to the fact that they possess strong buffer properties. The rate, direction, and degree of reaction change are dependent, of course, upon a number of different factors, some of the more important of which are the composition and concentration of the nutrient solutions, and the species, age, and activity of the plants. It is not the purpose of this paper, however, to consider the various factors involved in the relation of the plants to the reaction changes which they may be capable of bringing about in nutrient solutions in which they are grown, but to report briefly an experiment carried out for the purpose of comparing the various nutrient solutions, commonly used for plant cultures, with respect to the initial H ion concentrations of the solutions, and to study the reaction changes induced in them by contact with the roots of young wheat plants.

[1] Paper no. 68 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Physiology.

Procedure

Spring wheat of the Marquis variety was germinated on a net as described by SHIVE (**15**). Seedlings carefully selected for uniformity of size and vigor were transferred, when about 5 cm. tall, to SHIVE'S three-salt solution R_5C_2, after having been mounted in the double piece paraffined cork stoppers devised by TOTTINGHAM (**18**). The stoppers were of the proper size to fit quart fruit jars of colorless glass, which were used for culture vessels. Three seedlings were included in each culture. The cultures thus prepared were conducted during a time period of twenty-five days, with renewal of solutions every three or four days. When the seedlings were approximately thirty days old from the time of germination, cultures were selected in which the plants were about equal with respect to size and vigor in so far as this could be judged from careful observation. The plants of the selected cultures, without being removed from the cork stoppers in which they were mounted, were then taken from the three-salt solutions, the roots were washed by carefully dipping them several times into distilled water, allowed to drain, and were then placed in the nutrient solutions devised by the various authors. The glass jars to which the plants were transferred were like those from which they were removed. Each jar had a capacity of 900 cc.

The formulae of the nutrient solutions devised by the various authors are given in tables I and II in terms of gram-molecules per liter. The solutions made up according to the formulae in table I were corrected to a total osmotic concentration value of approximately 1.75 atmospheres by the method of the freezing point lowering, while those prepared according to the formulae in table II were similarly corrected to an approximate osmotic concentration value of 1.00 atmosphere. No iron was added to any of these solutions, except to those for which this element is mentioned in the formulae. The solutions devised by BIRNER and LUCANUS (**1**), CRONE (**2**), and SACHS (**13**) contained precipitates. In making the cryoscopic tests for the total osmotic concentration values of these solutions the precipitates were allowed to settle, after which samples of the supernatant solutions were drawn off by means of a pipette, the lowering of the freezing points determined, and the corrections made whenever necessary.

TABLE I

FORMULAE OF NUTRIENT SOLUTIONS COMMONLY USED FOR PLANT CULTURES, ALL WITH TOTAL OSMOTIC CONCENTRATION VALUE OF APPROXIMATELY 1.75 ATMOSPHERES AS DETERMINED BY METHOD OF FREEZING POINT LOWERING

Author of solution	Volume-molecular partial concentrations of salts employed											
	KH_2PO_4	$Ca_3(PO_4)_2$	$Ca(H_2PO_4)_2$	$Fe_3(PO_4)_2$	$Ca(NO_3)_2$	KNO_3	$NaNO_3$	$MgSO_4$	K_2SO_4	$CaSO_4$	KCl	NaCl
Birner and Lucanus (1)	0.0108	.....	.....	0.0043	0.0133	.....	.....	0.0061	.....	.....	.....	.....
Crone (2)	.....	0.0022	.....	0.0019	.....	0.0266	.....	0.0056	.....	0.0049	.....	.....
Detmer (3)	0.0035	.....	.....	.....	0.0149	.....	.....	0.0051	.....	.....	0.0083	.....
Hartwell, Wheeler, and Pember (5)	.....	.....	0.0027	.....	0.0136	.....	.....	0.0077	.....	.....	0.0075	.....
Knop (7)	0.0044	.....	.....	.....	0.0145	0.0059	.....	0.0050	.....	.....	.....	.....
Pfeffer (12)	0.0041	.....	.....	.....	0.0136	0.0056	.....	0.0046	.....	.....	0.0037	.....
Sachs (13)	.....	0.0025	.....	.....	.....	0.0155	.....	0.0065	.....	0.0075	.....	0.0076
Schimper (14)	0.0028	.....	.....	.....	0.0018	0.0048	.....	0.0040	.....	.....	.....	0.0083
Schreiner, Skinner (16)	.....	.....	0.0066	.....	.....	.....	0.0278	.....	0.0030	.....	.....	.....
Shive, R_5C_2 (15)	0.0180	.....	.....	.....	0.0052	.....	.....	0.0150	.....	.....	.....	.....
Tollens (17)	0.0038	.....	.....	.....	0.0128	0.0052	.....	0.0049	.....	.....	.....	0.0054
Tottingham, $T_3R_1C_4$ (18)	0.0108	.....	.....	.....	0.0101	0.0034	.....	0.0081	.....	.....	.....	.....

The initial H ion concentrations of the nutrient solutions were determined immediately before bringing the plant roots in contact with them. H ion measurements were then repeated for each solution throughout a time period of fifty-two hours, during which the solutions remained in contact with the roots of the growing wheat plants. Nine tests were made of each solution

TABLE II

FORMULAE OF SOME LIVINGSTON-TOTTINGHAM (9) THREE-SALT NUTRIENT SOLUTIONS (TYPES I–VI) AND OF TOTTINGHAM'S (18) SOLUTIONS $T_1R_1C_5$ AND $T_1R_3C_5$ MODIFIED (JONES AND SHIVE 6) BY SUBSTITUTING AMMONIUM SULPHATE FOR POTASSIUM NITRATE IN EQUIVALENT OSMOTIC CONCENTRATIONS; ALL SOLUTIONS HAD TOTAL OSMOTIC CONCENTRATION VALUE OF APPROXIMATELY 1.00 ATMOSPHERE

Type	Number	Volume-molecular partial concentrations									
		KH_2PO_4	$Ca(H_2PO_4)_2$	$Mg(H_2PO_4)_2$	$Ca(NO_3)_2$	$Mg(NO_3)_2$	KNO_3	$MgSO_4$	$CaSO_4$	K_2SO_4	$(NH_4)_2SO_4$
		Livingston-Tottingham three-salt solutions									
I.	R_3S_2	0.0072	.....	.....	0.0048	.....	.....	0.0072	.....	.....	.....
II.	R_5S_2	.....	.....	0.0019	0.0037	.....	.....	.....	.....	0.0094	.....
III.	R_4S_1	.....	0.0025	.....	.....	.....	0.0099	0.0074	.....	.....	.....
IV.	R_4S_1	.....	0.0019	.....	.....	0.0057	.....	.....	.....	0.0076	.....
V.	R_3S_4	.....	.....	0.0025	.....	.....	0.0074	.....	0.0098	.....	.....
VI.	R_4S_2	0.0093	.....	.....	.....	0.0047	.....	.....	0.0047	.....	.....
		Modified Tottingham solutions									
	$T_1R_1C_5$	0.0021	.....	.....	0.0073	.....	.....	0.0071	.....	.....	0.0014
	$T_1R_3C_5$	0.0021	.....	.....	0.0073	.....	.....	0.0024	.....	.....	0.0042

during this period of contact. The small quantities of solutions withdrawn from the culture jars for the purpose of making tests were not replaced, since only about 2 cc. of solution was required for each determination. The H ion concentrations were determined by the colorimetric method, using the double tube color standards described by GILLESPIE (4).

The formulae of the three-salt solutions given in table II were selected from series of the six type-solutions proposed by LIVINGSTON and TOTTINGHAM (9). One solution was selected from each of six series to represent the six different types.[2] The table also

[2] A detailed description of these six type solutions, together with directions for their preparation, may be found in a "plan for cooperative research on the salt requirements of representative agricultural plants prepared for a special committee of the Division of Biology and Agriculture of the National Research Council." Edited by B. E. LIVINGSTON, Baltimore. 1919.

contains the formulae of TOTTINGHAM'S (**18**) solutions $T_1R_1C_5$ and $T_1R_3C_5$ modified (JONES and SHIVE **6**) by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations. It has previously been shown (**6**) that in the TOTTINGHAM solutions when thus modified, the direction of the reaction change induced by contact with the roots of young wheat plants is usually the exact opposite of that in the unmodified solutions. It was because of this fact, and also because these modified solutions are capable of producing excellent growth of young wheat plants, that they were included in the experiment.

Discussion

The initial P_H values of the nutrient solutions, the formulae of which are given in table I, and the P_H values determined at intervals during the period of fifty-two hours throughout which the solutions remained in contact with the plant roots, are given in table III. It will be observed from the data of this table that the

TABLE III

P_H VALUES OF NUTRIENT SOLUTIONS DETERMINED AT INTERVALS DURING CONTACT WITH ROOTS OF GROWING WHEAT PLANTS

AUTHOR OF SOLUTION	P_H VALUES									
	INITIAL	Duration of intervals in hours								
		2	4	6	8.5	24.5	27	30.5	33	52
Birner and Lucanus...	4.3	4.5	4.6	4.7	4.8	5.0	5.0	5.2	5.2	5.5
Crone.....	6.6	6.7	6.6	6.5	6.5	6.5	6.5	6.5	6.5	6.6
Detmer.....	4.7	4.8	4.9	5.0	5.2	5.6	5.6	5.7	5.7	5.9
Hartwell, Wheeler, and Pember.....	4.0	4.0	4.1	4.5	4.4	4.7	4.7	5.0	5.3	5.7
Knop.....	4.6	4.7	4.8	5.0	5.1	5.3	5.3	5.4	5.5	5.7
Pfeffer.....	4.7	4.7	4.9	5.1	5.3	5.5	5.5	5.6	5.7	5.8
Sachs.....	6.7	6.8	6.6	6.5	6.5	6.5	6.5	6.5	6.5	6.6
Schimper.....	4.8	4.9	5.1	5.2	5.4	5.7	5.7	5.8	5.9	6.1
Schreiner and Skinner..	4.2	4.2	4.4	4.4	4.6	5.4	5.5	5.7	5.9	6.1
Shive, R_5C_2.....	4.5	4.6	4.6	4.6	4.7	4.8	4.8	5.0	5.1	5.3
Tollens.....	4.6	4.7	4.9	5.1	5.1	5.5	5.5	5.6	5.9	5.9
Tottingham, $T_3R_1C_4$..	4.6	4.7	4.8	4.9	5.0	5.2	5.2	5.3	5.4	5.5

initial P_H values of only two of these solutions are close to the neutral point. CRONE'S (**2**) solution has an initial P_H value of 6.6, and SACHS'S (**13**) solution has a corresponding initial value of 6.7.

The initial P_H values of all the other solutions range between 4.0 and 4.8.

The P_H values of CRONE'S and of SACHS'S solutions remained practically unaltered during the entire period of contact with the plant roots. This is probably what might be expected, since the initial P_H values of these solutions lie close to the neutral point, and since the maximum reaction change which the wheat plants are capable of producing in any of the solutions whose formulae appear in table I finally brings the H ion concentrations of these solutions very close to this point, either slightly below or slightly above a P_H value of 7.0, regardless of the initial H ion concentrations of the solutions.

The various solutions exhibit marked differences in the rates of reaction change in contact with the plant roots under similar experimental conditions. Of the solutions with initial P_H values below 5.0, SHIVE'S solution R_5C_2 exhibited the highest resistance to reaction change, while TOTTINGHAM'S solution $T_3R_1C_4$ showed only slightly lower buffer properties as indicated by resistance to reaction change produced by the plants during the fifty-two hour period of contact. On the same basis the solutions of SCHREINER and SKINNER (**16**), and of HARTWELL, WHEELER, and PEMBER (**5**) possess relatively low buffer properties. With respect to the solutions here considered, it appears in general that the resistance offered to reaction change resulting from contact with the roots of growing plants is dependent largely upon the volume-molecular proportions of the soluble phosphate salts contained in the solutions. Thus SHIVE'S solution R_5C_2, which contains the highest proportion of dihydrogen potassium phosphate, exhibited the highest buffer properties. This is in entire accord with the observations of MCCALL and HAAG (**10**), and of MEIER and HALSTEAD (**11**). In the present experiment, however, there is one striking exception to this general rule as exhibited by the solution of BIRNER and LUCANUS (**1**), which has a volume-molecular proportion of dihydrogen potassium phosphate equal to that in TOTTINGHAM'S solution $T_3R_1C_4$, and about two and one-half times higher than that in KNOP'S (**7**) solution or in PFEFFER'S (**12**) solution, yet these solutions showed a higher resistance to reaction change as influ-

enced by the growing wheat plants than did the solution of BIRNER and LUCANUS. These comparisons, of course, are made upon the assumption that the plants in all the cultures were approximately equal with respect to their ability to cause reaction change.

The P_H values recorded in table IV were determined in the same manner and at the same intervals during the same time period as were those given in table III. The initial H ion concentrations of the LIVINGSTON-TOTTINGHAM (9) solutions containing dihydrogen potassium phosphate are always much lower than

TABLE IV

P_H VALUES OF NUTRIENT SOLUTIONS DETERMINED AT INTERVALS DURING CONTACT WITH ROOTS OF GROWING WHEAT PLANTS

TYPE	NUMBER	P_H VALUES									
		INITIAL	Duration of intervals in hours								
			2	4	6	8.5	24.5	27	30.5	33	52
		Livingston-Tottingham three-salt solutions									
I.	R_3S_2	4.6	4.7	4.8	5.0	5.1	5.4	5.4	5.5	5.6	5.7
II.	R_5S_2	3.8	3.8	4.0	4.1	4.3	4.4	4.5	5.0	5.3	5.8
III.	R_4S_1	3.6	3.6	3.7	3.9	4.0	4.3	4.3	4.6	4.8	5.6
IV.	R_4S_1	3.7	3.8	3.9	4.0	4.1	4.3	4.3	4.5	4.7	5.6
V.	R_3S_4	3.6	3.6	3.7	3.8	3.9	4.3	4.3	4.3	4.4	5.4
VI.	R_4S_2	4.3	4.7	4.7	4.9	5.0	5.2	5.2	5.3	5.4	5.6
		Modified Tottingham solutions									
	$T_1R_1C_5$	4.8	4.9	4.9	5.0	5.0	4.7	4.6	4.6	4.6	4.2
	$T_1R_3C_5$	4.8	4.9	4.9	5.0	4.9	4.6	4.4	4.4	4.4	4.2

are those containing the corresponding calcium or magnesium salts. This fact is more certainly brought out by MCCALL and HAAG'S (10) table of P_H values determined for six complete series of the LIVINGSTON-TOTTINGHAM type-solutions described in a publication (8) prepared for a special committee of the Division of Biology and Agriculture of the National Research Council.

Mention should be made of the fact that the initial P_H values of the solutions here chosen as representatives of types III, IV, V, and VI, as given in table IV, are not in very close agreement with those determined for the same solutions by MCCALL and HAAG.

The initial P_H values of these solutions in the order given are 3.6, 3.7, 3.6, and 4.3, while McCALL and HAAG'S values are 4.1, 4.1, 4.3, and 4.7 in the same order. Disagreements of this kind are to be expected, of course, because of the different methods employed in determining the P_H values and in the preparation of the solutions, variations in the degree of purity of the salts used, differences in the temperatures of the solutions at the time when the measurements are made, etc. A good example of such discrepancies appears in the P_H values of a series of the LIVINGSTON-TOTTINGHAM solutions of type I as determined by McCALL and HAAG and by MEIER and HALSTEAD. These authors do not agree upon a single solution of a complete series of twenty-one, the values determined by the latter authors always being considerably but uniformly higher than those determined by the former.

It will be observed from the data of table IV that the maximum reaction changes produced by the plants during the fifty-two hour period of contact with the LIVINGSTON-TOTTINGHAM solutions with low initial P_H values are always considerably greater than are the corresponding changes in the solutions with higher initial P_H values, since the final P_H values of all these solutions show no very marked differences. Experience with these type-solutions has shown that the maximum reaction changes which young wheat plants are capable of producing in them finally always brings the P_H values of the solutions very close to the neutral point, regardless of the initial P_H values, although the time required to accomplish this may vary considerably with the different solutions, owing to differences in their buffer properties.

As has previously been shown (**6**), the direction of the reaction changes of the modified TOTTINGHAM solutions during contact with the roots of the young wheat plants is usually the opposite of that of the LIVINGSTON-TOTTINGHAM solutions and of the unmodified TOTTINGHAM (**18**) solutions under similar conditions. It will be observed, however, that the maximum reaction changes produced in these solutions by the plants during the fifty-two hour period of contact are not very great, the change in the H ion concentration of both solutions here considered being from $P_H = 4.8$ to $P_H = 4.2$, although the total osmotic concentration value of these solutions is only 1.0 atmosphere.

Experience with twenty representative solutions of TOTTINGHAM'S (18) complete series of eighty-four, modified as here described by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations, has shown that the P_H values of these solutions are not greatly altered by contact with the roots of young wheat plants between the ages of four and five weeks, the tendency always being toward a slight increase in the H ion concentration of the solutions during growth intervals of three or four days without renewal of the solutions. It is thus easily seen that for certain types of culture studies in which it is desirable to maintain the H ion concentrations of the nutrient media within comparatively narrow variation limits, solutions of this kind possess marked advantages over those in which the H ion concentrations are rapidly decreased by the action of the plants. The two solutions (modified TOTTINGHAM solutions $T_1R_1C_5$ and $T_1R_3C_5$) have the added advantage of high efficiency in the production of young wheat plants when iron in small amounts is supplied to the solutions in an insoluble form such as ferric phosphate. Soluble iron in the form of ferrous sulphate, even in small traces, has been shown (6) to be exceedingly toxic to the plants grown in these solutions.

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SULPHUR AND NITROGEN CONTENT OF ALFALFA GROWN UNDER VARIOUS CONDITIONS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY

E. H. HALL

Introduction

Various investigators show a considerable variation in the analyses of the sulphur content of alfalfa, as well as in the proportion of organic and inorganic sulphur in the crop. It was thought well to analyze alfalfa from a number of selected places to see to what degree these variations could be explained by different sources of the crop, and to what degree by the personal factor of applying the none too reliable fusion methods, which probably always involve more or less volatilization and loss of sulphur. The very extensive Oregon work (11) has shown that the acre yield of alfalfa is enormously increased on their plats by the application of any sulphur source, and that on the fertilized plats the alfalfa contains a very much higher percentage of protein. Determinations were made in each sample of the total and nitrate nitrogen in order to ascertain whether there is any correlation between the sulphur and nitrogen content of alfalfa from the various sources.

It has been long established that sulphur is one of the ten essential elements for the growth of plants. This is easy to understand when one recognizes that sulphur is an essential building material for all plant proteins, as well as for various odor and flavor producing organic compounds found in members of the mustard family, onions, etc. While much of the sulphur in plants is found in the form of these organic compounds, there is also some inorganic or sulphate sulphur present. The latter is generally considered an excess of absorption of sulphur over its utilization. It may be, however, that some free sulphate sulphur must be present as a building material in order to insure maximum protein synthesis and maximum growth. SCHERTZ found that in the older leaves of *Coleus Blumei* some free nitrate must be present as a building

material in order to prevent the decomposition of organic nitrogen compounds—proteins, chlorophyll, phospholipines, etc. A similar situation may hold in alfalfa for sulphate as a sulphur source.

On the basis of WOLFF'S old ash analyses of crop materials, agricultural scientists came to assume that so little sulphur was used by crops that there was no doubt that all soils furnished an abundance of this material. The magnitude of error likely to appear in the ashing method is well illustrated by the fact that WOLFF'S analyses showed that one hundred bushels of corn contain 0.2 lb. of sulphur, while analyses of the modern fusion methods show at least 8.5 lb. or 42.5 times as much (2). Ashing seems to drive practically all of the organic sulphur off into the air, and determines only the small amount of sulphur existing in the inorganic form.

The use of the fusion method (7) of determining the sulphur content of crops has quite changed the situation by showing that all crops are considerable users of sulphur, and some crops very heavy consumers of sulphur. This fact, together with the general low percentage of sulphur in soils and the large losses of sulphur from soils by leaching, has shown that the question of sulphur supply to crops needs serious consideration. The Oregon (11) and Washington (9, 14) stations have shown beyond doubt that alfalfa cannot be grown successfully on many lands of those states without the addition of a sulphur source. They commonly get increased tonnage amounting from 100 to 500 per cent by the use of gypsum or other sulphur sources. The protein content of the hay is also increased almost 2 per cent in some cases by the use of sulphur fertilizers. From these facts it seems probable that the marked benefits received from the use of land plaster on legumes and other high sulphur-using crops in eastern United States and England during the last 150 years are due to gypsum furnishing an excellent sulphur source (3).

HART and PETERSON (5) of Wisconsin, BROWN and KELLOGG (2) of Iowa, and SHEDD (12) of Kentucky have all emphasized the fact that a permanent fertility system must look after the sulphur supply of the soil as well as the so-called three fertilizer elements—nitrogen, potash, and phosphorus. Recent work is indicating that

the same is true of calcium, and some suggest that the same may sometimes be true for magnesium. It is not within the scope of this paper to give a full discussion of the present status of the sulphur fertilization problems in the United States. For a critical discussion of this problem the reader is referred to a paper recently written by CROCKER (3).

Methods

In order to study the chemical composition of hay produced in various localities, samples were secured from three different states, Kansas, Illinois, and Missouri. So far as it was possible, these samples were taken from several different places in the mow. This may not have constituted so good a representative sample as could have been secured from the field, but it was the best that could be obtained under the circumstances. The samples were dried for some time in the laboratory and then finely ground and the moisture determined. The samples aggregated about 100 gm. of the finely ground material. Aliquots of these samples were used for all the determinations. Table I gives a list of the samples, and, so far as possible, the types of soils and locality from which

TABLE I

Sample	Locality	Soil type	Crop
1.....	Circleville, Missouri	.....	Alfalfa
2.....	Meadville, Missouri	Grundy silt loam	Alfalfa
3.....	Brookfield, Missouri	Shelby loam	Sweet clover
4.....	Horton, Kansas	Silt loam	Alfalfa
5.....	Horton, Kansas	Brown silt loam	Alfalfa
6.....	Emporia, Kansas	Silt loam	Alfalfa
7.....	Emporia, Kansas	Silt loam	Alfalfa
8.....	Pratt, Kansas	.....	Alfalfa
9.....	Paris, Illinois	Brown silt loam	Alfalfa

they were taken. The following facts are necessary for a complete understanding of the table. The samples taken from Missouri were from farms that had never been under alfalfa cultivation until the last two years. The sweet clover had been harvested but once and was purely an experimental crop. The samples taken from Kansas were from some of the oldest and best alfalfa fields in the state. Some of these fields had produced maximum

crops for the past twelve years. Particular attention must be given to the samples from Paris, Illinois. Part of this field had been an old orchard where alfalfa had never been grown before. When the hay was ready to be harvested, parts of the field showed a difference in color, some being yellow and others being dark green. These samples when analyzed showed a marked chemical difference, as shown in the tables.

Several methods of sulphur determinations were tried before satisfactory results warranted the adoption of any particular one. The peroxide method as described in the Agricultural Chemists Bulletin no. 107 was finally adopted. The Osborn method caused a great deal of trouble by the igniting or foaming over of the material near the completion of the first fusion. This may have been due to too rapid heating of the crucible. This difficulty was better controlled in the official method because of the presence of sodium carbonate, which slowed down the reaction. The carbonate also caused some trouble at first in removing the residue from the crucible. This, however, was overcome by allowing a small stream of water to play on the residue after it had cooled just enough to prevent spattering. When the crucible was filled with water the material came out very easily. This treatment ruined the crucible in a very short time, but it shortened the process considerably.

There were two distinct crucial periods in the process. The first was when the sodium peroxide began to break down the material and ammonia was being liberated. This was the most critical because of the flashing which caused the loss of many determinations. Samples varied considerably in the flashing, perhaps owing to the different amounts of nitrate present. The second critical point occurred in the slow heating after the addition of the first 10 gm. of peroxide. The flame had to be regulated and the stirring so constant that the reaction did not become violent enough to burn up the sample or cause it to foam over the top. When these conditions were carefully controlled, the solutions were clear when neutralized, and the results obtained from a series of duplicate samples agreed as well as could be expected.

The dry fusion method is severely criticized by KOCH (STOCKHOLM 13) because not all the sulphur is secured by this method.

He states that in any dry fusion method there is some sulphur being lost in the fumes no matter how carefully the fusion is made. No experiments were made to test this statement, but the variation in results obtained from a number of analyses of the same sample leads one to believe that even with the official method some of the sulphur is not secure. It seems probable that in all determinations of the sulphur content of plant materials to date, the results are low because of the loss of sulphur in fusion. Some difficulty was experienced in controlling the fusion so as to prevent the reaction proceeding too rapidly. If the reaction becomes too violent, smoke is evolved in considerable quantities, and results obtained under these conditions are uniformly higher than those obtained when the fusion takes place at a moderate rate. At first the samples were considered lost although no flashing took place, but later these samples were saved, and in every case showed a higher value than those that were controlled perfectly, and agreed very closely in the final result. The rate of fusion may have a great deal to do with the amount secured from the sample. This point will be studied more extensively later. The evidence is that all the sulphur was not being obtained from the samples. KOCH advocates the use of perhydro, a very concentrated hydrogen peroxide, in the determination of sulphur, but unfortunately this substance has not been on the market since the war, as Germany was the sole manufacturer. OLSON (8) used the Parr bomb, and, when certain precautions are followed, claimed for it advantages in speed and in assurance against losses of sulphur. Modification of the method is reported in connection with more recent determinations (9, 10).

All the determinations were made on the air dry material, and the percentage of sulphur calculated to the oven dry weight as follows:

Number of grams in aliquot.....	1.25	1.25
Weight of $BaSO_4$ obtained.....	0.0328 gm.	0.0329 gm.
Percentage of sulphur in $BaSO_4$.....	13.73	.....
Percentage of moisture in sample....	5.82	5.82
Percentage of sulphur in sample.....	0.382	0.382

The BENEDICT method (4) was tried, but proved very unsatisfactory because of the extreme tendency of the material to sputter

at the time of fusion. This method is highly recommended by its author to give very close checks, even though they are lower than those of the peroxide method. BENEDICT attributes these high results in the peroxide method to silica, which is entirely lacking in his method. The writer experienced no trouble with silica in using the official method, for at no time was strong alkali allowed to stand in contact with glass without being neutralized. The ease with which the material was removed facilitated quick neutralization. There was never a weighable amount of silica found in the solution.

Sulphate sulphur determinations

The method of determining sulphate sulphur was the same as that used by AMES (1). Five grams of the dry material mixed with one per cent of hydrochloric acid were shaken in the mechanical shaker for three hours. The solution was filtered and an aliquot of 2.5 gm. taken. This was then treated with barium chloride and precipitation allowed to take place in the cold. After standing for at least forty-eight hours the sulphates were determined in the usual way. The averages of the determinations are given in table II.

TABLE II

Sample no.	Moisture	Weight $BaSO_4$	Total sulphur	Percentage total sulphur	Percentage sulphate sulphur	Total nitrogen	Nitrate nitrogen
	Circleville, Missouri (5 gm.)						
1	7.58	0.1304	0.0179	0.387	0.01	2.41	0.01
2	7.59	0.1320	0.0183	0.397	0.01	2.42	0.01
3	.....	0.1246	0.0182	0.371	.....	.....	.....
4	.....	0.1254	0.0171	0.373	.....	.....	.....
5	.....	0.1262	0.0173	0.375	.....	.....	.....
	Emporia, Kansas (1.25 gm.)						
1	5.81	0.0348	0.0047	0.405	0.013	2.32	.....
2	5.82	0.0369	0.0050	0.424	0.012	2.31	Trace
3	.....	0.0328	0.0045	0.382	.....	.....	.....
4	.....	0.0529	0.0045	0.383	.....	.....	.....
5	.....	0.0371	0.0051	0.432	.....	.....	.....

TABLE II—Continued

Sample no.	Moisture	Weight $BaSO_4$ /	Total sulphur	Percentage total sulphur	Percentage sulphate sulphur	Total nitrogen	Nitrate nitrogen
	Brookfield, Missouri						
1.....	8.57	0.0354	0.0048	0.425	0.024	2.09	0.01
2.....	8.43	0.0337	0.0045	0.390	0.027	1.97	0.01
3.....	.....	0.0319	0.0044	0.383	.....	.....	.....
	Horton, Kansas						
1.....	7.35	0.0430	0.0059	0.508	0.038	2.95	0.015
2.....	7.27	0.0476	0.0065	0.563	0.044	2.85	0.013
3.....	.....	0.0429	0.0059	0.507	.....	.....	.....
	Horton, Kansas						
1.....	8.45	0.0365	0.0051	0.440	0.02	2.66	0.01
2.....	8.54	0.0310	0.0041	0.374	0.027	2.71	.....
3.....	.....	0.0369	0.0051	0.440	.....	.....	.....
	Meadville, Missouri (1.25 gm.)						
1.....	8.81	0.0345	0.0047	0.408	0.02	2.39	Trace
2.....	8.83	0.0346	0.0048	0.409	0.024	2.36	.....
	Emporia, Kansas						
1.....	8.11	0.0345	0.0047	0.412	0.04	2.47	Trace
2.....	8.12	0.0346	0.0048	0.413	0.042	2.44	.....
	Pratt, Kansas						
1.....	5.82	0.0307	0.0042	0.358	0.012	2.38	Trace
2.....	5.88	0.0319	0.0044	0.370	0.011	2.36	.....
3.....	.....	0.0318	0.0044	0.370	.....	.....	.....
4.....	.....	0.0308	0.0042	0.358	.....	.....	.....
	Paris, Illinois (green alfalfa)						
1.....	7.08	0.0425	0.0058	0.503	.....	2.70	Trace
2.....	7.12	0.0414	0.0057	0.490	.....	2.86	.....
3.....	.....	0.0419	0.0058	0.495	.....	.....	.....
	Paris, Illinois (yellow alfalfa)						
1.....	5.81	0.0331	0.0045	0.386	.....	2.37	.....
2.....	5.94	0.0326	0.0045	0.380	.....	2.54	.....

Total nitrogen

Total nitrogen was determined by the ARNOLD-GUNNING method as modified to include nitrate nitrogen. This method is described by MATHEWS (6).

Nitrate nitrogen

The nitrate nitrogen was determined by the SCHLESING-WAGNER method as modified by KOCH. The method is described in detail by WOO (15) in his chemical study of *Amaranthus*. To test the accuracy of this method a five-tenths per cent solution of potassium nitrate was used. Theoretically, 2 cc. of this solution should give 2.22 cc. of gas calculated to standard conditions. The average result of several determinations was 2.15 cc., which was about 97 per cent of the calculated amount. The precaution necessary to insure the success of the process as described by WOO is that all the solutions must be entirely free from oxygen. The presence of oxygen tends to cut down the amount of gas absorbed, thus causing a low result.

In making the determinations, aliquots containing 5 gm. of the original samples were extracted with two 100 cc. of water for one-half hour each. This extract was then treated with lead acetate to precipitate the proteins which caused much trouble unless removed. The samples were made up to volume of 250 cc. and filtered, then 100 cc. of the clear solution representing 2 gm. of the dry material was concentrated on the steam bath to about 20 cc. and the NO gas determined. The following represents a determination as run in duplicate from a sample:

Aliquots in cc. (2 gm.)	100	
Total volume of gas evolved	1.66	cc.
Volume of unabsorbed gas	0.91	cc.
Volume of absorbed NO gas	0.75	cc.
Barometic pressure 749.7 mm., temperature	24.5	
Volume at standard conditions	0.68	cc.
Equivalent milligrams of KNO_3	0.0076	
Equivalent milligrams of NO_3	0.005	

Discussion

It is important to note the variation in the sulphur content of the hay from the widely separated districts. From the tabulated

results it will be seen that the amount of sulphur in the various hays analyzed runs from 7.4 to 11.16 lbs. per ton of hay. If an acre produces from 5 to 8 tons of hay annually, 37 to 90 lbs. of sulphur will be removed from each acre of soil each year. The maximum figures here are much higher than those of PETERSON, because of the very high sulphur content of the samples from Kansas. The analyses of the samples from Missouri and Illinois give results that agree more nearly with the results of PETERSON'S analyses of alfalfa from Wisconsin.

One other marked deviation from the results reported by PETERSON is seen in the low amount of sulphate sulphur obtained in these analyses. He found that the ratio of the organic to inorganic sulphur was practically unity. In table II the sulphate sulphur in no case exceeds 10 per cent of the total sulphur in the crop, and in the samples taken from Illinois there was no sulphate sulphur. On the average for all analyses the sulphate sulphur equals 4.35 per cent of the total sulphur. The following shows the percentage of the sulphur that existed in the inorganic form in the alfalfa collected from various regions:

	PER CENT
Paris, Illinois (1)	0.0
Paris, Illinois (2)	0.0
Circleville, Missouri	2.5
Emporia, Kansas (1)	3.0
Pratt, Kansas	3.2
Meadville, Missouri	5.3
Brookfield, Missouri (sweet clover)	6.2
Horton, Kansas	7.6
Emporia, Kansas (2)	9.9
Average	4.35

Summary

1. Alfalfa hay grown in various parts of the United States shows considerable difference in the percentage of total sulphur content, quite independent of sulphur fertilization.

2. In general, hay from fields with the heaviest crops contains the highest percentage of sulphur.

3. Good to excellent crops of alfalfa hay remove annually from thirty-seven to ninety pounds of sulphur per acre, an amount which would seem far in excess of the amount returned by rain.

4. In some samples all, and in every sample more than 90 per cent, of the total sulphur was in the organic form. There was none or little sulphur present in excess of the actual needs as building material.

This investigation was conducted under a research fellowship from the Gypsum Industries Association. The work was performed at the University of Chicago in the Hull Botanical Laboratory under the direction of Dr. WILLIAM CROCKER. The writer wishes to thank the Gypsum Industries Association for their kindness in furnishing the fellowship, and Dr. CROCKER for his advice and criticism. Thanks are also due Dr. FREDERICK KOCH for his advice and criticism of analytical methods.

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CURRENT LITERATURE

BOOK REVIEWS

Root systems

WEAVER[1] has made another notable contribution to our knowledge of root systems. Former investigations, noted in this journal,[2] included many root systems of grassland plants, and the present publication is confined to grassland vegetation and to the crop plants grown within its limits. The grasslands are considered under the three subdivisions of true prairie, mixed prairie, and short-grass plains.

The true prairie is characterized by tall sod-forming grasses growing in soil of rather abundant water content, with greater moisture in the subsoil. On the basis of root development, three general classes may be recognized in grassland vegetation. In the first the "working depth," or average depth reached by a large number of roots, is about 1.5 feet, with a maximum depth of 3.3 feet. The second class possesses roots with a working depth of 3.3 feet and a maximum of about 6 feet; while in the third class the working depth of the roots is usually 5–8 feet and the maximum penetration 8–12 feet, with a few species reaching an extreme of 15–20 feet. Examples of the three classes are *Aristida oligantha*, *Elymus canadensis*, and *Koeleria cristata;* *Andropogon scoparius*, *Bouteloua gracilis*, and *Grindelia squarrosa;* and *Andropogon furcatus*, *Aster multiflorus*, and *Panicum virgatum*. The deeper rooted species have few roots in the surface layers of the soil, showing a grouping of roots into more or less definite layers, thus reducing competition and permitting the growth of a larger number of species. In the short grass plains practically all plants have root systems well adapted for water absorption from surface soils. Two have roots with a working depth less than 2 feet, three have working depths of 2–4 feet, and three have a range of 4–7 feet. Examples of the three classes are *Opuntia polyacantha*, *Bulbilis dactyloides*, and *Psoralea tenuifolia*. Here the water supply is much more limited, especially in the subsoil. The soil and moisture conditions, as well as the vegetation in the mixed prairie, are intermediate between the true prairie and the short-grass plains. Compared with the true prairie, the plants are not as deeply rooted, but have usually developed a very efficient and widely spreading absorbing system in the surface soil.

The root systems of cereal crops grown at many stations in true and mixed prairie and short-grass plains were also examined. The comparative amount

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[1] WEAVER, JOHN E., Root development in the grassland formation. Carn. Inst. Wash. Publ. 292. pp. 151. *pls.* 23. *figs.* 37. 1920.

[2] BOT. GAZ. **69**:351–353. 1920.

of root development of cereals in each seems to be in true prairie 100 per cent, in mixed prairie 80–95 per cent, and in short-grass plains 51–79 per cent. The experimental data are given in the form of tables, drawings, and photographs, all of excellent quality. It is recognized that variations in root development are caused by various factors, such as the chemical and physical character of soils and the evaporating power of the air. The soil factors are most effective through water content and aeration. The water relations of the various habitats were examined by WEAVER by means of atmometers and by soil moisture determinations, the latter being interpreted by means of the wilting coefficient and hygroscopic coefficient. He calls attention to the recognized fact that many plants are able to continue to absorb water below the limits of the wilting coefficient. In fact, it is in the responses of different plants to the use of soil moisture lying between the limits of the wilting and the hygroscopic coefficients that differences appear which might be used with advantage for a most significant classification. All wilting, whether clearly manifest or not, takes place at about the same moisture content, that is, at the wilting coefficient. As the moisture passes below this point the hydromesophytes soon die, the mesophytes live for a somewhat longer time, while xerophytes or drought-resistant plants prolong their existence for a very considerable period, reducing the soil moisture to the hygroscopic coefficient. It seems rather clear from the work of ALWAY and others, however, that the water absorbed below the limits of the wilting coefficient is quite insufficient for growth and merely serves to sustain life, indicating that the term "growth water" has been correctly used by the reviewer and others for the amount of soil moisture in excess of that indicated by the wilting coefficient.

While all of WEAVER'S investigations of root development have been of the highest order, this report shows a decided advance, for the accumulation of data has become sufficient to permit some significant generalizations. Among other things he points out that as our knowledge of root development in various associations increases it will render more accurate our interpretation of the indicator significance of the natural vegetation. Thus the contribution he has made to the science of ecology becomes most useful in the practice of agriculture.—GEO. D. FULLER.

Sturtevant's notes on edible plants

The purpose of this large volume,[3] as indicated in the preface, is that new knowledge may be available as follows: (1) the original home of many esculents is given for the first time; (2) new landmarks in the histories of edible plants are pointed out; (3) an effort is made to mention all cultivated esculents; (4) although the book contains much new information as to the history of the

[3] HEDRICK, U. P. (editor), STURTEVANT'S notes on edible plants. Report N.Y. Agric. Exper. Sta. for 1919. 2:4to. pp. vii+686. Albany: J. B. Lyon Co., State printers. 1919. $2.75.

food plants of the Old World, it is especially full and accurate in the discussion of the esculents of the New World; (5) STURTEVANT presents much new information on the variations that have been produced in plants by cultivation; (6) the book adds much to geographical botany; (7) many data are contributed toward the study of acclimatization.

The material contained in the book was compiled from the notes and manuscripts of the late Dr. E. LEWIS STURTEVANT, first Director of the New York Agricultural Experimental Station, and represents the labors of at least a quarter of a century. The editor and his assistants have had no light task in selecting from the large mass of partially organized material, and in verifying and organizing the bibliography, which includes some 6000 citations contained in 500 publications. This task of organization seems to have been well done. The information is arrayed in encyclopedic entries under the scientific name of the plant. To further facilitate reference there is an index of common names. The articles vary in length from a few lines for many of the less important plants to six or eight pages for such plants as beet, potato, tomato, and strawberry, and twelve pages for squash, pumpkin, and corn.

A portrait of STURTEVANT, a biographical sketch, and a bibliography of his writings add to the interest of the volume. Its comprehensive nature, including reference to some 3000 plants, its close attention to historical and geographic data, and its numerous citations of literature make it invaluable for reference. It greatly expands and often corrects details of the knowledge formerly available through such works as DE CANDOLLE'S *Origin of cultivated plants*. It is excellently printed, remarkably low priced, and is available for purchase from the New York Agricultural Experiment Station, Geneva, N.Y.—GEO. D. FULLER.

Devonian floras

ARBER'S[4] posthumous volume, which was sent to the press by the deceased author's wife and his friend D. H. SCOTT, gives a very welcome general survey of the Devonian floras. There is comparatively little known of the Devonian plants, and a great deal of what was formerly attributed to Devonian turned out to be of a later geologic age. On the other hand, the great discoveries of lower Devonian plants at Rhynie in Scotland, which represent the oldest known type of land plants, had given a renewed interest to Devonian paleobotany.

ARBER divides the fossil plants of the Devonian formation into two floras. The first and older division, which he calls *Psilophyton* flora, is represented by those low pteridophytes of which some types had already been described by Sir WILLIAM DAWSON in 1859, and whose affinities have remained so doubtful. The latest addition to this group is the newly discovered plants from Rhynie.

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[4] ARBER, E. A. NEWELL, Devonian floras, a study of the origin of Cormophyta. 8vo. pp. xiv+100. *figs.* 47. Cambridge. 1921.

The second flora characterizes the middle and upper Devonian, and is called by ARBER *Archaeopteris* flora, because this fern predominates.

Other very interesting early types occurred in the upper Devonian, like *Archaeosigillaria*, *Protolepidodendron*, and numerous other earlier representatives of *Sphenophyllum*, *Sphenopteris*, and *Pseudbornia*.

This fascinating and very instructive little volume, whose value is increased by an extensive bibliography on the subject, forms a suitable monument for ARBER, who has been removed from the world of science at too early an age.—A. C. NOÉ.

North American slime-moulds

In 1899 MACBRIDE'S *The North American slime-moulds* was published, which was the first comprehensive presentation of this interesting group for American botanists. A brief review of that work was published in this journal.[5] A second edition has just appeared,[6] the first edition "having been exhausted long ago." It corrects certain errata of the first edition, but chiefly it incorporates the results of research during recent years. The painstaking work and clear statement which characterize MACBRIDE have resulted in a book of unusual quality. It is intended especially for American students, and therefore discusses chiefly American species, but it also includes brief descriptions of other forms, and refers to many extra-limital species now generally recognized. To many botanists it will probably give a new perspective of a group of organisms often dismissed with too little attention.—J. M. C.

MINOR NOTICES

Manual of woody plants.—TRELEASE[7] has published a second, revised edition of his small pocket manual of the woody plants used for decorative purposes. The intention of the author "is to make it possible for any careful observer to learn the generic and usually the specific name of any hardy tree, shrub, or woody climber that he is likely to find cultivated in the United States." The need of a new issue indicates that the manual has found a constituency. In the revised edition certain errors have been corrected, and the scope of the book has been enlarged by including a few additional types.—J. M. C.

British forestry.—A handy little volume by HANSON[8] gives in a non-technical manner the general principles of forestry as practiced in the British Isles and adjacent parts of Europe. In addition to plain directions for nursery

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[5] BOT. GAZ. **29**:74. 1900.

[6] MACBRIDE, THOMAS H., The North American slime-moulds. A descriptive list of all species of Myxomycetes hitherto reported from the continent of North America, with notes on some extra-limital species. 8vo. pp. xvii+299. *pls. 23*. 1922.

[7] TRELEASE, WILLIAM, Plant materials of decorative gardening: The woody plants. 2d ed. 16mo. pp. xliii+177. Urbana: Published by the author. 1921. $1.00.

[8] HANSON, C. O., Forestry for woodmen. 12mo. pp. 238. *pls. 13. figs. 15*. 2d ed. Oxford. 1921.

and forest management, sawing and planting, felling and measurement, there are chapters on forest animals, birds, insects, weeds, and fungi. The final chapter deals with the uses of British timber. The book is in an easily readable style and seems to give a good idea of forestry as practiced in Great Britain.—GEO. D. FULLER.

NOTES FOR STUDENTS

Vegetation and climate.—The most recent phase of ecology, which finds expression in attempts to introduce quantitative methods for the investigation of vegetation and of the factors that determine its nature and distribution, has received a notable contribution in a recent volume by LIVINGSTON and SHREVE.[9] "The existence, limits, and movements of plant communities are controlled by physical conditions" is stated as a fundamental law of plant geography, and an attempt is made to map the distribution both of various physical factors and of a considerable number of plants and plant communities. Data for the latter are obtained from a variety of sources, and more particularly from SHREVE's well known map of the vegetation areas of the United States. In fact a generalized form of this map is employed as a basis upon which to display the distribution of climatic factors. In addition to this, ranges of various plant species and groups of species, such as deciduous, microphyllous, and broad-leaved evergreen trees, are delimited. Here emphasis is placed upon the lack of anything like an adequate knowledge of the ecological distribution, based on relative abundance, dominance, or density of stand, of any considerable number of plants even in such a well explored land as our own.

A general discussion of the influence of the environment on plant life classifies environmental factors which are important in distribution as: (1) moisture conditions, (2) temperature conditions, (3) light conditions, (4) chemical conditions, (5) mechanical conditions. These are considered in turn. In discussing the supply of water to vegetation the "residual soil moisture content" of the soil is not regarded as a soil constant, in spite of many data tending to prove that within very considerable limits its constancy holds. No mention is made of wilting or hygroscopic coefficients, nor of the notable contributions of such well known investigators as ALWAY, SHULL, KEEN, and BOUYOUCOS. These omissions constitute the most serious defect in an otherwise admirable and comprehensive volume.

The tabulation of the climatological data, used for the construction of the maps, in a form that makes them available for future investigators is highly to be commended. As an example of such accumulations of exact information regarding conditions limiting growth and distribution, the table of frost data may be cited. Here for 1803 different stations the altitude, number of years of record, average date of the last frost in spring, and the earliest in the

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[9] LIVINGSTON, B. E., and SHREVE, F., The distribution of vegetation in the United States as related to climatic conditions. Carn. Inst. Wash. Publ. 284; pp. xvi+590. *pls.* 73. *figs.* 74. 1921.

fall, together with the length of the average frostless season, are given in readily accessible form, the resulting tables covering thirty-three pages. These data are plotted as isoclimatic lines on the map of the United States. Among the factors thus tabulated and mapped are: temperature efficiencies for the frostless season expressed as (1) remainder indices, (2) expotential indices, and (3) physiological indices; absolute temperature maxima and minima; average daily temperature for the coldest and hottest weeks of the year; mean daily precipitation for the frostless season together with the number of rainy and dry days for same; normal annual precipitation; atmospheric evaporating power; ratios of precipitation to evaporation; aqueous vapor pressure; relative air humidity; wind velocity; sunlight; and moisture temperature indices.

It is recognized that there are decided difficulties in establishing correlations of these isoclimatic areas with the distribution of plant species, growth forms, and vegetational areas, but even here the efforts of the authors have met with considerable success. The statement of climatic extremes for various vegetational features, in 128 tables covering 86 pages, certainly gives more exact information than was ever before available regarding the conditions under which various plant communities and plant species have developed. A very decided addition to our knowledge of the exact conditions that probably determine general vegetational areas is also provided in the plotting of the comparative ranges and intensities of twelve leading climatic conditions for nine such areas, for the life-zones of Merriam, and for over thirty plant species.

The book shows the uniformly good printing of text and maps characteristic of the publications of the Carnegie Institution of Washington, and seems reasonably free from errors of typography and in the use of specific names. It will be indispensable to all ecologists who wish to take account of climatic factors, and will become increasingly useful as increasing knowledge permits more accurate interpretation of such factors and their closer correlations with the resulting displays of plant life.—GEO. D. FULLER.

Anatomy and biology of gymnosperm leaves.—While there have been several investigations of leaves in various groups of gymnosperms, there has been no comprehensive study of the entire line. Consequently, a recent work by FEUSTEL[10] will be welcomed by those who wish to find, in compact form, a survey of the literature of the subject. The author states frankly that his work is only a summary of the literature, not an investigation; but his observations, especially along biological lines, and the comparative presentation of anatomical features are suggestive. The various orders are treated separately.

CYCADOFILICALES.—The term Pteridospermae is used for this order. The mode of treatment is similar in the other orders. In general appearance the leaves are fernlike, but the internal structure shows a mixture of fern and

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[10] FEUSTEL, HERM., Anatomie und biologie der Gymnospermenblätter. Beih. Bot. Centralbl. **38**:177–257. 1921.

cycad characters. He notes the multiple leaf traces of the Medullosae, the concentric bundles in the rachis of *Lyginodendron* and *Heterangium*, and in the leaf of *Sutcliffia;* and among the cycad characters, the double leaf trace of *Lyginodendron*, and the secretory canal system. The leaves belong to the leathery type of recent ferns. The thickness and inrolled margins are xeromorphic characters, and the prevalent hypodermal sclerenchyma is xerophytic.

CYCADALES.—The review of the literature of the cycad leaf is particularly thorough, probably because there have been two rather extensive investigations. In the various genera and species, the shapes of cells of the epidermis, the stomata, the parenchyma, the thick-walled cells, and the vascular system are treated under separate headings. The xerophytic features are emphasized. The leaf structure is so characteristic in the different genera that a taxonomic key, based upon leaves, is presented. Since no study has been made of the leaves of *Microcycas*, this genus is not mentioned. Doubtless most of the investigations have been made upon leaves taken from greenhouse specimens. While the general structure is probably about the same as in plants in their native habitats, we should expect to find the xerophytic characters more pronounced in plants exposed to the extreme xerophytic conditions than in greenhouse plants, which are more or less shaded and are frequently watered.

BENNETTITALES.—So little is known of the internal structure of the mature leaf that this section is very brief, but there is a mixture of fern and cycad characters, and, according to FEUSTEL, some angiosperm characters.

CORDAITALES.—This order is treated under the separate headings Poroxyleae, Pityeae, and Cordaiteae; but since no leaves are known in the Pityeae, the study deals only with the other two groups. Resemblances to some of the Cycadofilicales and to some of the recent cycads are pointed out, but it is very questionable whether the similarities are due to relationship. Resemblances between the leaves of *Cordaites* and some of the Coniferales, especially *Agathis*, seem more striking.

GINKGOALES.—The heterophyllous leaves of *Ginkgo* are significant, the lobed and divided character being retained from the ancient forms. The structure of the leaf, with its long petiole, broad blade, and soft consistency, is not very xerophytic, but indicates that *Ginkgo* in its phylogeny has come from a climate with long wet periods.

CONIFERALES.—The structure and biology of the leaf of *Pinus* are treated in great detail as a type of the order, and the other genera are considered from the standpoint of comparative morphology and biology. The leaves of all the conifers, by their form, structure, and consistency, are protected against wind and rain. They are both xeromorphic and xerophytic. Several ecological hypotheses are advanced to account for the geographical distribution of the group.

GNETALES.—Naturally, the genera of this group are treated separately; but, in spite of the striking differences, the three genera show more resemblances to each other than to the rest of the gymnosperms. The leaves of

Gnetum resemble those of angiosperms in their internal structure as well as in their general appearance.

The conclusion for the entire group of gymnosperms is that the leaves belong to a single xerophytic type, with *Ginkgo* and *Gnetum* as the only exceptions. The literature list is very incomplete, because it was not thought necessary to repeat references which can be found in standard texts.—C. J. CHAMBERLAIN.

Respiration of thermophiles.—The respiratory activity of the thermophile fungi, *Thermoascus aurantiacus*, *Anixia spadicea*, and others, has been studied by NOACK,[11] who finds that the high respiratory activity is directly related to the rapid growth rate of these organisms, and that it is merely a consequence of the high temperature, not due to specific constitution or peculiar enzyme equipment. The economic coefficient for young cultures is 1.8, and about 3.6 for older ones. The respiratory quotient with changing oxygen supply and different growth rates from changed sources of carbon remains near one, so that the only peculiarity is the high respiration. From a comparison of the temperature coefficient of respiration in thermophiles, which is about 1.7 within the temperature limits for growth (35°–55°), with that of *Penicillium*, which is about 2 at 15°–25° C., NOACK concludes that the thermophiles show a restricted respiration. Thus, *Thermoascus* produces 310 per cent of its dry weight of CO_2 in 24 hours. If the respiratory rate of *Penicillium* at 25° C. were quadrupled by a rise to 45° C., however, it would produce 532 per cent of its dry weight of CO_2 in 24 hours. From this consideration of the VAN'T HOFF rule, and the absence of abnormal behavior in respiration and growth, he concludes that the high respiration of thermophiles is merely a temperature consequence, and is really somewhat restricted for that temperature.

With regard to this use of the VAN'T HOFF rule, and the finding of a lower temperature coefficient of respiration for thermophile fungi at 45° C. than for *Penicillium* at 25° C., attention is called to a recent paper by MATISSE,[12] who criticizes the use of the VAN'T HOFF rule, and urges the adoption by biologists of the ARRHENIUS temperature law instead. The formula for the VAN'T HOFF rule is incompatible with that developed by ARRHENIUS, and the latter is now accepted universally by physical chemists. The curves developed from each formula are much alike at low temperatures, but the ARRHENIUS formula shows that as the temperature goes higher, the value of Q_{10} decreases. The lower temperature coefficient for thermophiles is exactly what one would expect according to the ARRHENIUS temperature law, and the argument that thermophiles show a restricted respiration for that temperature (45°) is probably not justified.—C. A. SHULL.

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[11] NOACK, KURT, Die Betriebstoffwechsel der thermophilen Pilze. Jahrb. Wiss. Bot. **95**:413–466. 1920.

[12] MATISSE, GEORGES, La loi d'Arrhenius contre la règle du coefficient de température. Archiv. Int. Physiol. **16**:461–466. 1921.

Salts and permeability to acids.—BRENNER[13] finds by the use of a deplasmolitic method on red cabbage that neutral salts modify the toxicity of acids. The following table shows the killing concentration of HCl, four hours exposure, following plasmolysis by the various salts:

Plasmolytic agent	Critical conc. of HCl in mols.	H ion conc. of critical sol.
NaCl 2.2 per cent	1/1000	8.91×10^{-4}
KNO_3 3.75 per cent	1/800	1.29×10^{-3}
KCl 2.8 per cent	1/600	1.38×10^{-3}
K_2SO_4 5.0 per cent	1/400	4.68×10^{-4}
$Mg(NO_3)_2+6$ aq. 8.8 per cent	1/1000	1.09×10^{-3}
$MgCl_2+6$ aq. 7.0 per cent	1/400	3.16×10^{-3}
$MgSO_4+7$ aq. 16.1 per cent	1/250	1.12×10^{-3}
$Ca(NO_3)_2+4$ aq. 6.5 per cent	1/500	1.95×10^{-3}
$CaCl_2+6$ aq. 6.2 per cent	1/250	5.50×10^{-3}
Dextrose	1/700	8.90×10^{-4}
Saccharose	1/700	8.71×10^{-4}

The author emphasizes the fact that salts antagonize the toxic action of strong mineral acid, H ion, just as they have long been known to do with other salts. The antagonistic action of salts toward H ions is due to the joint action of cations and anions of the salts. By change of color in the anthocyanin of the cells used, the author determined that acids enter uninjured cells very slowly, and that the effect of salts in reducing this entrance corresponds to their antitoxic effects. In cells that are killed by acids, NaCl, KCl, and KNO_3 favor the exosmose of anthocyanin, and salts of earth alkali delay it very much. Of the plasmolytic agents Mg salts proved very toxic. In $Mg(NO_3)_2$ no cells were alive after twenty-four hours, and in $MgCl_2$ and $MgSO_4$ very few. The salts of alkalis were only slightly less toxic, except for KCl, which showed many cells alive after two days. In $CaCl_2$ the cells would remain alive and plasmolyzed for a much longer time, some of them for twenty-one days. The author emphasizes the toxic action of pure salts and the balanced or non-toxic nature of mixtures of salts.—WM. CROCKER.

Soil moisture.—A new classification of soil moisture, based upon its behavior in freezing, appears to be founded upon scientific principles and to give a deeper insight into the actual condition of such water, its movement, and its relationship to plants. BOUYOUCOS[14] has found that a portion of the soil moisture freezes readily near 0° C., another portion only when a temperature of −4° C. is reached, and a third portion does not freeze at all. The first

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[13] BRENNER, W., Über die Wirkung von Neutralsalzen auf die Säureresisteng, Permeabilität und Lebensdauer der Protoplasten. Ber. Deutsch Bot. Gesells. 38: 277–285. 1921.

[14] BOUYOUCOS, G., A new classification of soil moisture. Soil Science 11:33–47. 1921.

portion is called "free" water, to distinguish it from the remaining "unfree" water. The portion of the latter capable of being frozen is regarded as capillary-adsorbed, while that which does not freeze at all is the combined water or the water of solid solution and of hydration.

The physiological and ecological significance of such a classification is indicated by showing that a close relationship exists between the unfree water and the wilting coefficient of BRIGGS and SHANTZ, and between the combined water and the hygroscopic coefficient. The new classification, together with the relationship of the different classes of soil moisture to plants, may be concisely expressed as follows, gravitational water being the same as in older systems of classification:

1. Gravitational			superavailable
2. Free			very available
3. Unfree	Capillary-adsorbed		slightly available
	Combined	water of solid solution water of hydration	unavailable

The method of measuring the relative amounts of these various forms of water in the soil is known as the dilatometer method, and is relatively simple, being based upon the expansion of water upon freezing. This method would also seem to offer a convenient, rapid, and accurate method of determining the wilting coefficient.—GEO. D. FULLER.

Nutrients for Rhizopus.—Miss DUNN[15] has studied the effect of various concentrations and proportions of nutrients upon BLAKESLEE's two races (male and female) of *Rhizopus nigricans*. The salts KH_2PO_4, NH_4NO_3, $MgSO_4$, and $FePO_4$ were used in various proportions, and total concentration with glucose or glycerine as the carbon source. Apparently calcium is not needed by this plant, and it makes no use of nitrate as a nitrogen source, but uses the NH_4 ion. Under the conditions of this investigation "the activity of the organisms appears clearly to be controlled by a combination of (*a*) salt proportions (or perhaps ion proportions), (*b*) total salt concentration, and (*c*) dextrose concentration. When these three conditions are poorly balanced for the growth of these races, the solution may sometimes be greatly improved by altering just one of the conditions, but it is frequently necessary to alter two conditions simultaneously to obtain good physiological balance. The solution representing the best combination has the following characteristics: (*a*) Molecular salt proportions: KH_2PO_4 6.0:NH_4NO_3 1.0 : $MgSO_4$ 1.1 : $FePO_4$ a mere trace. (*b*) Total salt concentration, equivalent to a calculated osmotic value of 14.5 atmospheres. (*c*) Dextrose concentration, 1.0 gram-mol. per liter." There was no consistent difference in dry weight production between the male and female races when grown on dextrose, but when grown with glycerine as the

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[15] DUNN, GRACE A., A comparative study of the two races of *Rhizopus nigricans*. Physiol. Researches **2**:301–339. 1921.

carbon source, the male uniformly gave the higher yield. In all dextrose cultures where the yields were medium or high, the male showed much more sporangium production than the female, and the female was more vigorous in mycelial production. The male seems to have a somewhat higher maximum temperature than the female. The optimum for the female also seems to be somewhat lower.—Wm. Crocker.

Amylase of Rhizopus.—Harter[16] has made a study of the amylase of *Rhizopus tritici*, particularly the effect of various factors on its secretion and action. The best growth of the fungus, and also secretion of amylase, as indicated by the amount of hydrolysis of starch, was obtained with Czapeck's nutrient solution, when ammonium nitrate was the source of the nitrogen, and starch the source of the carbon. Poorer results were obtained when sodium nitrate was used as the source of the nitrogen, and either cane sugar or glucose as the carbon source. Sweet potato bouillon, however, which contained both glucose and starch, gave the best results of all. The nutrient solution best for the growth of the fungus was also best for the secretion of the enzyme. The best temperature for the action of the enzyme was 45° C., and its action was practically nil at 60°. The secretion of the enzyme, as measured by the hydrolytic power of a unit weight of the enzyme powder, was much less when the fungus was grown at 40° C., the maximum temperature for its growth, than when it was grown at 9° C., which represents about the minimum temperature for its growth. While most of the experiments, the data from which are recorded in this paper, have been tried out by other workers, using other species of fungi, this seems to be the first time that *Rhizopus tritici* has been used for such an investigation.—S. V. Eaton.

Plants of Mississippi.—The greater part of a useful little volume by Lowe[17] is occupied by an annotated list of the vascular plants of Mississippi, compiled from the Allison and Tracey herbaria and from the field work and collections of the author and his assistant, Thomas L. Bailey. A feature of the work which should appeal to the general reader is an introduction comprising an elementary discussion of the problems of plant ecology expressed in non-technical language. Botanists will be more interested in the division of the state into the ten following topographic and floristic regions: (1) Tennessee River hills, (2) Northwestern prairie belt, (3) Pontotoc ridges, (4) Flatwoods, (5) North central plateau, (6) Jackson prairie belt, (7) Loess or bluff hills, (8) Yazoo-Mississippi delta, (9) Long leaf pine belt, (10) Coastal pine meadows. The topography, soil, and vegetation of each of these subdivisions are briefly described and lists of characteristic species given. The usefulness of the volume would be decidedly increased by supplementing the table of contents with an adequate index.—Geo. D. Fuller.

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[16] Harter, L. L., Amylase of *Rhizopus tritici*, with a consideration of its secretion and action. Jour. Agric. Res. **20**:761–786. 1921.

[17] Lowe, E. N., Plants of Mississippi. Miss. Geol. Surv. Bull. 17. pp. 292. 1921.

Bahaman endemics.—The Bahama Islands have been found to possess an endemic flora of some 185 species, or rather more than fourteen per cent of the entire plant population. There is but a single endemic genus, however, *Neobracea*, belonging to the Apocynaceae. A careful analysis of the situation by TAYLOR[18] shows that the distribution of these forms does not coincide with the "age and area" hypothesis of WILLIS, nor do the distribution and growth forms of the endemic differ materially from those of the non-endemic species. Moreover, the comparatively youthful land surface seems to preclude the idea of any considerable number of relic species. TAYLOR is thus forced to the conclusion that the endemics are due largely to the rather direct influence of the somewhat peculiar set of external factors that include sterile and often saline soil, deficient rainfall, strong trade winds varied by violent hurricanes, and possibly certain other factors.—GEO. D. FULLER.

Action of lichens on glass.—Doubt has sometimes been cast on the capacity of lichens to disintegrate the harder rocks. In this connection Miss MELLOR[19] reports the occurrence of twenty-two forms growing on the glass of church windows in France. The plants not only etched the glass, but produced pits up to 0.5 cm. in diameter and as much as 1.6 mm. deep, in the process chipping off fine fragments which became imbedded in their tissues. The action is explained as a purely mechanical result of the solution in rain water of the CO_2 excreted as a result of the respiration of the lichens, and is very slow, but the conclusion is drawn that plants able to attack glass in this way would have a similar disorganizing effect on rocks. In fact, one of the species found growing on glass was also found growing on slate, in which situation fine chips of slate were incorporated in the thallus.—G. W. MARTIN.

Zoölogisch-Botanische Gesellschaft.—This society is one of the oldest and most famous of the natural science societies of Europe. In order to continue its existence, it is compelled to sell some of its herbarium collections. In view of this need we publish the following notice received from Vienna:

"For sale—a valuable collection of mosses containing more than 1000 European species, including about 12,000 fine specimens collected by famous bryologists, as SCHIMPER, WILSON, DE NOTARIS, LINDBERG, JURATZKA, MOLENDO, LORENTZ, BREIDLER, many of them being types. The collection also includes several hundred members of old rare exsiccatae, as, for example, RABENHORST, Bryotheca Europaea. Offers should be sent to the Zoölogisch-Botanische Gesellschaft, III/3, Mechelgasse 2, Vienna, Austria."

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[18] TAYLOR, NORMAN, Endemism in the Bahama flora. Ann. Botany **35**:523–532. 1921.

[19] MELLOR, ETHEL, Les lichens vitricoles et leur action mécanique sur less vitraux d'église. Comptes Rend. Acad. Sci. **173**:1106–1108. 1921.

Embryology.—SOUÈGES, in continuation of his work on the embryology of Angiosperms, has published several recent contributions. *Rheum Emodi*[20] is presented as a further representative of the Polygonaceae. *Chenopodium Bonus-Henricus*[21] is the subject of an unusually full treatment. In the presentation of the embryogeny of the Labiatae, *Glechoma hederacea* and *Lamium purpureum* form the subject of one paper,[22] and in the second paper[23] *Mentha viridis* is used. The Scrophulariaceae are represented by *Veronica arvensis*.[24] The presentation of the embryogeny of *Urtica pilulifera*[25] is completed with two papers.—J. M. C.

Overgrowth of stumps.—The overgrowth of the upper ends of stumps of *Pseudotsuga* and of *Abies grandis* has been observed by PEMBERTON[16] in British Columbia. Careful excavation showed that in every case root grafting with living trees existed, the connected roots providing an avenue for food conduction to the stump, and this he believes to be the true explanation of the interesting phenomenon.—GEO. D. FULLER.

Addisonia.—The fourth number completing Vol. 5 of this publication appeared in December 1920, and the first number of Vol. 6 appeared in March 1921. The fine colored illustrations and popular descriptions form a collection that should be in every botanical library.—J. M. C.

Potassium and growth of plants.—SMITH and BUTLER[27] have published a report of rather extensive work on the effect of potassium on the growth of plants. It confirms all the old findings and gives quantitative data on several new plants.—WM. CROCKER.

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[20] SOUÈGES, R., Recherches sur l'embryogénie des Polygonacées (suite). Bull. Soc. Bot. France **67**:75–85. 1920.

[21] ———, Développement de l'embryon chez le *Chenopodium Bonus-Henricus* L. *Ibid.* **67**:233–257. *figs. 40.* 1920.

[22] ———, Embryogénie des Labiées. Développement de l'embryon chez le *Glechoma hederacea* L. et *Lamium purpureum* L. Compt. Rend 1921: 48–50.

[23] ———, Développement de l'embryon chez le *Mentha viridis* L. *Ibid.* 1921: 1057, 1058.

[24] ———, Embryogénie des Scrofulariacées. Développement de l'embryon chez le *Veronica arvensis* L. *Ibid.* 1921: 703–705.

[25] ———, Développement de l'embryon chez l'*Urtica pilulifera* L. Bull. Soc. Bot. France **68**: 172–188; 280–294. *figs. 57.* 1921.

[26] PEMBERTON, C. C., Overgrowth of stumps of conifers. Can. Field Nat. **35**:81–87. *figs. 4.* 1921.

[27] SMITH, T. O., and BUTLER, O., Relation of potassium to growth in plants. Ann. Botany **35**:189–225. 1921.

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THE

BOTANICAL GAZETTE

June 1922

VARIATIONS IN CYTOLOGY AND GROSS MORPHOLOGY OF TARAXACUM

II. SENESCENCE, REJUVENESCENCE, AND LEAF VARIATION IN TARAXACUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 295

PAUL BIGELOW SEARS

(WITH NINE FIGURES)

The variability of *Taraxacum* has frequently been recorded in botanical literature without receiving much elucidation. It is less often an object of investigation than of conjecture and discussion. A few botanists have studied the effect of environmental factors upon *Taraxacum*, but their results have not cleared away taxonomic difficulties. Systematic botanists have either looked upon the variations in this genus as fluctuations or slight mutations, and then proceeded to lump or split as judgment might dictate. That life history exerts a profound influence upon form seems generally to have escaped attention.

The 1910 supplement of *Index Kewensis* (12) lists 152 new species as described within five years, mainly from Scandinavia. Leaving aside such overwhelming evidence of polymorphy, it is interesting to note that the painstaking monograph of HANDEL-MAZETTI (10) admits fifty-seven species. These fall into eleven sections. Of these eleven sections four are so distinctive that none of their species have been mistaken by preceding students for either *T. vulgare* or *T. laevigatum*. In each of the remaining sections species have

been confused at various times with one or the other of the familiar species named, and designated accordingly in publications. Beyond question some of this confusion has been due to bad taxonomy. There is ample reason to believe that more of it has been caused by lack of definite boundaries between species.

Although the two common species mentioned are parthenogenetic, sexual species are known and hybrids may exist. Aside from this possibility, any explanations offered for the overlap between species have been so admittedly hypothetical (10) that they need not be rehearsed at this point. It is necessary, however, to take inventory of the precise information available with respect to variation in *Taraxacum* and its causes. It is also necessary to examine the nature of the criteria used in differentiating species and species groups.

ROOT.—Generally referred to as perennial, without modification, it has long been known to branch multicipitally (4), and to split into separate individuals (5).

STEM.—Usually represented only by a growing point; in the absence of light internodes develop (20), giving rise to a vertical rhizome (cf. fig. 4).

LEAVES.—Although taxonomists admit great variability and the frequency of overlap between leaf characters of different species, it is a fact that leaf characters are extensively used in species descriptions and often in distinctions. "Habit" as indicated by leaf orientation is also so used (3), despite a lack of evidence that the light responses of *Taraxacum* differ from those of other plants. In a saturated atmosphere greatly elongated leaves are produced (20). The reverse is true, that dry air shortens the longitudinal components of the leaf, although WIESNER's statement that it slightly increases dissection is open to question. Arctic alpine conditions result in a symmetrical dwarfing of the leaves of transplants (2). Check and transplant in this experiment were of equal age, and show little difference in degree of leaf dissection in BONNIER's original figures. Salt itself is not the direct cause of succulence in leaves of saline soil species of *Taraxacum* (11). The rate of production of leaves in plants of identical age varies with the habitat, being less in strong sunlight than in shade (20).

HANDEL-MAZETTI (10) notes a marked difference in the rate at which oldest leaves die away and leave their bases, considering it a specific characteristic.

As to segmentation, GOEBEL (9) classifies *Taraxacum* with those plants which produce first entire, then dissected leaves, but quotes evidence that segmentation is "richer" in temperate than arctic forms, and in "well-nourished" plants than in "half-starved" meadow-moor forms. DEVARIGNY, as quoted by MORGAN (14), places his own construction upon the matter by attributing the dissection to dry soil conditions and entire leaves to hydrophytic environment. FRANK (8) states that leaf development is basipetal, the tip segment developing first. STORK (17) found that uniform culture conditions upon seedlings (of identical age) produce rather uniform leaf type, regardless of leaf form extremes in the parents.

SCAPE.—The normal course of changes in length and position throughout flowering and fruiting are well known (15). FRANK states that in alpine species the scape is longer in crevice plants than in those growing exposed.

INFLORESCENCE.—Fasciation is admittedly a nonheritable character, and was determined by SCHORBATOW (16) to be due to mechanical pressure during the period of most active flowering, "the third year."

BRACTS.—In taxonomic works these rank highly as criteria, the characteristics used being number, color, size, form, and position. Nevertheless, considerable variability is admitted. HANDEL-MAZETTI notes that infection by *Synchitrium taraxaci* converted the outer bracts of *T. vulgare* into those typical for *T. alpinum*. He also speaks of the great variation in corniculi in many species. FERNALD (7) found very complete intergrades between *T. vulgare* and *T. palustre* in bracts, as well as other characters. STORK reports that uniform culture conditions produce great uniformity in seedlings from parents with widely different bract characters.

FLOWERS.—HANDEL-MAZETTI uses approximate flower number as a specific criterion, and states that flower color is undoubtedly a trustworthy character, although useless in dealing with herbarium

material. The presence or absence of pollen, used by several Scandinavian authors as a specific character, is considered by the authority just quoted to be highly variable within the species, unless the observations of other men were erroneous.

ACHENES.—These are generally regarded as affording the most critical taxonomic characters, that is, in size, form, color, beak length, pappus color, ribbing, and tubercles. Actually size is no more exempt from the laws of fluctuation in *Taraxacum* than in any plant, while form is subject to considerable modifications by pressure during development. HANDEL-MAZETTI, however, states that uniform culture conditions have less effect upon achene form than upon leaf and bract characters. Colors vary from dark brown to clear green in *T. officinale* according to SCHORBATOW, who states that the two extremes are fixed in inheritance, but whether he includes *T. laevigatum* under the other species is not clear. In STORK's cultures, chosen from parents varying as widely as possible in achene as in other characters, the progeny were either red or gray fruited. Color does not develop until the outer cells die, and in red fruits is due to homogentisic acid which is absent in gray ones (10). Freezing may prevent its appearance in *T. laevigatum* (17). Beak length is notoriously interfered with by injury, whether by freezing or mowing. Pappus color is said by HANDEL-MAZETTI to be due to diffraction phenomena and not pigment. The small species group characterized by definite pappus color is therefore likely to be a distinct one. Ribbing is due to the number of stereome bundles beneath the epidermis.

Summarizing, it is seen that while bracts and achenes are conceded to afford the critical characters, the leaves are actually given great weight in spite of their variability and the uncertainty concerning its nature. There is also not a little evidence for bract variability. Pappus color seems dependable, and achene color ought to be generally so, barring environmental extremes.

Observations

METHOD.—Several years of desultory observation in connection with other phases of this work having failed to throw the necessary light upon the problem of leaf variation, it seemed best to conduct

thorough studies of entire plants excavated from various habitats. After some fifty plants had thus been secured and studied, several hundred additional spud collections were made for confirmatory purposes. Subsequently all conclusions were tested rigorously by field observations extending over a period of fifteen months. By comparison of root development, color and texture of cortical rind, number and nature of withered petiole zones, and general condition of rosette, it was found that the age of a given plant could be told with considerable accuracy. With this technique as a basis, attention was centered upon the development of life history details and leaf form changes.

LIFE HISTORY.—This procedure revealed an intimate connection between leaf variability and life history. The first consideration is that strict accuracy forbids speaking of *Taraxacum* as a perennial plant without qualification. As stated by STORK, *Taraxacum* ordinarily does not bloom the first year. Blossoming occurs freely in both species throughout the second year. At the end of the period of most active flowering (autumn of second year or spring of third) the vigorous production of flower buds forces apart the inner leaves of the original rosette with their axillary growing points. From such growing points arise numerous secondary rosettes. As a rule several of these persist, functioning essentially as new individuals, in spite of their common root connection. Throughout the length of the root each secondary rosette has its own strand of vascular tissue. The individuality of the parent rosette can only be maintained by the cortical tissues, and these rather generally become cleft by fission and cork ingrowth. Observation indicates that such cleavage is hastened by conditions favoring rapid growth. Cleavage tends to become complete throughout the root length.

LEAF FORM.—The relations between this life history and leaf form is intimate and direct. The first leaves of a seedling (juvenile leaves) tend to be entire and smooth (fig. 1), later becoming more dissected and generally more hairy (fig. 2). In *T. laevigatum* this dissection in general reaches a higher degree than in *T. vulgare*, although the latter species, under conditions favoring luxuriant growth and numerous leaves, finally may attain an extreme degree

of dissection. When multicipital branching occurs, the new secondary rosettes consist of leaves of juvenile form, that is, tending to be smooth and entire (fig. 3). Each daughter rosette which persists then repeats the history of the parent rosette with respect to leaf form, as well as with respect to flowering habits and eventual

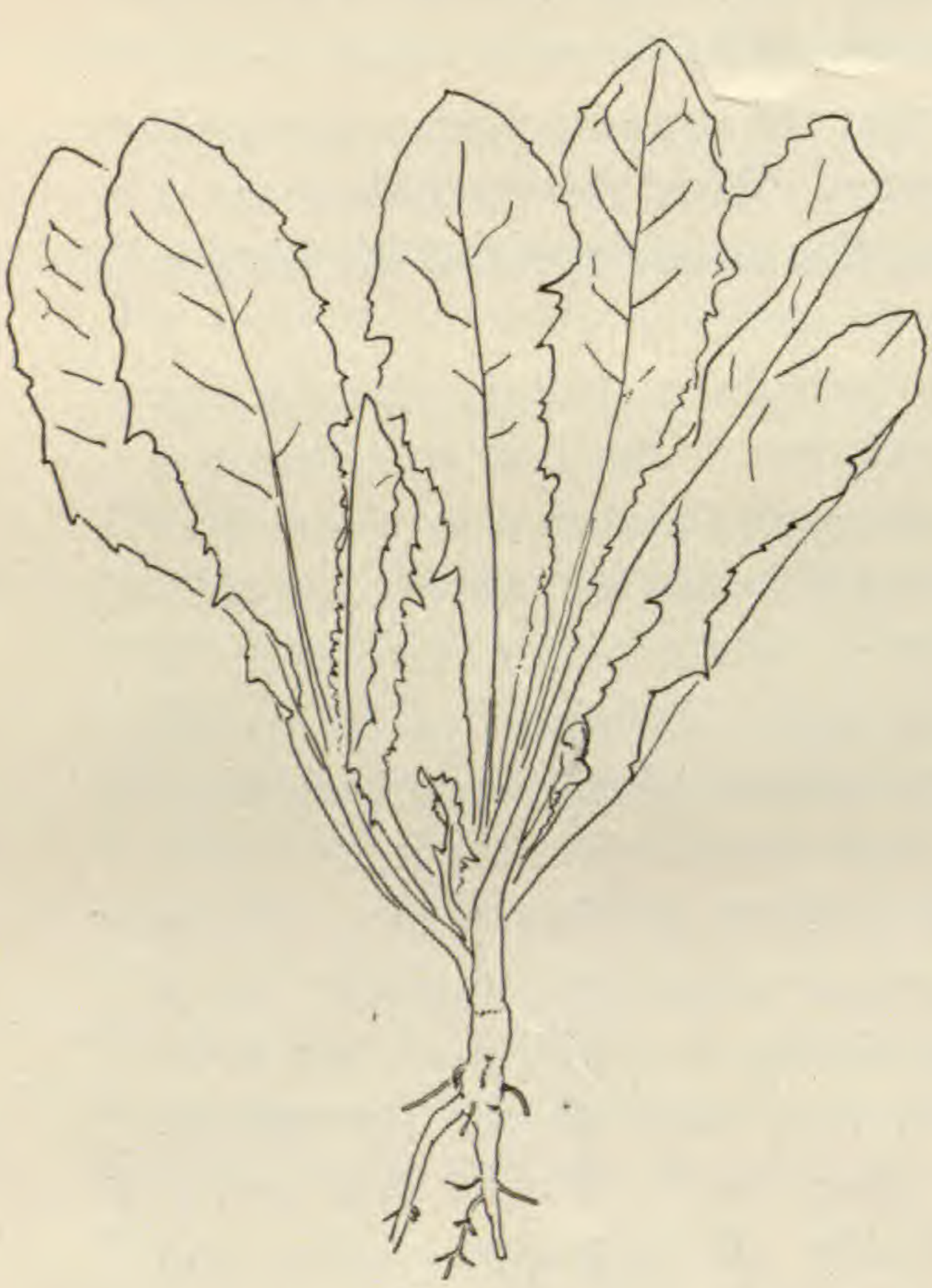

FIG. 1

FIG. 2

FIGS. 1, 2.—Fig. 1, young seedling showing relatively entire leaf form and unbranched crown; fig. 2, seedling at end of flowering period showing highly dissected leaf form, scars of juvenile leaves of first year, and beginnings of root fission.

rejuvenation (fig. 4). Certain modifying factors must be considered. For example, the supply of reserve food in rejuvenating roots results in rosettes that are often unusually vigorous and decidedly different in appearance from seedlings of the same degree of leaf dissection. Again, it not infrequently happens that secondary rosettes on the same root are of different ages, and hence different leaf form (fig. 4). This is bound to confuse an observer who does not excavate the plant and inspect it carefully.

In the next place, *Taraxacum* fruits are produced throughout the growing season and germinate readily. Very early spring seedlings may be ready to bloom before frost, and then resume blooming the following spring, equipped with highly dissected leaves. Later seedlings will not bloom until the following spring,

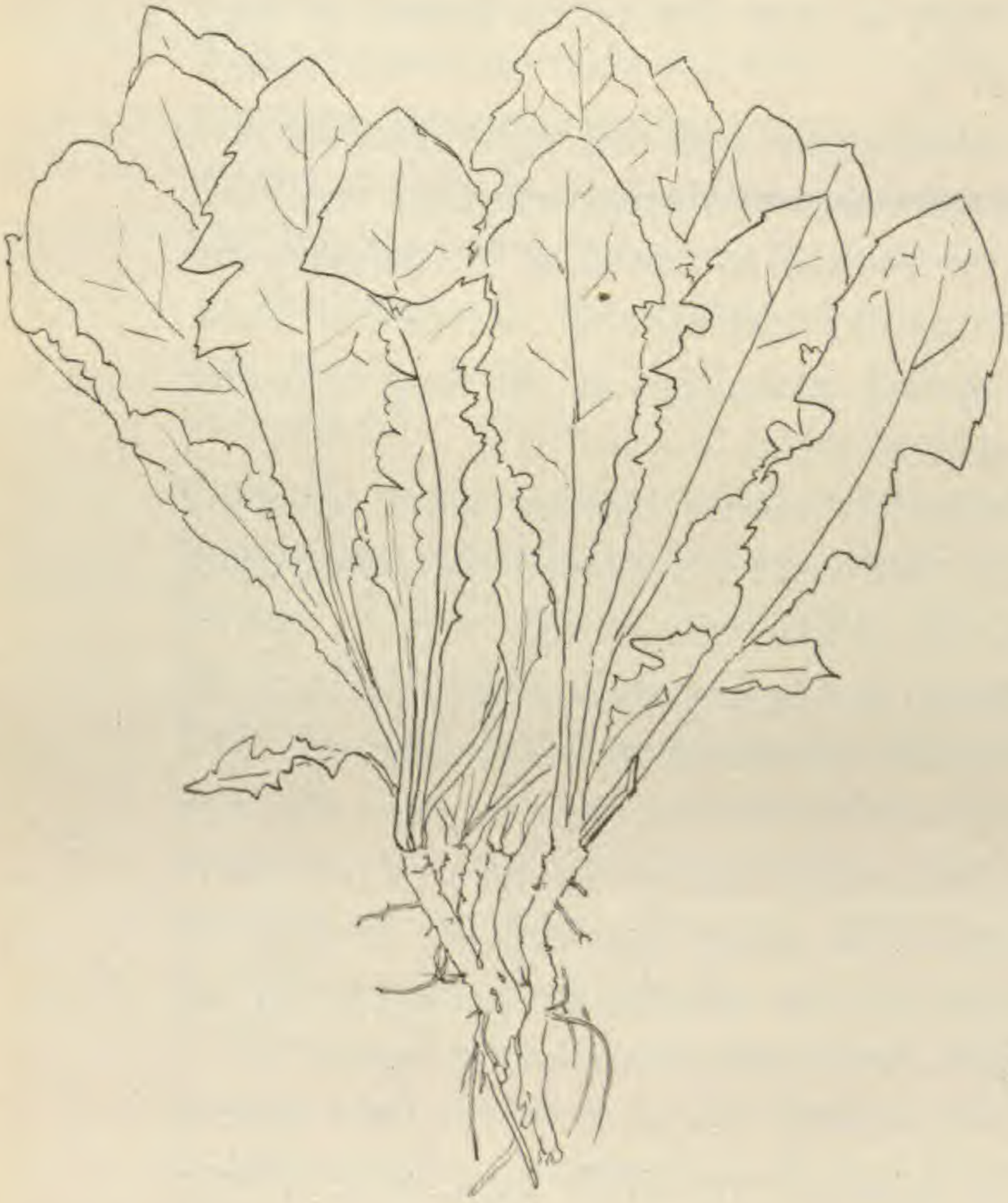

FIG. 3

FIG. 4

FIGS. 3, 4.—Fig. 3, plant during first season following rejuvenation, showing juvenile leaf form on each secondary rosette; degree of root fission rather unusual; fig. 4, rejuvenation of buried crown, showing formation of vertical rhizomes (note leaf scars), which secondary thickening will transform into roots; note presence of rosettes of second year (leaves highly dissected) and of first year (leaves entire).

beginning with somewhat less dissected leaves. Fall seedlings frequently seem to start blooming the next spring with almost entire leaves. In other words, the winter rest period permits many young plants to bloom which would otherwise require a greater degree of maturity or senescence.

The same relations hold true of rejuvenated plants. Secondary rosettes which have started early begin blooming the following

spring with very much segmented leaves. Those started late in the year begin to bloom the next spring with nearly entire leaves. It is not inconceivable that *T. palustre* consists of vigorous secondary rosettes of *T. vulgare* which have rejuvenated in autumn, or for some other reason flowered in more juvenile condition than usual. If occasional pure stands of such a type are to be found, it may even be suspected that ecological conditions there favor fall germination and establishment of *Taraxacum* and fall rejuvenation as a consequence. More or less entire-leaved forms, whether regarded as distinct species, varieties, or ecological forms of *T. vulgare*, are generally arctic, alpine, or temperate hydrophytic. None of these habitats favors very early spring germination or long growing season before blooming the second year.

Another source of modification comes during good growing weather in the case of plants that have completed their flowering cycle. Rejuvenation by multicipital branching occurs before the last flower scapes are gone, giving a combination of juvenile leaves with the scapes which characterize senescence. The senescent leaves, present before rejuvenation, very rapidly die and decay. This circumstance, that early leaves die as new ones are produced, is an important one. The plant thus rapidly loosens the bond connecting it with the earlier condition, resulting in an isolation of pseudotypes which are really transient phases.

Field observations have successfully applied WIESNER'S finding of the effect of moist and dry atmosphere, while experiments have verified the truth of it. The effect of a xerophytic habitat is not to increase the amount of dissection, but to shorten leaves at all stages of senescence. Juvenile leaves become nearly orbicular, whereas they may be almost spatulate in a habitat with low transpiration. Senescent leaves in a xerophytic habitat are so shortened that lobes and incisions become sharply triangular. Senescent leaves in a non-xerophytic habitat are much more graceful and very different in appearance.

ACHENES.—Aside from the failure of red pigment to develop in injured fruits of *T. laevigatum*, achene color seems to be rather a fundamental character, varying in degree but not in kind. Homogentisic acid is a highly specific substance derived from tyrosin, and neither was found in non-red fruits, according to HANDEL-

MAZETTI, already quoted. As to details of achene form, it is possible to find in a field of *T. vulgare* and *T. laevigatum* achenes varying from nearly smooth to almost shaggy. As a rule the form seems consistent in the individual plant.

FLOWERS.—Color is markedly influenced by pigmentation of styles as well as of petals, and by presence or absence of ripe pollen. That flower number per head may not always be relied upon as a criterion becomes evident from field studies. Depauperate plants whose species is unquestionable may produce a surprisingly small number, as few as fifty.

POLLEN.—In the spring of 1921 at Lincoln, Nebraska, pollenless plants, both red and gray fruited, were found, the former in abundance. Microscopic examination showed that pollen development had been arrested before the grains had separated. Certain interesting correlations were noted in the red fruited pollenless forms. The leaves are invariably dissected less than the maximum for *T. laevigatum*, and the inner bracts are twelve or thirteen in number, containing in all eighteen to twenty-two corniculi on their greenish tips. It was at first believed that this represents a distinct genetic type, and such indeed may be the case. Search revealed a number of transitional forms, however, with scant pollen, fifteen to eighteen reddish bracts, and leaves considerably dissected. *T. laevigatum* itself has copious pollen, eighteen to twenty red tipped bracts each bearing a corniculus, and leaves heavily dissected. In one case a combination of the two extremes was obtained on different rosettes of the same old root. Quantitative studies, quoted by the courtesy of Mr. H. PEGLER, show that the degree of leaf dissection in the pollenless forms increases as one passes from peripheral to central leaves, and lies exactly between the increasing dissection of a young seedling of *T. laevigatum* and the fluctuating dissection of a typical adult of the same species. It is not unlikely, therefore, that this is even a more interesting case of isolation of pseudotype than is the *T. palustre* form of *T. vulgare*.

BRACTS.—Aside from the case just described, bracts of both species have been found to vary notably in the degree of development of the corniculi, in number between twelve and twenty-two, and in color.

REGENERATION.—Mere removal of older competing leaves does not alter the degree of senescent dissection of those subsequently produced. Removal of all leaf incepts, down to undifferentiated meristem, results in production of juvenile leaves. The rhizomes produced by etiolation produce juvenile leaves when cut and placed in a moist chamber; in fact, after secondary thickening begins, pressure crushes the pith, and all rhizomes become essentially roots. Study of developing leaves shows that the juvenile leaves are produced by a disproportionate development of tip segment at the expense of the rest, while dissected leaves result from a more or less uniform development of all segments.

Quantitative studies

METHOD.—More precise analysis of the rôle of senescence as a cause of leaf dissection being desired, quantitative verification of these observations was obtained from studies of plants growing under widely different natural conditions. Prints of each successive leaf in each rosette studied were outlined by bounding polygons, and area of both leaf and polygon taken by means of a planimeter. The real area of each leaf was then divided into the difference between real and ideal (polygon) area. This, it will be seen, gives a percentage expression for the degree of dissection of each leaf.

It was finally deemed necessary, in order properly to delimit the problem, to investigate some of the correlations which more obviously suggested themselves. On the whole, these center about various phases of conductive efficiency of the xylem. Root cross-sections at various levels were studied to determine whether any correlation could be noted between cross-section areas and relative age of xylem tubes. Next the tube diameters were carefully measured at the base of successive leaf petioles in a number of rosettes. These measurements were made with an eyepiece micrometer graduated to intervals of approximately 3.33 μ. Since water conducting efficiency of a given cross-section of capillary tube is a function of the fourth power of its radius (18), the total capacity of each leaf was obtained by getting the sum, $(\text{diameter}/2)^4$, of all its xylem tubes. Mean capacity represents this figure divided by the number of tubes in a given leaf.

The ratio of total capacity to leaf area was also obtained in an effort to see whether it might be correlated with degree of dis-

section. Leaf area being given in terms of square millimeters, and capacity figured in terms of fourth powers of micromillimeters, the latter have been multiplied by 10^{-12} to get both sets in terms of a common unit. For the sake of compactness these figures are multiplied by 10^{8} before tabulation, as noted in tables. The foregoing data will be found listed in tables I–VA and plotted in figs. 5 and 6. Here are also included counts of the number of vein islets per unit area of leaf, taken at mid-distance between margin and midrib and averaged from several counts in each case.

TABLE I

DIAMETER FREQUENCIES OF XYLEM TUBES FOR SUCCESSIVE LEAVES OF SEEDLING PLANT *SA;* EACH UNIT REPRESENTS 3.3 μ

Leaf no.	Diameters						
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
1	1	2	3	7	11	2	1
2	3	9	12	9	7	2	2
3	15	6	6	3	2	.....	.....
4	2	4	12	5	3	5	7
5	1	4	16	7	5	.....	.....

TABLE I A

OTHER DATA FOR SUCCESSIVE LEAVES OF SEEDLING PLANT *SA*

	Leaf nos.				
	1	2	3	4	5
Leaf area	.....	250	125	150	100
Total xylem capacity $\times 10^{8}$	1.37	1.52	0.32	2.47	0.73
Mean xylem capacity $\times 10^{8}$	0.0510	0.0346	0.0101	0.0652	0.0222
$\frac{\text{Total capacity} \times 10^{8}}{\text{Leaf area}}$	.....	0.0061	0.0025	0.0165	0.0073

TABLE II

DIAMETER FREQUENCIES OF XYLEM TUBES FOR SUCCESSIVE LEAVES OF SEEDLING PLANT *SB;* EACH UNIT REPRESENTS 3.3 μ

Leaf no.	Diameters															
	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5
1	....	....	3	5	9	7	7	2	5	6	5	12	4	6	....	1
2	....	1	6	5	16	10	9	8	11	5	2	1	....	....	....	....
3	....	1	8	12	12	11	11	4	7	2	3	2	....	....	....	....
4	1	5	18	25	34	15	7	11	12	1	1	....	....	....	....	....
5	2	9	19	12	15	24	12	2	....	....	....	....	....	....	....	....
6	3	10	7	9	17	12	7	2	....	....	....	....	....	....	....	....
7	....	5	6	6	14	10	10	4	4	1	....	....	....	....	....	....
8	....	6	5	6	6	10	11	2	....	....	....	....	....	....	....	....

TABLE II A

OTHER DATA FOR SUCCESSIVE LEAVES OF SEEDLING PLANT *SB*

	Leaf nos.							
	1	2	3	4	5	6	7	8
Leaf area	1335	896	1232	1575	1058	806	.....	.....
Leaf dissection	0.01	0.01	0.02	0.08	0.12	.....	.....	.....
Total xylem capacity $\times 10^8$	57.24	18.71	16.36	17.10	7.33	4.86	8.14	4.62
Mean xylem capacity $\times 10^8$	0.7950	0.2529	0.2241	0.1315	0.0772	0.0726	0.1358	0.1005
Total capacity $\times 10^8$ / Leaf area	0.0428	0.0208	0.0132	0.0108	0.0069	0.0060	.....	.....
Vein islets per unit leaf area	13	12	12	12	10	9	.....	.....

TABLE III

DIAMETER FREQUENCIES OF XYLEM TUBES FOR SUCCESSIVE LEAVES OF SEEDLING PLANT *SC;* EACH UNIT REPRESENTS 3.3 μ

Leaf no.	Diameters												
	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0
1	.....	3	10	5	15	7	16	2	15	2	5	1	.....
2	.....	5	17	14	39	21	34	10	28	8	13	4	.....
3	1	2	11	4	16	17	9	10	13	6	3	2	1
4	.....	6	4	8	24	5	20	4	17	1	2	.....	.....
5	2	3	14	11	27	16	11	6	5	.....	.....	.....	.....
6	.....	3	12	10	18	15	14	6	6	.....	.....	.....	.....
7	.....	5	11	13	16	16	20	4	10	3	2	.....	.....
8	2	10	15	10	23	20	17	6	4	1	.....	.....	.....
9	4	6	10	13	29	18	1	1	.....	.....	.....	.....	.....
10	4	9	11	16	19	6	3	.....	.....	.....	.....	.....	.....
11	4	8	11	17	15	14	5	2	.....	.....	.....	.....	.....
12	2	9	10	14	10	7	13	3	1	.....	.....	.....	.....

TABLE IV

DIAMETER FREQUENCIES OF XYLEM TUBES FOR SUCCESSIVE LEAVES OF SEEDLING PLANT *SD;* EACH UNIT REPRESENTS 3.3 μ

Leaf no.	Diameters										
	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
5	3	12	14	9	16	4	3	.....	.....	.....	.....
6	2	10	22	17	18	6	2	.....	.....	.....	.....
7	1	11	31	9	13	5	.....	.....	.....	.....	.....
8	.....	15	17	12	22	14	10	2	.....	.....	.....
9	1	9	34	13	26	6	4	.....	.....	.....	.....
10	2	8	16	18	10	4	3	.....	.....	.....	.....
11	4	10	20	11	13	4	1	.....	.....	.....	.....
12	2	3	11	6	21	7	6	.....	.....	.....	.....
13	4	3	6	6	15	12	9	5	5	3	.....
14	.....	2	5	9	11	11	14	5	5	.....	.....
15	.....	1	6	9	16	9	11	4	7	1	1
16	.....	2	7	8	17	8	13	.....	7	.....	.....
17	1	.....	12	3	11	13	9	.....	3	.....	.....
18	1	2	10	5	10	5	8	3	.....	.....	.....

TABLE III A

OTHER DATA FOR SUCCESSIVE LEAVES OF SEEDLING PLANT *SC*

	Leaf nos.											
	1	2	3	4	5	6	7	8	9	10	11	12
Leaf area	.....	.....	2013	1581	1425	1290	1155	1387	993	.....	.....	.....
Leaf dissection	.....	.....	0.05	0.12	0.18	0.15	0.22	0.20	0.36	.....	.....	.....
Total xylem capacity $\times 10^8$	20.72	41.00	25.98	18.43	10.50	10.85	17.46	12.13	4.91	3.15	4.85	6.05
Mean xylem capacity $\times 10^8$	0.2558	0.2124	0.2734	.02025	0.1106	0.1292	0.1746	0.1123	0.0599	0.0463	0.0638	0.0876
Total capacity / Leaf area	.....	.....	0.0129	0.0116	0.0073	0.0084	0.0151	0.0087	0.0049	.....	.....	.....
Vein islets per unit leaf area	7	7	8	8	9	11	12	.....	.....	.....	.....	.....

TABLE IV A

OTHER DATA FOR SUCCESSIVE LEAVES OF SEEDLING PLANT *SD*

	Leaf nos.													
	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Leaf area	400	270	425	787	709	535	387	554	645	967	903	580	541	309
Leaf dissection	0.04	0.11	0.07	0.09	0.15	0.18	0.23	0.30	0.31	0.43	0.42	0.64	0.54	0.72
Total xylem capacity $\times 10^8$	2.55	3.03	2.06	6.23	3.95	2.46	2.08	3.64	10.39	9.05	10.86	8.24	5.63	4.03
Mean xylem capacity $\times 10^8$	0.0418	0.0394	0.0295	0.0678	0.0424	0.0403	0.0331	0.0650	0.1522	0.1460	0.1671	0.1330	0.1083	0.0916
Total capacity / Leaf area	0.0063	0.0112	0.0048	0.0079	0.0055	0.0046	0.0053	0.0065	0.0161	0.0093	0.0120	0.0142	0.0104	0.0130
Vein islets per unit leaf area	17	16	18	11	13	12	.....	12	9	8	9	10	9	.....

TABLE V

DIAMETER FREQUENCIES OF XYLEM TUBES FOR SUCCESSIVE LEAVES OF REJUVENATED PLANT *R*; EACH UNIT REPRESENTS 3.3 μ

Leaf no.	Diameters															
	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5
2	....	2	13	13	19	5	8	6	13	7	12	3	9	4	6	1
4	....	8	18	10	32	11	25	8	17	5	13	2	10	2	1	....
6	....	5	10	7	22	14	24	15	25	11	6	2	....	....	....	....
8	1	4	10	10	16	14	20	11	15	7	8	....	1	....	....	....
10	....	9	23	14	13	12	17	15	16	5	3	....	....	....	....	....
12	....	3	6	9	11	5	12	3	16	7	6	....	3	....	....	....
14	....	....	7	7	11	5	9	8	10	3	8	3	3	....	....	....
16	....	....	6	4	11	1	10	3	6	5	7	3	....	....	....	....

For comparative purposes, distortion figures have been worked out so far as trustworthy available data permit. These are based upon changes produced by senescence (original), depauperation (**2**), and moisture (**20**). It is to be regretted that the only established facts with regard to light are its axiomatic effects upon leaf position and the effect of darkness in stimulating the production of internodes. The method used in working out these figures should be apparent from inspection, and can be found adequately discussed by THOMPSON (**19**).

In addition to these methods, microchemical studies were made throughout the summer of 1921 on tissues of *T. vulgare* plants in all stages of senescence and rejuvenescence.

RESULTS.—In general it will be noted that the percentage of dissection in various plants of *T. vulgare* tends to rise at a uniform rate. Studies of second year flowering plants indicate that after a value of 0.5 is attained, the degree of dissection fluctuates about that as an approximate mean until late in the flowering period. Then, as previously noted, if many leaves have been produced, the curve may rise to a higher value.

With respect to *T. laevigatum*, fewer determinations are at hand and none are listed. All that have been made indicate that the rate of increase of dissection is not so rapid, but continues until a value of about 0.8 is reached, fluctuating about this value during the adult period proper.

That the increase in degree of dissection is not merely a veiled expression of progressive decrease in leaf area is evident from fig. 5.

TABLE VA

OTHER DATA FOR SUCCESSIVE LEAVES OF REJUVENATED PLANT *R*

	Leaf nos.														
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Leaf area	1806	.....	.....	1584	1741	1322	.....	2735	2138	2432	2025	1896	1064	739	870
Leaf dissection	0.13	.....	.....	0.25	0.30	0.41	.....	0.41	0.40	0.49	0.50	0.44	0.51	0.49	0.57
Total xylem capacity $\times 10^8$	70.86	.....	64.70	.....	41.02	.....	32.27	.....	25.10	.....	28.80	.....	30.21	.....	21.31
Mean xylem capacity $\times 10^8$	0.5856	.....	0.3994	.....	0.2909	.....	0.2758	.....	0.1976	.....	0.3556	.....	0.4083	.....	0.3806
Total capacity $\times 10^8$ / Leaf area	0.0392	.....	.....	.....	0.0235	.....	.....	.....	0.0117	.....	0.0142	.....	0.0283	.....	0.0245
Vein islets per unit leaf area	.....	.....	15	10	11	11	14	9	12	.....	13	.....	16	15	.....

It will be noted that absolute leaf area decreases rather uniformly in *SC*, a seedling plant grown under apparently favorable conditions. On the other hand, *SD*, a seedling that started under hard conditions (tight, dry clay), shows a gradual uniform increase. Of course these facts are established easily by familiar observation, but they are mentioned here to show the positive necessity of studies based upon field material growing under varying natural conditions. Not only here, but in connection with studies of xylem capacity, very erroneous ideas of senescent correlation might have been obtained by limiting studies to uniformly grown culture material, as a glance at the graphs will show.

Total and mean xylem duct capacity measured and computed as stated show a seeming correlation with degree of senescence in the cases of *SB* and *SC* seedlings, and somewhat less in the case of *R*, a secondary rosette. The possibility that this correlation is real, however, is quite upset by data on the depauperate seedlings *SA* and *SD*.

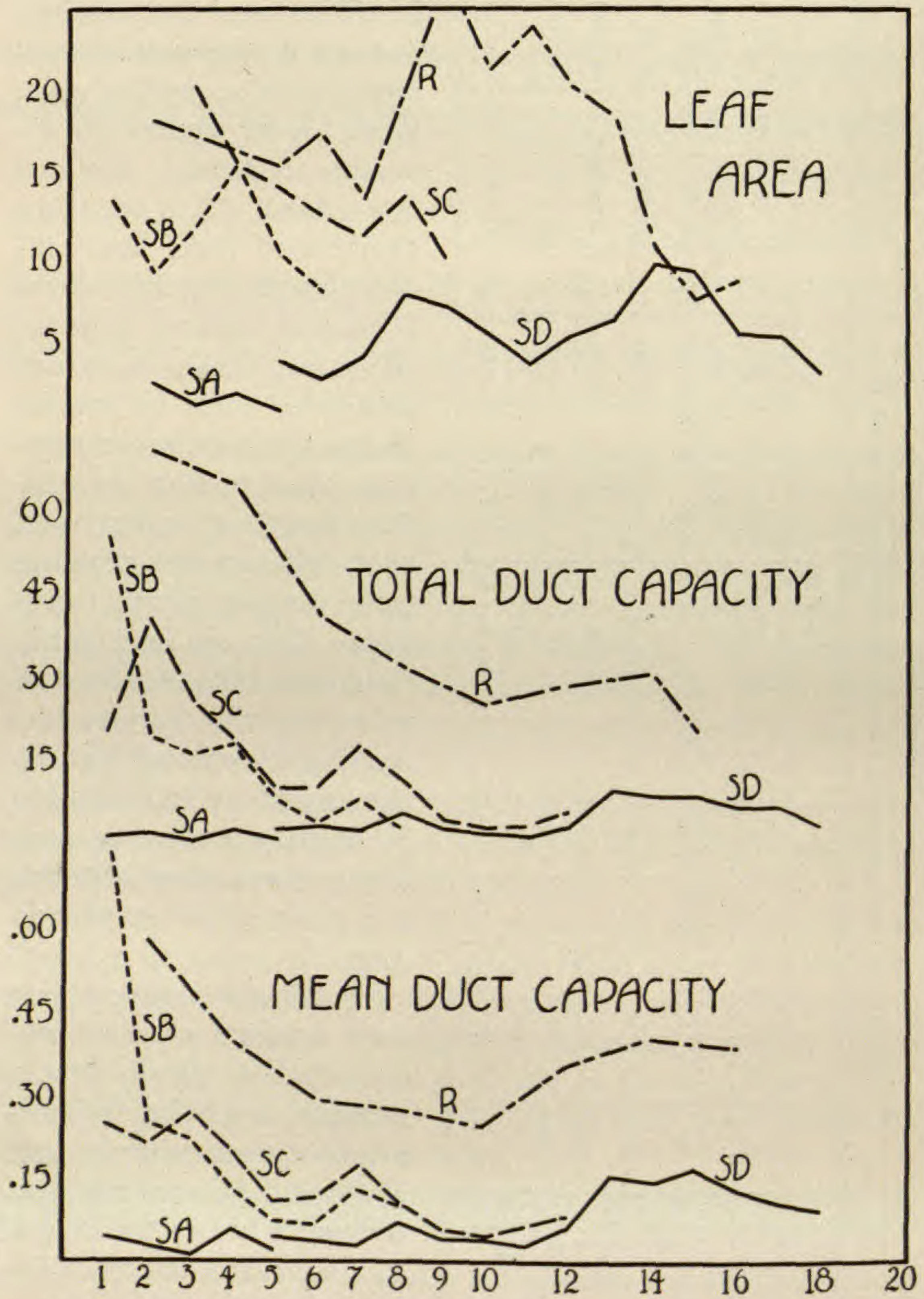

FIG. 5.—Abscissae represent successive leaves of various rosettes, ordinates the respective characters measured in each set of graphs; *S*, seedlings; *R*, rejuvenated rosette.

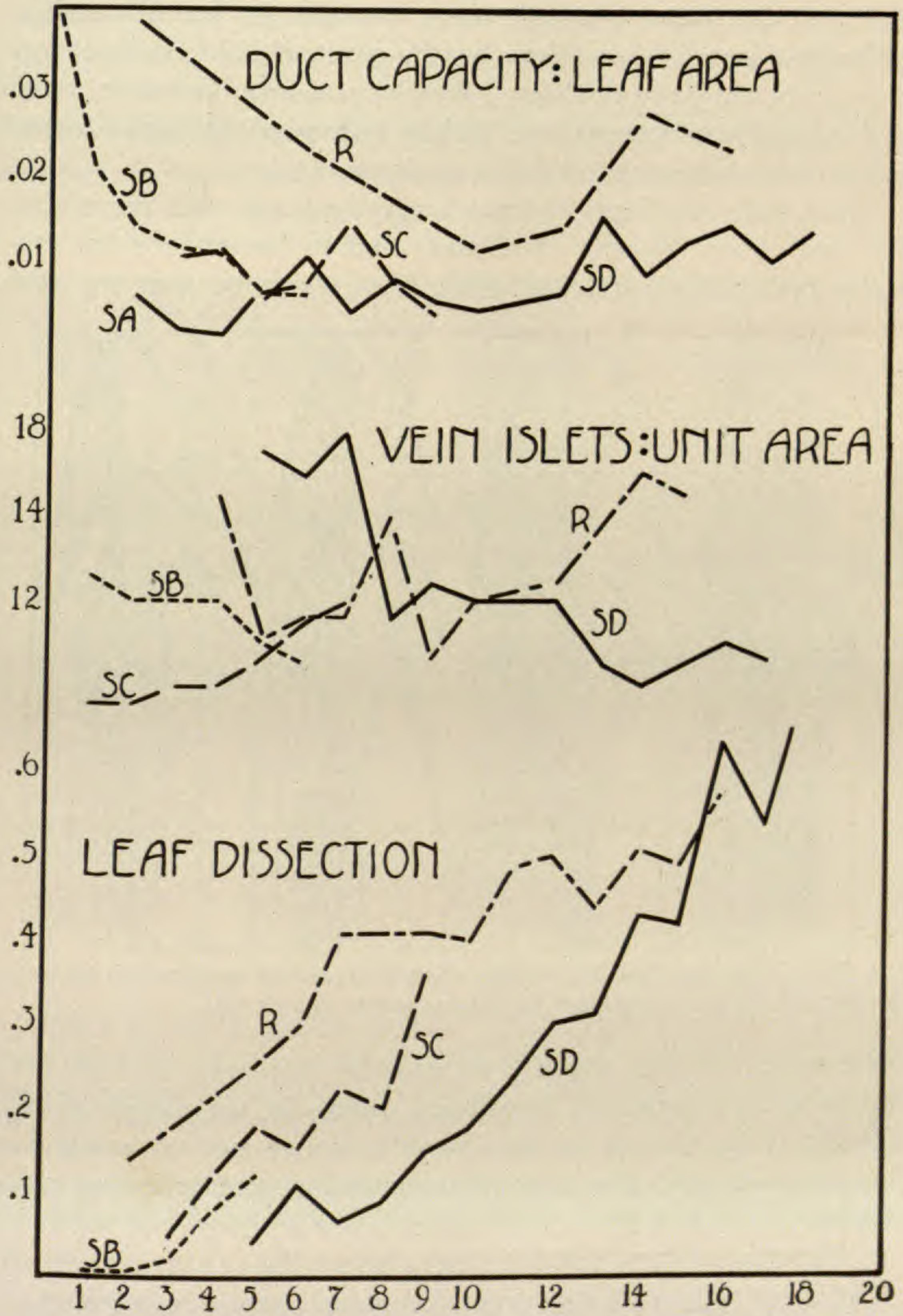

FIG. 6.—See explanation of fig. 5

Both of the latter showed steady increase in leaf dissection, although through an accident to the print of *SA* its dissection data could not be worked out. As with leaf area, therefore, total and mean duct capacity are plainly influenced by some factor other than senescence, doubtless environmental.

Essentially the same statement may be made with respect to the ratio (duct capacity / leaf area) and to BENEDICT'S (1) criterion (vein islets / unit leaf area), from which we may at least

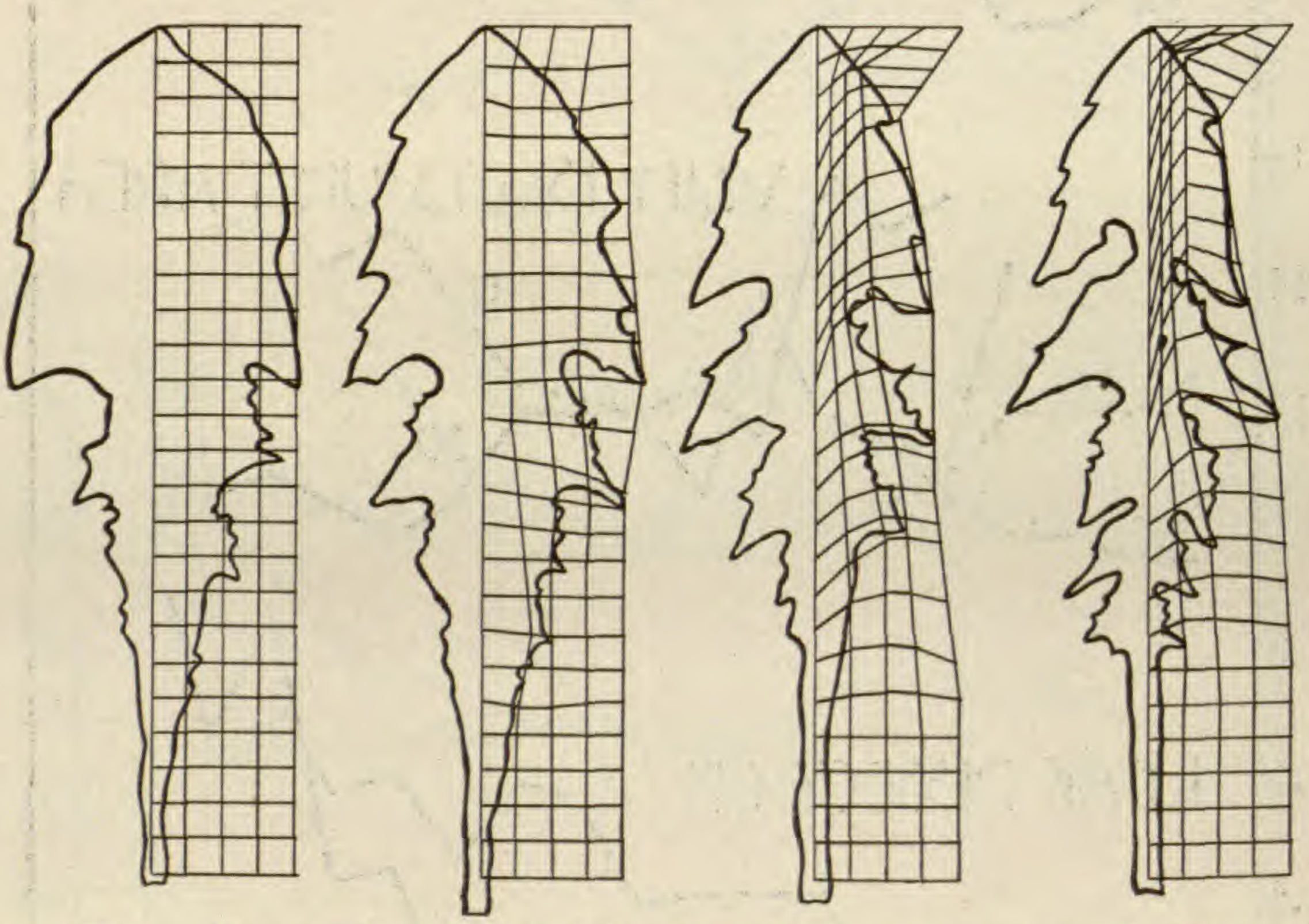

FIG. 7.—Showing, from left to right, effect of senescence on successive leaves of rosette; coordinates run through homologous points in each case.

with safety conclude that the criterion for senescence in *Vitis* certainly is no criterion in *T. vulgare*. Since the completion of the present work, ENSIGN (6) has shown a similar lack of correlation between vein islet area and age in several genera, including even *Vitis*.

Distortion figures for senescent change (fig. 7) are chiefly of value in showing that the successive sets of forms in a rosette can be homologized. They likewise show, what seems clear from inspection of ordinary leaves, that the increase in degree of dissection is the result of an increasing degree of inhibition of the tip

segment and of expression of the following segments. Fig. 8, derived from BONNIER'S (2) transplanting experiment, shows in leaves of corresponding age a rather uniform reduction of coordinates throughout, that is, more or less symmetrical reduction of leaf size. Numerous observations of the writer upon potted specimens of the same age, growing side by side but in pots of different

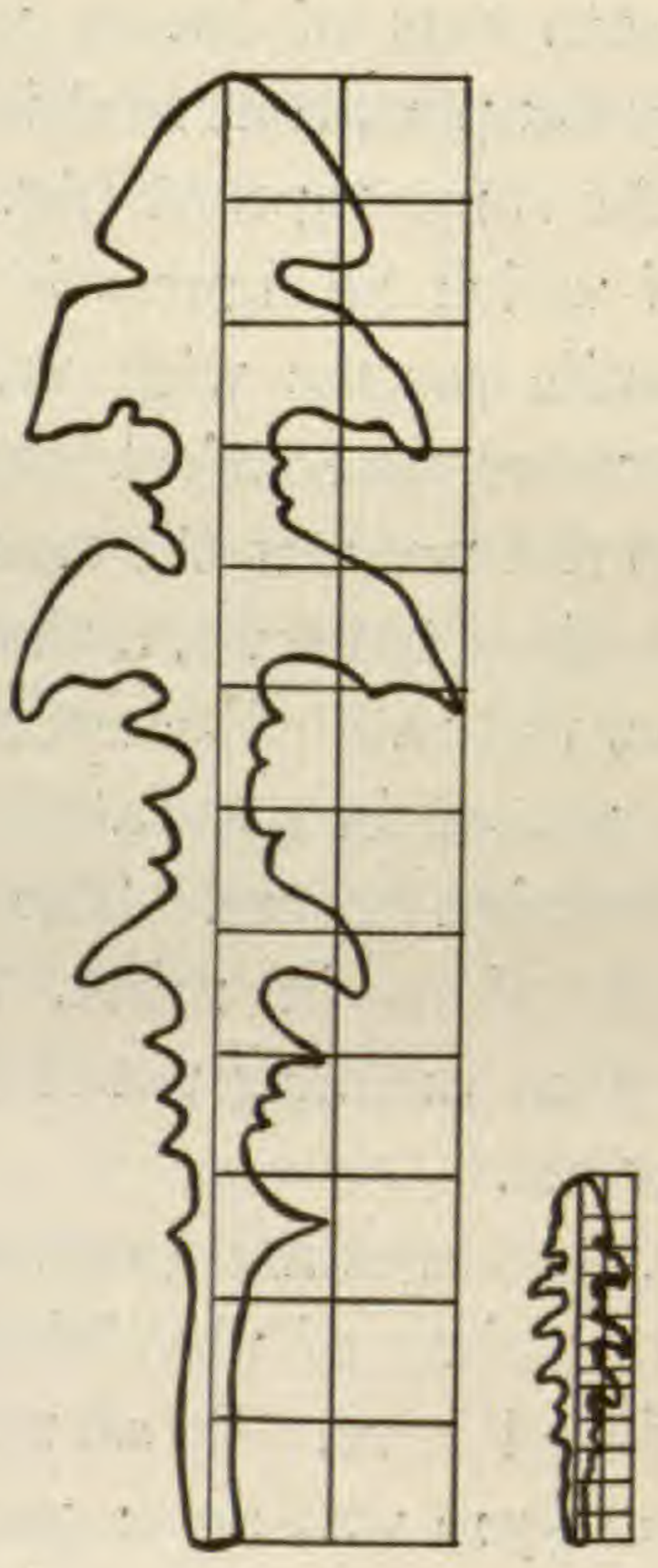

FIG. 8

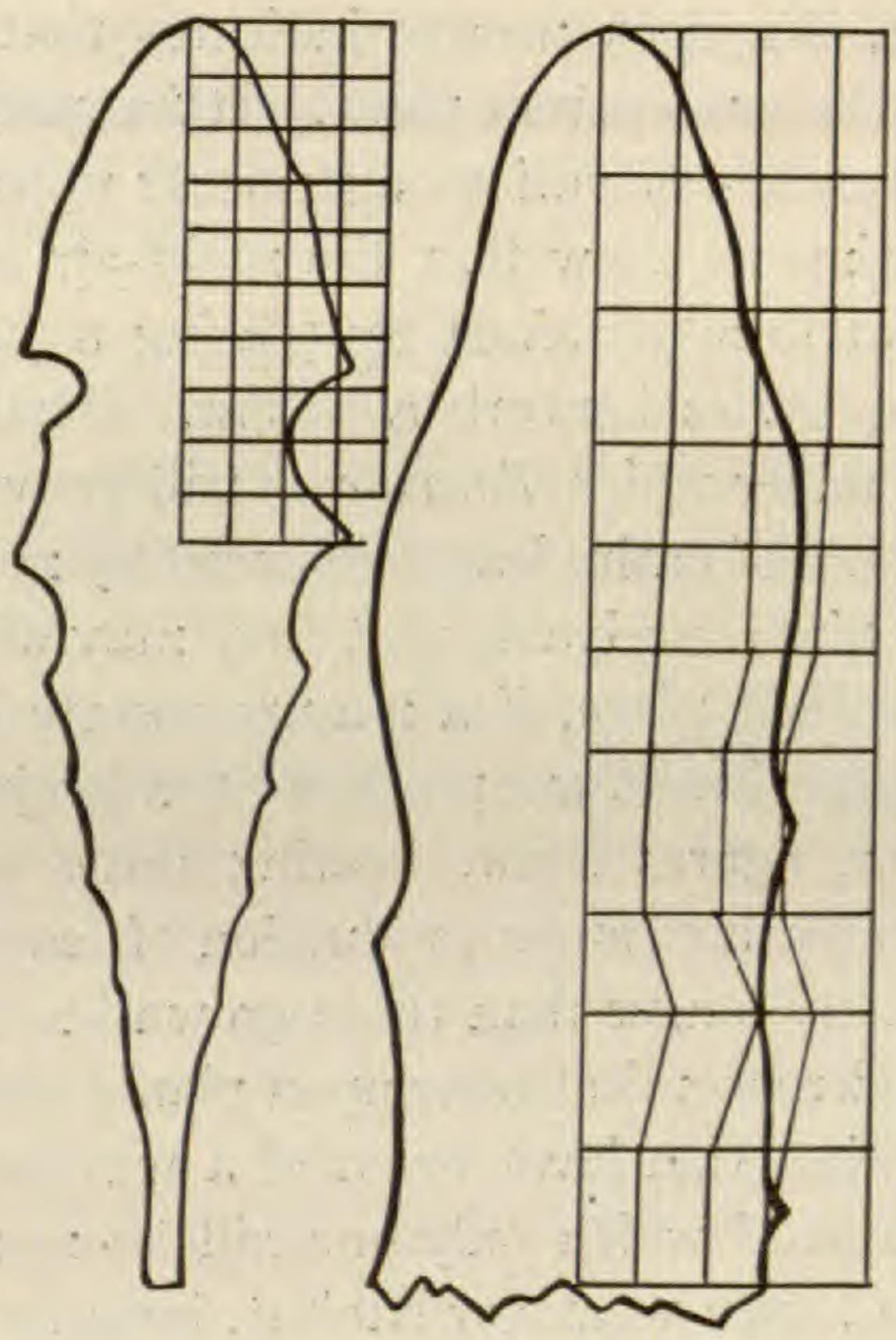

FIG. 9

FIGS. 8, 9.—Fig. 8, showing relatively uniform dwarfing brought about by alpine conditions (right) as compared with lowland (left); after BONNIER; fig. 9, showing great increase, particularly in length, produced by saturated (right) as compared with somewhat dry air (left); after WIESNER.

sizes, indicate that symmetrical dwarfing (depauperation) does occur without greatly influencing leaf form. Under such conditions the depauperate specimens produce markedly fewer leaves, and as a consequence may be ready to bloom before dissection has progressed quite so far as in normal thrifty specimens. Facts of this nature must be thoroughly understood before "alpine" forms of *Taraxacum* are pronounced distinct species, or the generalization

drawn that unfavorable conditions per se tend to produce entire leaves.

WIESNER'S figures of the effect of increased moisture, while in general borne out by the writer's observations, must be accepted with reserve upon one detail. It is by no means certain that the slight degree of dissection visible in the smaller dry-air leaves can be attributed to environmental factors (fig. 9). As previously noted, WIESNER merely indicates that both sets of leaves were from the same parent plant. It frequently happens that multicipital branches of various ages occur upon the same taproot (fig. 4). Experiments show that the moist-air leaf which he figures is the typical form obtained by placing a juvenile rosette with undissected leaves beneath a belljar. Furthermore, the driest conditions under which *Taraxacum* will grow do not prevent the normal sequence of entire leaves followed by more dissected ones, although it is quite conceivable that they may affect the rate of senescence. In the last place, if a mature rosette be placed in saturated air, dissected leaves are produced until rejuvenation occurs. The distortion figures show, bearing these reservations in mind, that saturated air causes production of leaves that are larger and considerably longer than those grown in drier air.

Microchemical tests upon plants of various ages and degrees of leaf dissection have revealed a number of points of physiological interest, of which only one will be mentioned here. In all plants tested, from whatever habitat, juvenile leaf form was accompanied by high nitrate tests and little reserve carbohydrate. This is not unique; unpublished studies of ECKERSON, working with KRAUS at Wisconsin, show that leaf dissection of tomato is increased by scarcity of nitrate as compared with carbohydrate. It is moreover of interest to recall LONG'S (13) finding, that inner leaves of *Taraxacum* rosettes are richer in photosynthate than outer.

In conclusion, it is to be noted that a rather extensive survey of published species descriptions reveals that a considerable number of them will fit variants of either *T. vulgare* or *T. laevigatum* caused by the interplay of the factors that have been described. While this statement is presented upon the writer's responsibility, without detailed data, it may readily be verified in one case by an inspection

of the figure and description of *Leontodon latilobum* given in BRITTON and BROWN'S *Illustrated flora*, 2d ed. It is clearly a juvenile rosette and hence not a valid type for comparison. Possibly it is flowering in the juvenile condition because of an autumnal start followed by a winter rest, as previously described. It certainly may be a legitimate distinct species, and so may a great many others, but it is not too much to suggest that taraxacologists have been ignoring grave sources of error, and to insist that future diagnoses be placed upon a satisfactory physiological basis.

Summary

1. Senescent and rejuvenescent change in leaf form is a prime factor in producing variations in *Taraxacum vulgare* and *T. laevigatum*.

2. Senescence produces a steadily increasing degree of dissection, and frequently of hairiness; rejuvenescence restores the unsegmented juvenile seedling leaf form.

3. These changes are independent of changes in (*a*) absolute leaf area, (*b*) total xylem duct capacity in successive leaves, (*c*) mean xylem duct capacity in successive leaves, (*d*) ratio of total capacity to leaf area, and (*e*) number of vein islets per unit area of leaf.

4. Senescence, however, is accompanied by a marked increase in carbohydrate-nitrogen ratio and rejuvenescence by its abrupt decrease.

5. Environmental factors are of secondary importance in so far as studied. Moist atmosphere causes elongation of leaves. Time of seeding may govern time of flowering and rejuvenation; this in turn may affect degree of dissection in plants at flowering time.

6. Pure stands of distinctive variants in certain cases may be explained by environmental control of seed germination time.

7. The majority of so-called specific characters in *Taraxacum* are subject to extreme fluctuation.

8. These fluctuations in other than leaf characters are in some cases due to senescent change, in others to environmental factors, but mostly to causes not known.

9. Such fluctuations, together with senescent and ecological changes in leaf form, are potent enough when working upon the

two common species to produce phenotypes duplicating many so-called species.

10. Many such species may be valid, but so far the physiological evidence has not been obtained.

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ANATOMY OF EQUISETUM GIGANTEUM

ISABEL M. P. BROWNE

(WITH SEVEN FIGURES)

I. Material

While studying the anatomy of the cone of *Equisetum giganteum* serial preparations were made of some of the upper nodes of the fertile branches. These branches are small and usually possess eleven ribs and bundles. They were collected in Chili by Professor R. C. MCLEAN, who most kindly handed them over to me. Serial transverse sections have also been prepared from the upper part of a large young main stem of *E. giganteum* from the West Indies, for which I am indebted to Professor F. W. OLIVER. This series of sections extended from a level distinctly below one node to a point near the upper limit of the next node. The complete internode was about 1 cm. in length, and would doubtless have elongated much more. This stem had thirty-two bundles and ribs.

So far as I am aware, all accounts of the anatomy of *E. giganteum* since MILDE'S (11) in 1867, are based upon a short description by GWYNNE-VAUGHAN (7) that appeared in 1901. As my observations differ in some points from his, and in others supplement and confirm the details given by him, it seems advisable to publish a short account of the specimens studied.

II. Node of young main stem

Taking first the large young vegetative stem, the internode possesses a wide central cavity, about 6 mm. in diameter, the total diameter of the stem being about 7.75 mm. The bundles are oval in shape, with the longer axis directed radially, and each is surrounded by a separate endodermis.[1] The vallecular canals are about the size of the bundles and of much the same shape as these. Under each rib of the stem is a tooth of sclerenchymatous fibers,

[1] In none of the internodes examined was any trace observed of the common outer sheath of the bundles figured by MILDE (*pl. 21, fig. 4*).

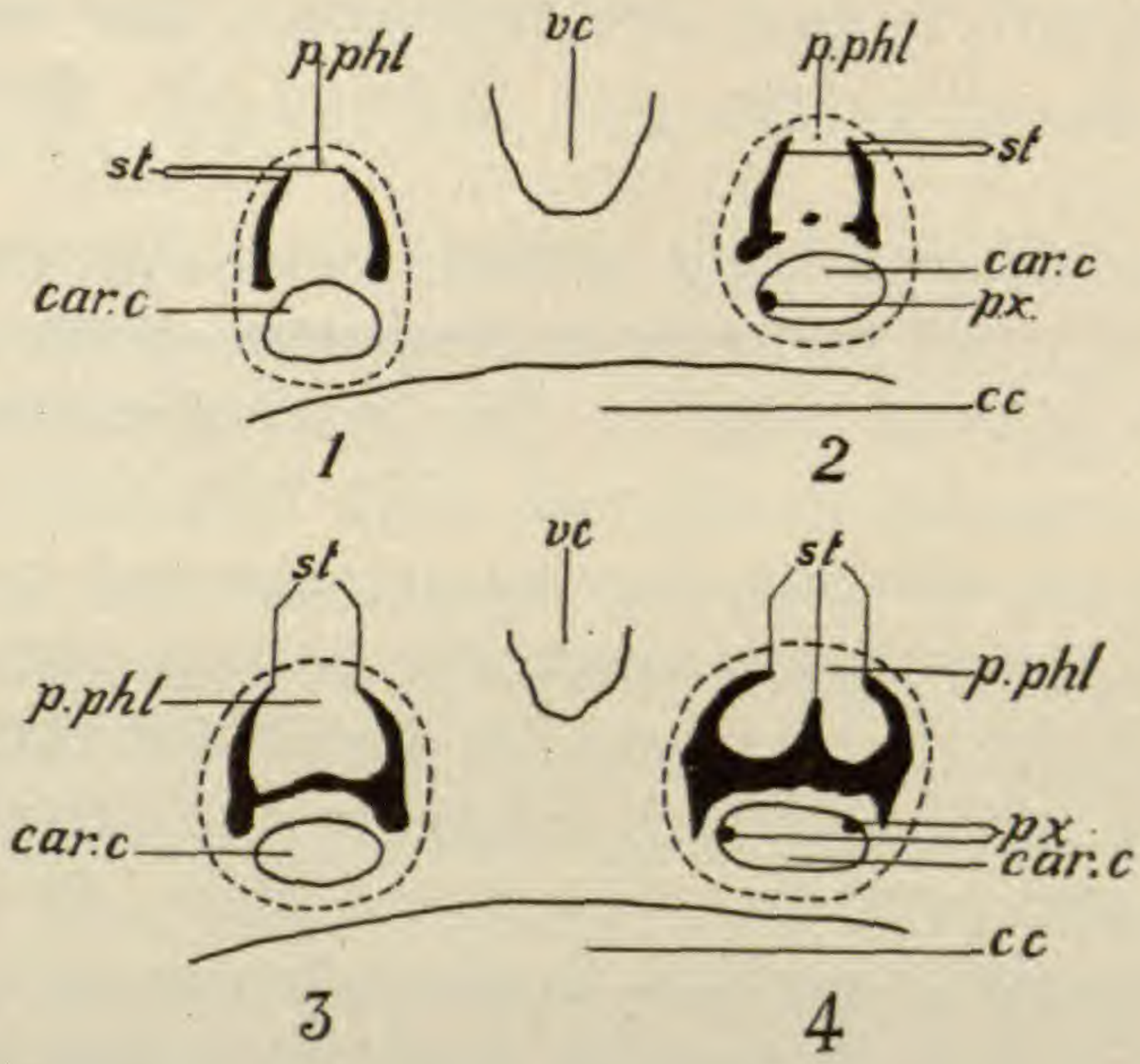
projecting inward to within one to four cells of the endodermis surrounding the bundles.

The carinal canals are large, especially considering the youth of the specimen, and occupy about one-third of the bundle. The remains of one or two tracheids may adhere to the edges of the carinal canal, but all trace of the protoxylem has frequently disappeared over long stretches. The metaxylem forms two lateral, nearly parallel bands that converge slightly toward the periphery of the bundle. Usually each band consists of a single row of tracheids, although locally there are often two tracheids lying side by side. In any case the band of metaxylem is markedly narrower toward the periphery of the bundle, because the tracheids here are always much smaller. The tracheids, of which each band contains from seven to fifteen, usually about twelve, increase steadily in size toward the interior of the bundle. This "internodal" condition of the bundle, which in essentials is that characteristic of the whole genus, is shown diagrammatically in fig. 1, stage 1.

The node was slightly oblique, so that a section apparently nearly accurately transverse of the individual strands showed many different stages of nodal development. In the diagrammatic figures advantage has been taken of the opportunity to show two or three neighboring bundles in successive stages.

The increase in the amount of metaxylem first becomes apparent about 1200–1600 μ below the level of the actual departure of a trace. The additional tracheids are situated between the inner ends of the bands of metaxylem. There is no regularity in their order of development. Sometimes the first to lignify are those in contact with the tracheids of the lateral bands, but at other times the first additional tracheids to be formed lie in the middle of the parenchyma between the bands (fig. 1, stage 2). These tracheids are much smaller than the relatively large xylem elements situated near the inner ends of the lateral bands on which they abut. Generally the crossbar which is thus formed is at first only one tracheid deep, but it may attain locally a depth of two or three tracheids, even before the bar is complete. *Pari passu* with its formation, other relatively large tracheids develop in contact with the inner

ends and flanks of the lateral bands of metaxylem. This phase is represented in fig. 1, stage 3. During these and the two subsequent stages figured, certain partially torn elements of protoxylem may be found adhering to the carinal canal. Their presence is



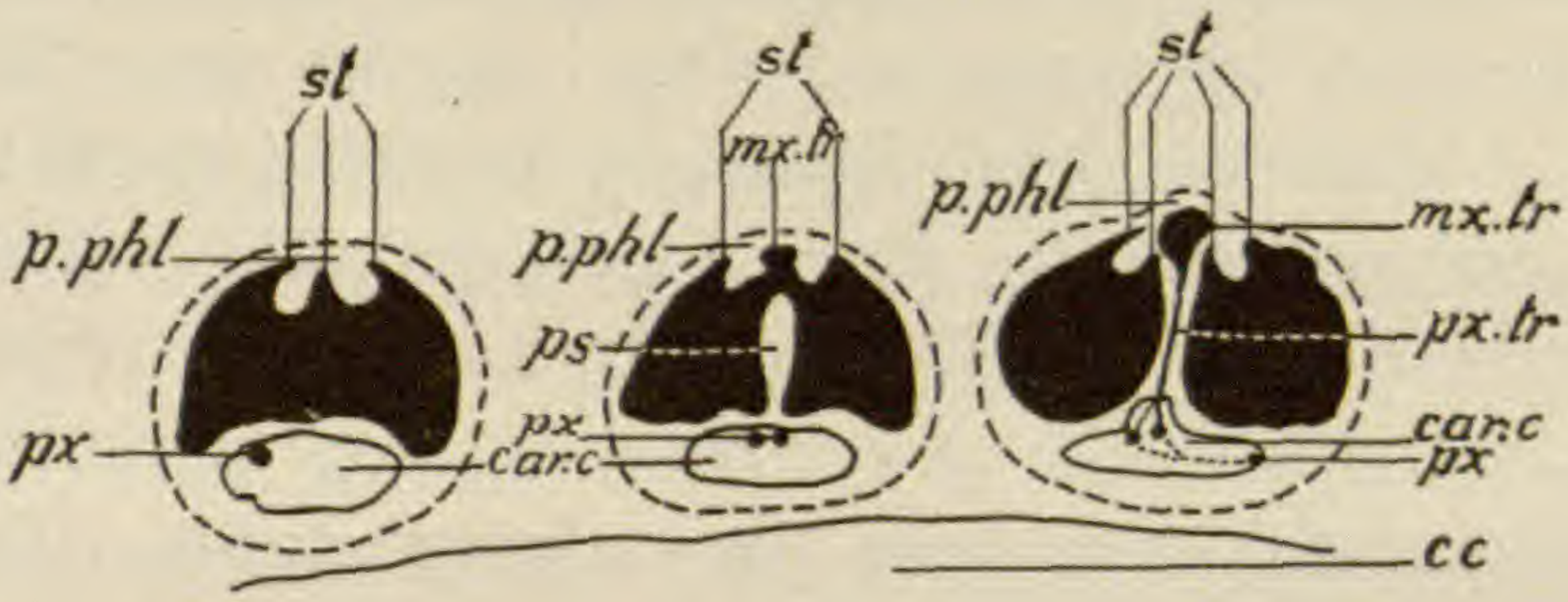


FIG. 1.—Stages 1–7, showing changes undergone by individual bundles in lower part of nodal region (xylem shown as black and endodermis as broken line): *cc*, central cavity of axis; *vc*, vallecular canal; *car.c*, carinal canal; *px*, protoxylem; *st*, small tracheids of metaxylem; *p.phl*, protophloem; *px.tr*, protoxylem of trace; *mx.tr*, metaxylem of trace; *ps*, parenchymatous sheath surrounding departing protoxylem of trace; ×120.

very inconstant; they may be absent from many consecutive sections, nor is their frequency correlated with the increase in xylem which continues as the node is approached.

As we pass upward, the tracheids of the crossbar increase in number and size, especially in its median region, where the increase

leads to the formation of an outwardly projecting tooth (fig. 1, stage 4). The tracheids at the outer edge of the tooth are very small, and have exactly the same appearance as those at the outer edge of the parallel bands of metaxylem (*st* of the figures). Their thickening appears to be a fine reticulum. The more internal elements of the metaxylem, both those of the crossbar, which becomes radially deeper, and those at the thickened bases of the former internodal lateral bands, assume more and more the appearance of typical nodal tracheids of *Equisetum;* some of them are already much widened radially. The bundles increase in width, but are still separated by intervals wider than themselves. The carinal canals are still large, but become narrower radially. The median tooth of metaxylem eventually projects nearly as far as the ends of the lateral bands and the concavities between it, and then becomes much shallower, owing to the great increase in number of nodal tracheids. This is the phase shown in fig. 1, stage 5. The small concavities in the xylem on each side of the median tooth seem to be occupied chiefly by metaxphloem.

Soon after this stage has been reached, the smaller outer tracheids of the median tooth begin to project more and more, and the tooth becomes less acute in outline. At the same time two to four rows of tracheids, lying internally to these small projecting tracheids, are replaced by parenchymatous cells. In this way the inner part of the bundle is divided by a narrow parenchymatous gully, continuous with the two or three layers of parenchyma that separate the carinal canal from the nodal tracheids. The carinal canal is now markedly narrower radially, and usually contains at its outer edge, opposite the parenchymatous gully, the remains of two or three partially disorganized tracheids. This condition is represented diagrammatically in fig. 1, stage 6. Almost immediately after the formation of the parenchymatous gully a few of the partially disorganized tracheids of the protoxylem may be seen to bend outward from the carinal canal, which here bulges a little outward. These tracheids now, no longer disorganized, run through the parenchymatous gully and fuse with the small oval mass of tracheids that forms the upward continuation of the small tracheids at the apex of the median tooth of metaxylem. These latter

elements, constituting the metaxylem of the trace, are more numerous than the protoxylem elements that pass out into the leaf. The metaxylem of the trace detaches itself from the nodal tracheids of the bundle before the phloem of the trace is set free from that of the axis. The endodermis of the bundle is beginning to bulge out somewhat opposite the trace that is preparing to depart. During its passage through the bundle (fig. 1, stage 7) the protoxylem of the trace is very clearly distinguishable under the microscope from the metaxylem, because, in a transverse section of the axis, the protoxylem running out from the carinal canal is cut almost longitudinally, while the metaxylem, some of the elements of which are not much larger, is moving so slowly and steeply upward and outward that its tracheids are cut almost transversely. When the junction of the protoxylem with the metaxylem has been effected, the tracheids of the former bend sharply upward and pursue the same steeply oblique course as the elements of the metaxylem. At this level the protoxylem is again disorganized, so that the trace contains a small protoxylem canal. This at first lies at the inner edge of the wood, but soon becomes somewhat internal in position, so that the xylem of the trace becomes mesarch almost at once. Before the trace is set free its protoxylem has assumed an approximately central position.

When the protoxylem has passed through the parenchymatous gully, two to four rows of parenchymatous cells, resembling those occurring below and on the sides of the departing protoxylem, are found above the latter. Thus the protoxylem runs through the nodal tracheids surrounded by a sort of parenchymatous sheath.[2] Immediately above the cells of this sheath nodal tracheids again form, leaving, however, a parenchymatous notch opposite the point of departure of the xylem of the trace.

Concurrently with the appearance of these tracheids the carinal canal becomes much narrower radially, and frequently contains a certain number of somewhat torn tracheids. It is usually at this stage that the nodal xylem of adjacent bundles becomes confluent. This union may take place in two ways. Where (as was

[2] Such a parenchymatous gully has also been observed at the nodes of *E. palustre* and *E. hiemale*.

the case at the points of junction of by far the greater number of bundles) the stele of a branch was inserted on the axial stele, the bundles of the latter first became united by their outer ends. Here the nodal xylem unites to form a sort of arch (fig. 2, stage 8*b*). The curve of this arch constitutes the outer and lower half of the continuous hollow central cylinder of the branch at its oblique insertion on the axial stele.[3] At a slightly higher level the more internal nodal xylem also becomes confluent, usually at a point about halfway between the periphery of the nodal xylem and its inner edge (fig. 3, stage 9). The tracheids at and near this junction

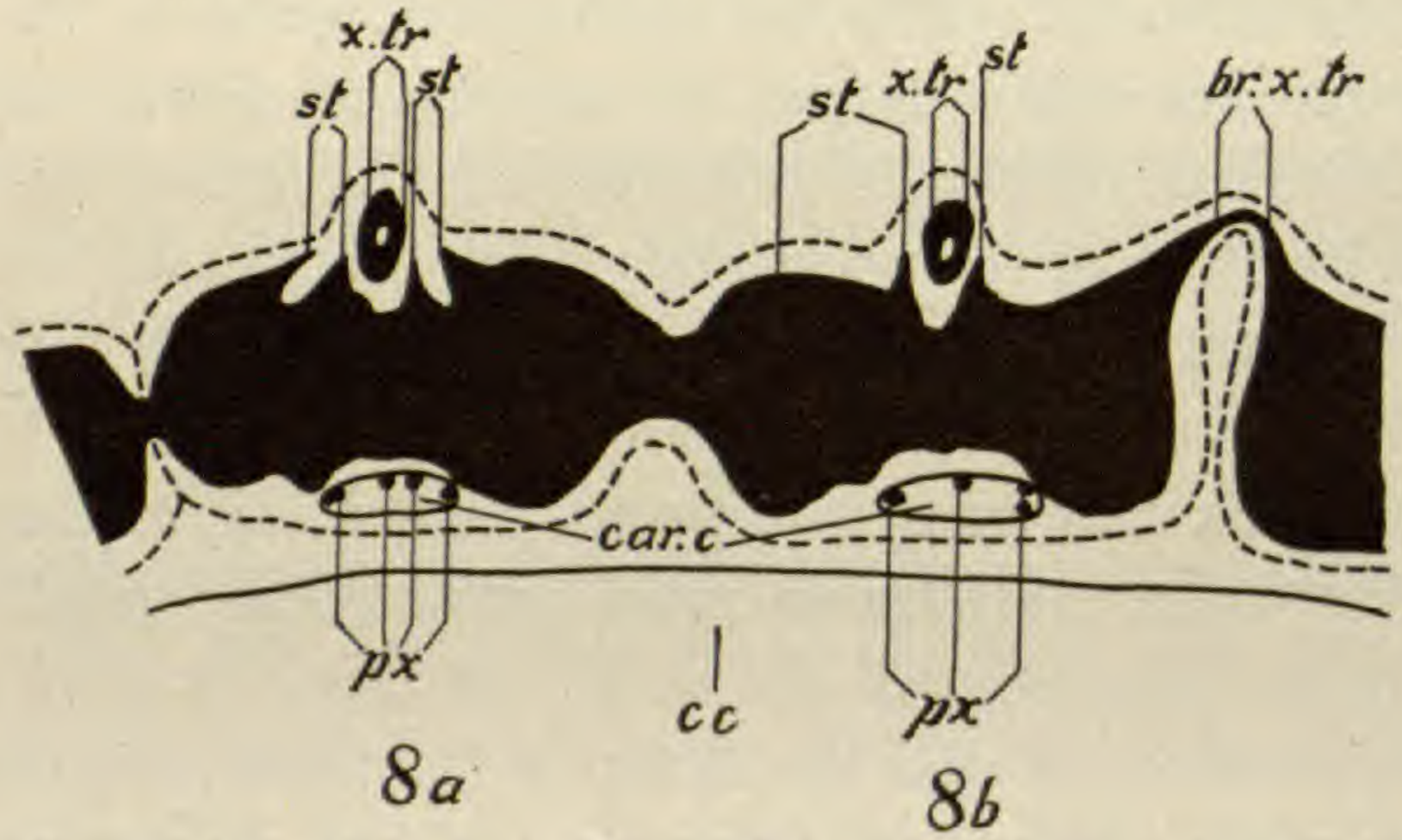


FIG. 2.—Stages 8*a* and *b*: *x.tr* xylem of trace; *br.x.tr*, xylem of branch trace; other lettering as in fig. 1; × about 120.

mark the insertion of the inner higher portion of the continuous ring of wood of the branch. At this level, however, the lower outer edge of the siphonostele is no longer visible, so that no single section shows the complete siphonostele of the branch and the continuity of its "pith" with that of the stem. Where no branch is given off, the bundles become laterally united in their middle regions by a narrow neck of xylem, as shown on both sides of the bundle in fig. 2, stage 8*a*. This neck of xylem rapidly thickens. The special endodermes lie outside the nodal tracheids, and their fusion precedes the junction of the xylem of adjacent bundles.[4] As we pass upward certain cells on the adjacent sides

[3] For a fuller description of the insertion of the vascular system of the branch on that of the stem, see PFITZER (**12**, pp. 329–330, *pl. 20, figs. 19, 20, 23*).

[4] A fuller account of the behavior of the special endodermes at the nodes will be found in PFITZER'S (**12**) description of their distribution at the nodes of *E. limosum* and *E. litorale*.

of two endodermal sheaths are replaced by single endodermal cells, common to both bundles. A little higher still these single endodermal cells are replaced by nodal tracheids, and the special endodermes by the common inner and outer endodermes typical of the vegetative nodes throughout *Equisetum*. When a branch is to be given off, the inner endodermis projects outward in a loop inside the arch of xylem representing the outer, lower insertion of the ramular siphonostele (fig. 2, stage 8*b*). In such a case the endodermal cells at the inner narrow end of the loop fuse before the junction of the nodal tracheids in this region. The endo-

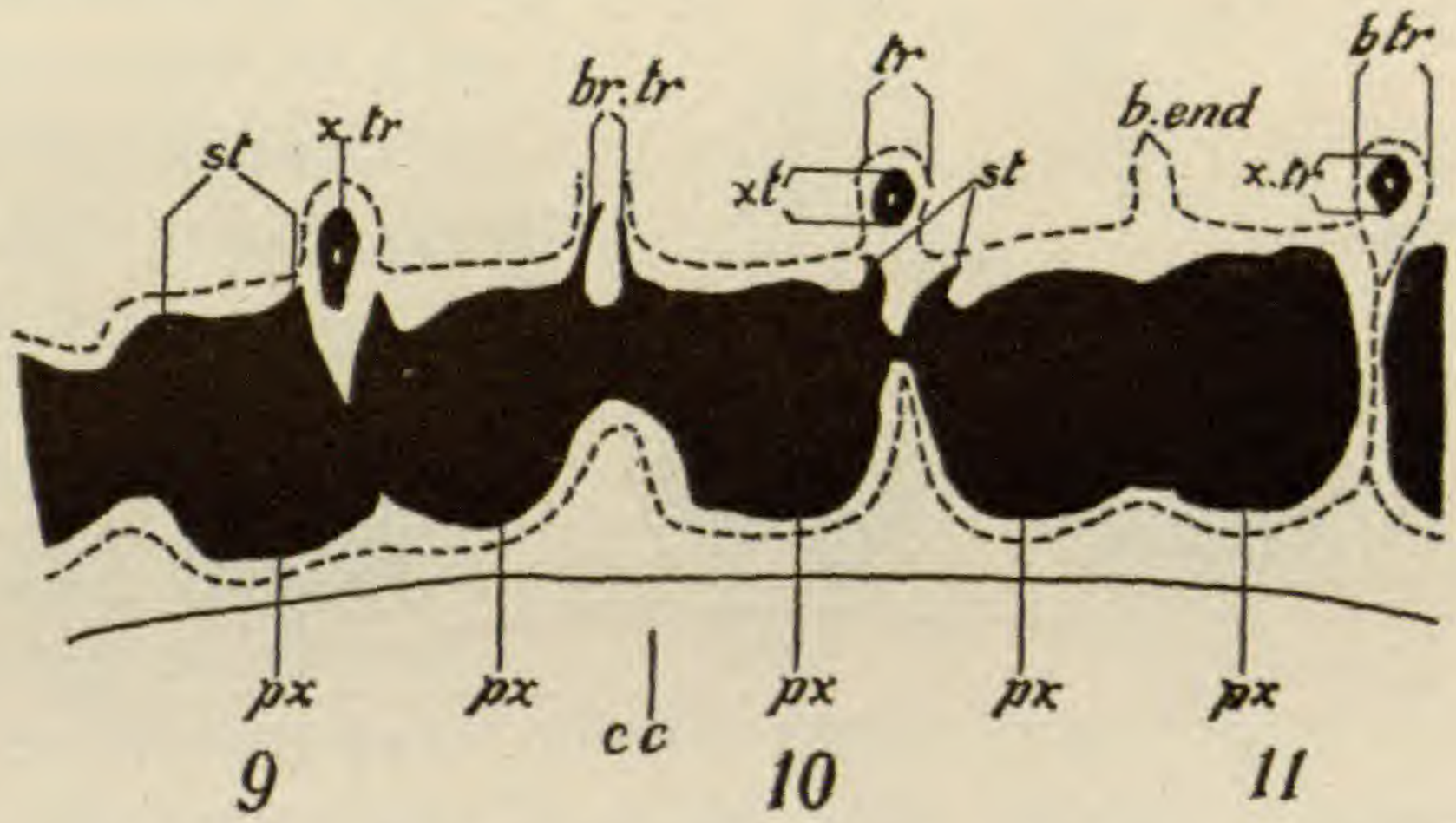


FIG. 3.—Stages 9–11; *br.x*, xylem of branch; *b.end*, endodermis of branch; other lettering as in figs. 1 and 2; × about 120.

dermis of the branch is thus in continuity with the inner endodermis of the axis.

To return to the carinal canals, these dwindle rapidly after the nodal tracheids have spread over the parenchymatous sheath, and soon disappear. As most of the medianly situated protoxylem tracheids have departed into the leaf, this region consists chiefly of parenchyma, in which are sometimes found a few tracheids that die out as we pass upward. A few of the protoxylem elements, however, forming the upward continuation of the elements at the sides of the carinal canals, may be seen persisting as two little groups on each side of the parenchymatous sinus that replaces the carinal canal. At the same time the parenchymatous depression in the outer edge of the nodal wood, opposite the departing trace, deepens, as is shown in fig. 3, stages 9 and 10. Meanwhile the inner endodermis becomes involuted into the parenchymatous

sinus at the inner edge of the xylem (stage 10). This sinus lies opposite that in the outer edge of the metaxylem, and the latter is broken in this region by the deepening of both notches; through the break inner and outer endodermes become confluent. Soon the endodermis becomes double in the region of involution, and two separate bundles are reconstituted, alternating with those of the internode below. The inner part of the endodermis of the departing trace arises as a duplication of that part of the invaginated endodermis that constitutes the bundle sheath of the new bundles at the sides of the parenchymatous depression in the outer edge of the nodal xylem. It is at this moment that the

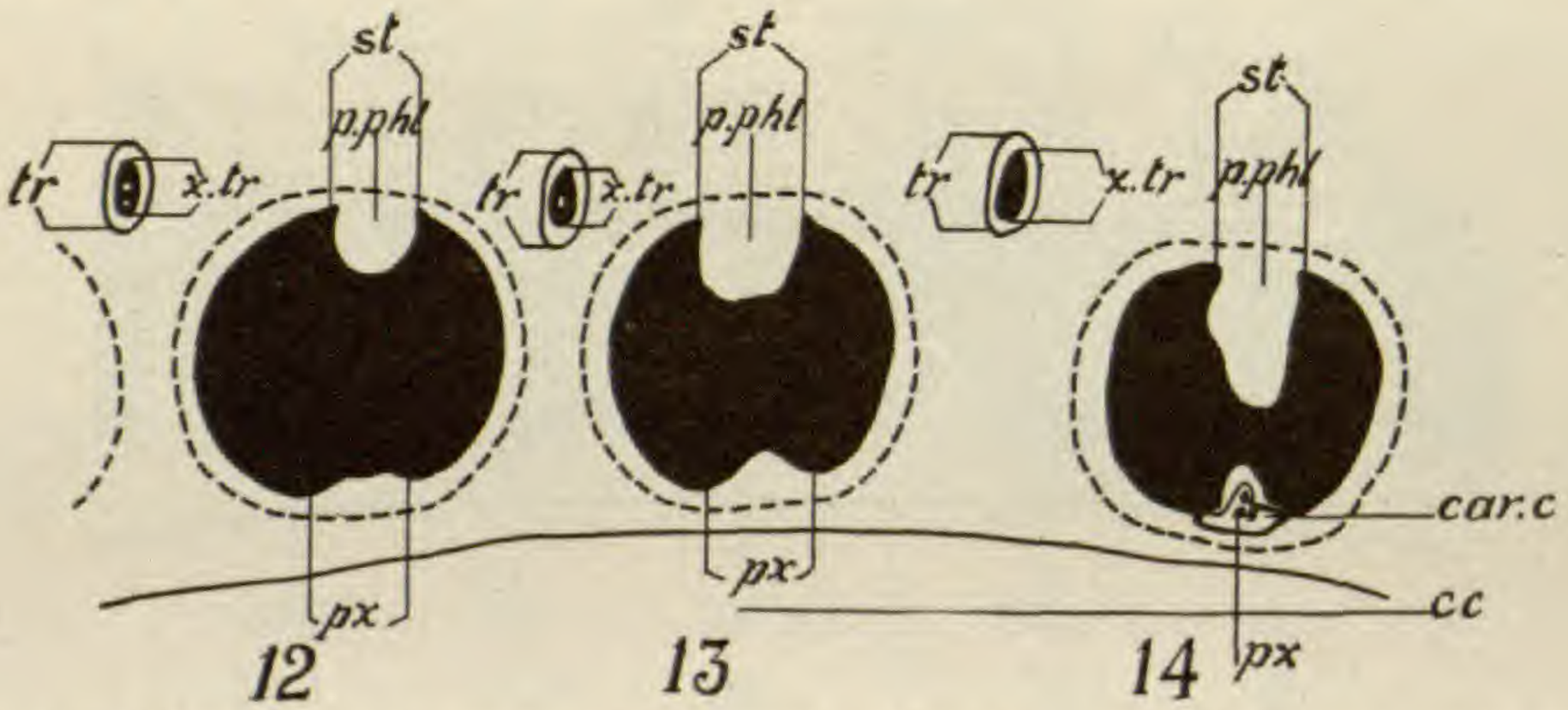


FIG. 4.—Stages 12–14: *en.tr*, endodermis of trace; other lettering as in preceding figures; × about 120.

leaf trace finally becomes free from the axial tissues (fig. 3, stage 11).

As we pass upward above the departure of the traces, the nodal xylem of the newly constituted bundles diminishes in amount, and these become more and more widely separated (fig. 4, stages 12–14). Each of them carries with it two small groups of protoxylem, situated laterally at the inner limit of the nodal xylem. Traced downward, these groups are continuous with the protoxylem of two neighboring but independent bundles of the internode below. As the nodal xylem diminishes, the two groups of protoxylem adherent to it are brought nearer to one another, each becoming farther and farther removed from its sister strand, now included in a neighboring bundle. Just before, or when the new bundles become free from one another, a slight depression appears in the outer edge of the xylem, in the median region of each new

bundle (figs. 3 and 4, stages 11, 12). This deepens rather rapidly, and another shallower parenchymatous depression appears opposite it on the inner edge of the metaxylem, between the two groups of protoxylem (fig. 4, stages 12, 13). In this inner indentation a carinal canal now forms, or, in other words, the protoxylem strands arising from different bundles of the internode below approach one another, and, partly by an increase in their number, become united into a single group (fig. 4, stage 14). By this time the metaxylem has decreased considerably in amount, and forms a deeply lobed mass, the two lobes being separated by the deepening parenchymatous sinus in the outer edge of the xylem. The lobes become less and less massive, and are soon only connected by a narrow crossbar. When this breaks, as it does a little higher up, the structure of the bundle (fig. 5, stages 15, 16) vividly recalls stages 1 and 2 of fig. 1, although the metaxylem is as yet more extensive than in these. By a decrease in the number of tracheids, however, the bundles soon assume the internodal appearance shown in fig. 1, stage 1.

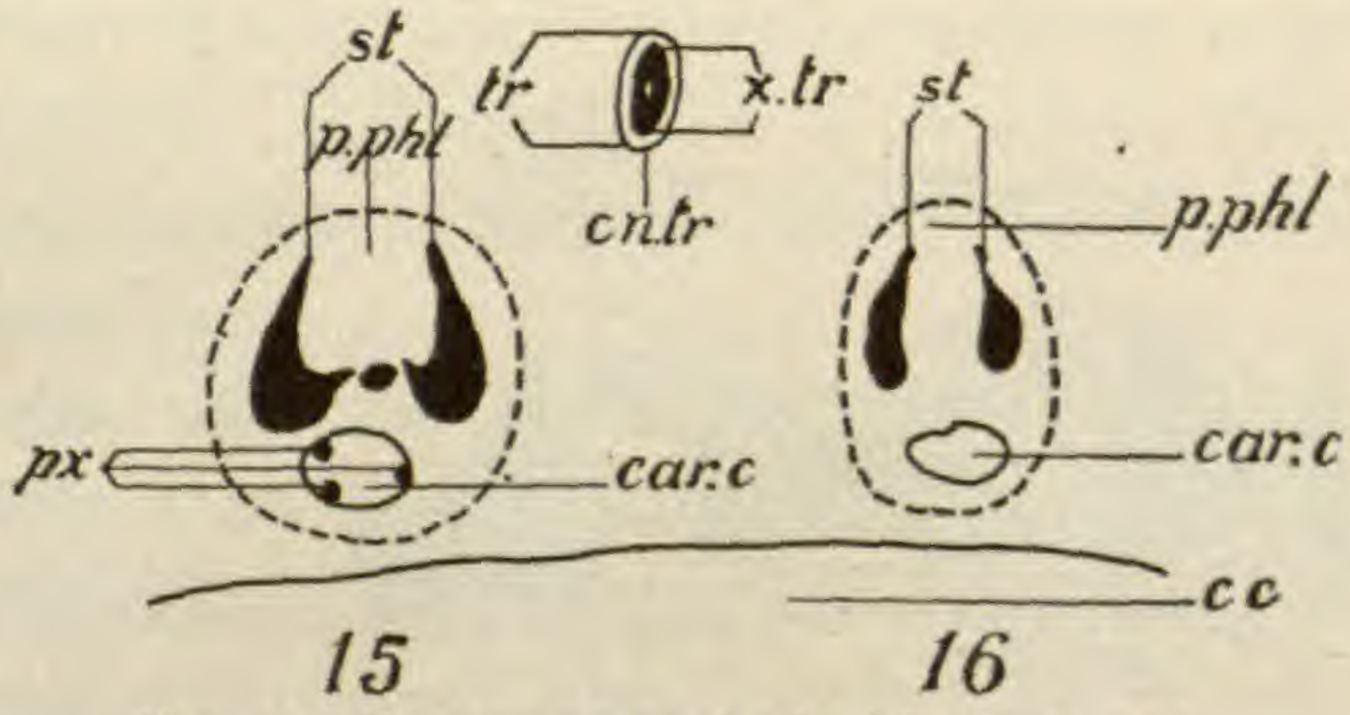


FIG. 5.—Stages 15 and 16; × about 120

As in the cone-bearing stem of *E. limosum* described by the writer (3), so at this node of *E. giganteum* an inverted cone of relatively thick-walled cells, staining deeply with Bismarck brown, hangs down from the upper limit of the node for a little distance into the internode.

III. Nodes of smaller fertile branches

The upper nodes of the cone-bearing branches of *E. giganteum*, although resembling in essentials the node of the main stem previously described, were on a much smaller scale, and showed one or two points of difference. The branches at my disposal all possessed eleven ribs and bundles, and their steles were but little over a millimeter in diameter. There is no definite diaphragm, neither is there an inverted cone of persistent tissue hanging down

from the node; but the central cavity is much narrowed in the nodal region, and the stele itself decreases somewhat in width, as is well seen in longitudinal sections. Sometimes no branches are initiated at these nodes, but in other cases one or two branches may be initiated, even at the uppermost vegetative node. As we approach the node the lateral bands of metaxylem of each bundle become united, as in the bundles of the large node already described. The bundles, at the corresponding heights, have much the appearance of those shown in fig. 1, stages 1–3, except that by the time we reach stage 3 the carinal canal is being replaced by a group of slightly disorganized tracheids. The median tooth of xylem seen in stage 4 of fig. 1 is not developed, although in some of the bundles the crossbar is slightly thicker in its median region. As in the larger axis, the crossbar is separated from the carinal canal by two or three layers of parenchyma. Some of the protoxylem elements pass out very gradually, carrying with them such of the metaxylem elements of the crossbar as lie outside them. The same parenchymatous sheath is formed for the passing out of the protoxylem. This sheath, which is relatively wide and conspicuous, is here directed obliquely upward, since the protoxylem, which lies in it, passes very slowly through the bundle. As there is here no median tooth of metaxylem, the metaxylem of the trace is more deeply situated in the bundle and nearer to the protoxylem, so that the protoxylem and metaxylem of the trace form, in most of their course through the bundle, a single, radially elongated mass of similarly oriented tracheids. This stage is shown in fig. 6, stage 1, a stage corresponding more or less with stage 7 of fig. 1. While passing through the bundle, the metaxylem of the trace remains in contact at its outer end with the nodal xylem of the

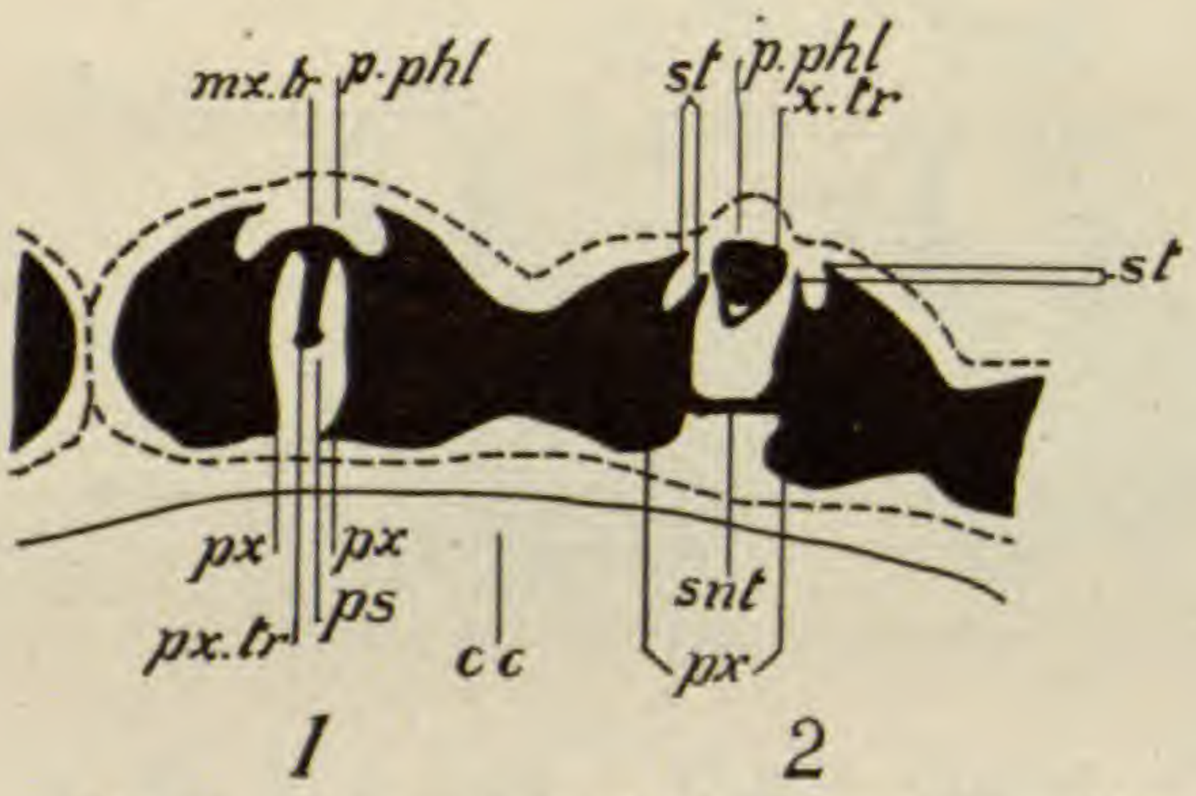


FIG. 6.—Stages 1 and 2; these stages show appearance of bundles of small cone-bearing branch of *E. giganteum* at levels of node corresponding to those shown for bundles of main axis in stages 7 and 8 of figs. 1 and 2; more highly magnified.

bundle. When the xylem of the trace detaches itself from the axial wood, it forms an isosceles triangle, sometimes containing one or two parenchymatous cells. At the inwardly directed apex of the triangle lies the protoxylem. In most cases at least, a few of the tracheids at the sides of the wide, outwardly directed base of the triangle are derived from the peripheral nodal xylem of the bundle. By the time the trace has become free and provided with a separate endodermis, its xylem has become or is becoming slightly mesarch. As the trace moves out, a bar of metaxylem, usually only one cell in depth, forms across the inner end of the parenchymatous sheath (fig. 6, stage 3). These tracheids very soon die out in passing upward. Although few in number, they seem to represent the much greater extent of median supranodal xylem of stages 8*a* and 8*b* of fig. 2.

It should be pointed out that, as in the node of the larger axis, some elements of the protoxylem, although here only a very few, persist on either side of those that depart to the trace. Each of the reconstituted bundles possesses two groups, derived respectively from the two bundles alternating with it in the internode below. Owing to the smaller size of the bundles, these two groups of protoxylem are nearer to one another than in the bundles of the main axis. They soon unite, forming with additional elements the protoxylem strands of the new internode. These groups of protoxylem traversing the node are very small and inconspicuous, and might easily be overlooked or taken for the narrow ends of nodal tracheids. It is possible that sometimes one of them may die out and not be continued in the internode above, but this could not be satisfactorily determined from the material.

IV. Branches initiated on main stem

One point in connection with the numerous branches initiated on the large young axis of *E. giganteum* deserves mention. None of these branches, the cells of which were active in process of division, had yet broken through the tissues of the leaf sheath of the parent axis. As they alternate with the teeth of this sheath, their median line coincides with the commissural furrow, or line of congenital fusion of two leaves. This furrow projects markedly

inward, causing a slight depression in the ochreola or lowest sheath of the branch. The ochreola, which is much better developed on the side away from the parent axis, possesses five teeth, forming in the lower part of the sheath five inconspicuous blunt ribs. The two which lie most externally, right and left of the slight median depression, are by far the most developed. Even in this early stage, in most cases at least, both possess a small vascular bundle containing two or three small tracheids. Cases have previously been recorded and figured in which the ochreola is penetrated by one vascular bundle (8, 11).

V. Young internode of main stem

The base of the leaf sheath is concrescent with the axis. At the level at which the former becomes free in the large young axis the tissues of the main stem have the appearance of being very young. The cells mostly have large nuclei and are rich in contents; no metaxylem has been differentiated, nor is the bundle sheath recognizable; most of the protoxylem still persists. The cortical cells below the ribs of the stem, which later develop as sclerenchymatous fibers, still have unthickened walls. As we pass upward in the internode the tissues gradually assume a more mature appearance. This should be expected, since it is known that the metaxylem of an internode undergoes lignification from above downward (1). In this lower end of a young internode, therefore, we have an anatomically incompletely differentiated portion of the axis of *E. giganteum*, the absence of which prevented GWYNNE-VAUGHAN from definitely establishing the direction of lignification of the lateral metaxylem in this species. He suspected that the direction of lignification in *Equisetum* generally was centripetal in the lateral strands of metaxylem. He held that this was indicated by the constantly smaller size of the peripheral tracheids in *E. giganteum*, the species in which the lateral metaxylem is most abundantly developed.

Near the base of the young internode, at the point at which the leaf sheath detaches itself from the axis, no metaxylem had been differentiated. As we pass upward one or two laterally situated tracheids soon make their appearance, but in most of the bundles it

is only about 1 mm. higher up that the lateral metaxylem consists of a number of tracheids more or less equivalent to the number found in a mature internodal bundle. The serial sections were 14 μ in thickness, and thirty-two bundles of a section often showed different stages of the differentiation of the laterally situated tracheids. It was possible, therefore, to examine very numerous examples of incompletely differentiated metaxylem. Some irregularities were observed, but no doubt was possible that in the very great majority of cases the small outer tracheids are the first to become lignified. Thus in this species, as GWYNNE-VAUGHAN suspected, the metaxylem develops centripetally. Not infrequently, after the lignification of two or three small elements at the periphery of the wood, the next element to be lignified is larger and much more deeply seated. Commonly, however, the lignification proceeds more or less regularly from without inward.

VI. General considerations

Conflicting statements have appeared as to whether the protoxylem of *Equisetum* persisted through the nodes or disappeared at this level. JEFFREY (**9**), in 1899, wrote that in the nodal region the vascular tissue was massive and entirely devoid of typical protoxylem elements, and in comparing the node of *Equisetum* with the description given by WILLIAMSON and SCOTT of that of a Calamite, he states that in the recent genus the protoxylem comes to an end below the node, and that it is absent from the inside of the nodal wood. LUDWIGS (**10**) also appears to regard the protoxylem as disappearing at the nodes. He writes as follows:

> At the node the protoxylem passes out as a bundle into the leaf, the groups of metaxylem approach one another and, uniting with the xylem of the next internode, completely fill up the carinal canal. In the position of the latter we find a large number of vessels with reticulately thickened walls, whereas the elements of the protoxylem show annular thickenings.

More recently Miss BARRATT (**1**) has asserted that the protoxylem does not traverse the node. In 1901, however, GWYNNE-VAUGHAN described the forking of the leaf trace protoxylem of *Equisetum* at the node and the fusion of each fork with the adjacent fork of protoxylem of a neighboring bundle. In 1908 BOWER (**2**)

adopted GWYNNE-VAUGHAN'S conclusions as to the basis of his description of the course of the bundles of *Equisetum*, illustrating this course by means of a hitherto unpublished diagram constructed by the latter.[5] QUÉVA (14) in his very careful researches

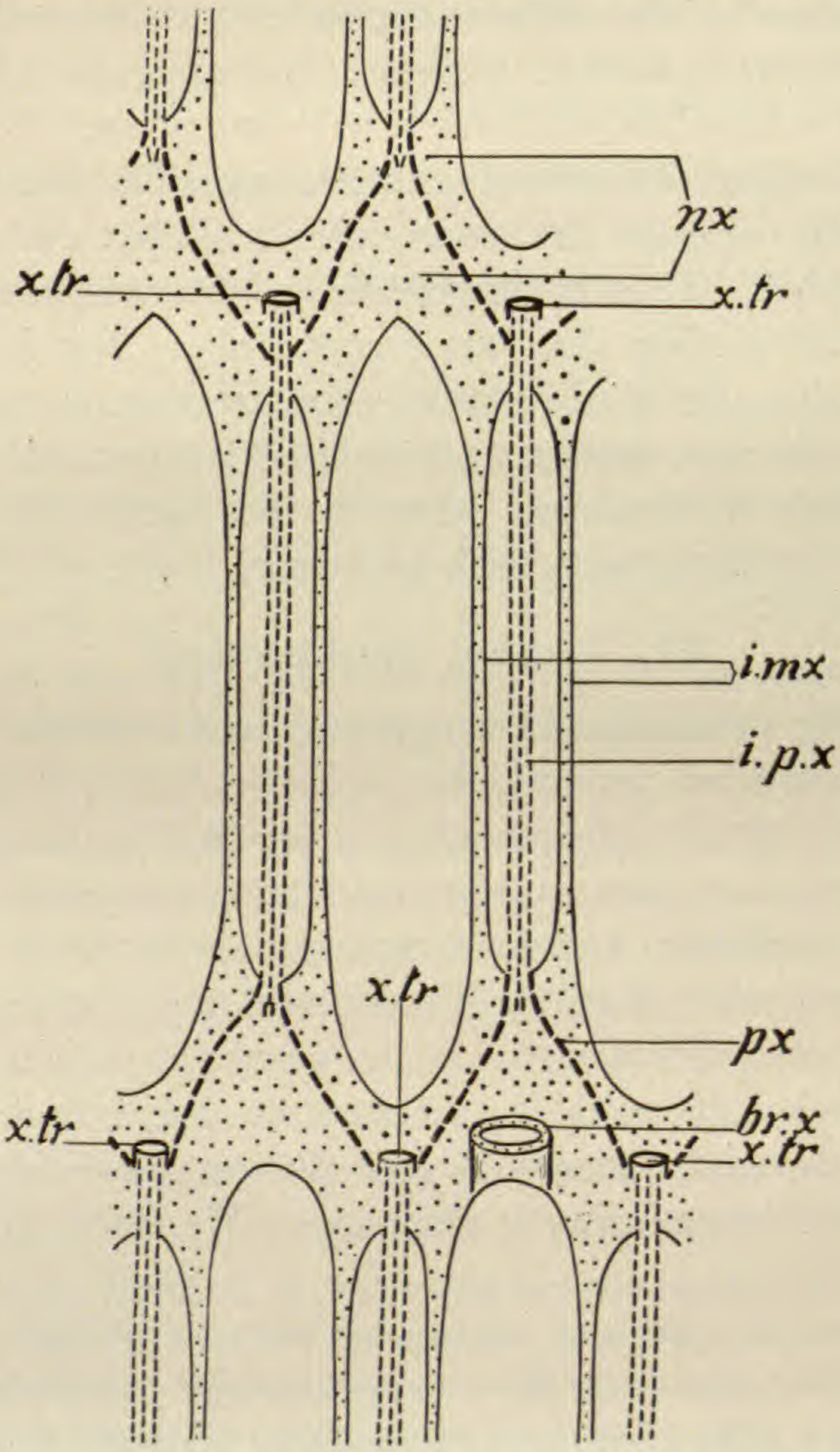


FIG. 7.—Diagram showing distribution of xylem of *E. giganteum* (protoxylem indicated by broken vertical lines, metaxylem by dotted surface): *x.tr*, xylem of trace; *br.x*, xylem of branch; *pr*, protoxylem; *i.p.x*, protoxylem of internode; *nx*, nodal xylem; *i.mx*, internodal metaxylem.

[5] This diagram is in disagreement with the comparable fig. 7 of the present paper in that in it the lateral internodal strands are made to persist through the node. As GWYNNE-VAUGHAN'S diagram was constructed only to show the course of the strands of the internodal bundle at the node, the distribution of the nodal xylem is not indicated in it.

gave no written description of the course of the protoxylem above the departure of the xylem of the trace. In describing the nodal xylem of *E. maximum*, however, he states that at the inner edge of each of the masses of nodal wood some protoxylem elements (his trachées) are always found. These axial protoxylem elements are shown before the lignification of the nodal tracheids in his figs. 16 and 17, and after the differentiation of the nodal wood in his figs. 18 and 19 (all of *E. maximum*). Moreover, his fig. 22 of *E. litorale*, in which two traces[6] may be seen, already some way out in the cortex, shows very clearly the protoxylem persisting from the bundles of the internode below. In this figure the protoxylem of each of these bundles has been divided into two groups of tracheids, and these sister groups have already diverged somewhat from one another.[7] EAMES (**6**) also regards the protoxylem as persistent, for in discussing the nodal, or as he calls it, the supranodal wood, he states: "Certainly its innermost elements are protoxylem, many radial sections show this condition clearly." Later, in summarizing the nodal structure of *Equisetum*, he states: "The carinal canal becomes discontinuous as it approaches the node. The protoxylem occupying that space enlarges, extending radially, then passes upward and forms the innermost tracheids of the supranodal wood." From these statements it would seem that EAMES holds that the protoxylem persists through the node, although hardly as distinct strands; he seems rather to regard the inner elements of nodal xylem generally as protoxylem.

The description of the nodal region of *E. giganteum* here given entirely confirms, so far as the course of the protoxylem is concerned, GWYNNE-VAUGHAN'S account. This might have been expected, as this author alone appears to have worked on the nodes of *E. giganteum*. It is, however, in agreement with QUÉVA'S less complete account of the protoxylem at the node, and in particular with his figures of *E. maximum* and *E. litorale*.

[6] Only a portion of the trace on the left is included in QUÉVA'S figure.

[7] In view of QUÉVA'S figures just mentioned, and of his allusion to the presence of protoxylem at the inner edge of the nodal wood, I cannot agree with Miss BARRATT'S statement that he had noted that the xylem of each internode develops quite independently, only linking up subsequently by the development of the nodal tracheids.

Examination of serial sections through nodes of a cone-bearing branch of *E. palustre* and of a fertile stem of *E. sylvaticum* showed that the continuity of the protoxylem strands through the node, their forkings and fusions, could very well be followed in these species. In both the course of the protoxylem at the node corresponds with that of the protoxylem in *E. giganteum*. The distinction between nodal wood and protoxylem is very clear, although the innermost nodal tracheids are relatively small. In both species the persistent protoxylem is sometimes locally separated from the inner edge of the nodal wood by a parenchymatous cell or two. In *E. sylvaticum* the persistent tracheids of protoxylem are relatively numerous, and some of the medianly situated ones may persist for a little distance above the departure of the tracheids of the trace. In a young node of a fertile stem of *E. arvense* that had not yet appeared above ground it was possible to observe the persistence of the protoxylem and indications of its forkings and fusions at the node. The phenomena, however, were not so clear.

Serial sections through a vegetative node of the cone-bearing branch of *E. debile* showed that in this species also two small groups of protoxylem, consisting of a few tracheids only, persisted after the departure of the trace. On the breaking up of the nodal xylem these two small strands entered separate but adjacent bundles. Each of the newly constituted bundles, therefore, possessed two of these small strands of protoxylem. These, however, did not always fuse to form the protoxylem strand of the internode. Frequently one, sometimes both, seemed to die out. In the latter case the protoxylem of the upper internode was unconnected (except by nodal tracheids) with that of the lower internode. In other cases a few elements of one or both branches of protoxylem linked up the protoxylem of one internode with that of the other.

In the specimens of *E. hiemale* of which I have examined serial sections, the protoxylem disappears at the nodes; in other words, the whole of it passes out into the trace. In this species the internodal bundles are relatively far apart, and the bundles of successive internodes are formed by the oblique course and fusion of adjacent halves of neighboring bundles. During the oblique course of the halves it is easy to see that all the tracheids com-

posing them are reticulately thickened. In nodes of the fertile stem of *E. maximum* I was able to trace the forking of the persistent protoxylem, the divergence of the forks, and their passage into separate though neighboring bundles. Each of the newly constituted bundles thus contained two small groups of protoxylem, but in no case could a connection between these forks and the protoxylem of the internode above be observed; they seemed always to die out.

As we are dealing in *Equisetum* with plants showing a reduced vascular system, it is probable that the dying out of part or all of the protoxylem of one internode without coming into connection with the protoxylem of the next is a derivative character, and that the course of the protoxylem described for *E. giganteum* is primitive within the genus. In this connection it may be pointed out that in the fertile stem of *E. maximum*, although the bundles are of much the same width as those of the large young axis of *E. giganteum* described in this paper, the height and the radial extent of the nodal wood of the latter, even in its young condition, were nearly twice as great as the height and depth of the nodal wood at the nodes of the fertile stems of *E. maximum* studied. Moreover, it has been shown (4) that the cone of *E. maximum* has a much reduced vascular system.

It is possible that at the nodes of the sterile stems of *E. arvense* and *E. maximum* none of the protoxylem, at least in typical cases, persists above the departure of the tracheids of the trace. This appears to be the view of JEFFREY and Miss BARRATT. On the other hand, QUÉVA's figures seem to show that sometimes at least the protoxylem persists, at any rate for some distance above the departure of the tracheids to the trace. Such protoxylem as persists at the node is small in amount and often inconspicuous, so that in many cases it may well have passed unnoticed. When, however, as in *E. hiemale*, the protoxylem disappears completely at the level of the departure of the tracheids of the trace, this would seem to be due to a further reduction along the lines exemplified at the nodes of the fertile axes of *E. debile* and *E. maximum*.

Passing from the consideration of the protoxylem to that of the metaxylem, we again meet with a conflict of evidence. In 1890

POIRAULT (13) asserted that the metaxylem developed centripetally, and eleven years later GWYNNE-VAUGHAN was led by his study of the vascular system of *E. giganteum* to adopt a similar view, although in the absence of an incompletely differentiated portion of the axis of this species he considered the centripetal development of the xylem as not established. As has been pointed out, a study of the young internode of *E. giganteum* confirms GWYNNE-VAUGHAN'S opinion, and it seems clear that in this species the direction of lignification, although subject to slight irregularities, is from without inward. On the other hand, QUÉVA in 1907 showed clearly that in *E. maximum* the lateral metaxylem of the internode was differentiated centrifugally. EAMES claimed that although there is a good deal of irregularity in the direction of its lignification, the internodal metaxylem was differentiated centrifugally in the majority of the bundles of *E. maximum*, *E. arvense*, and *E. hiemale*. This has recently been confirmed by Miss BARRATT for the first two species. Serial sections of the internodes of young cone-bearing branches of *E. arvense*, *E. limosum*, and *E. debile*, in which the lateral metaxylem was incompletely differentiated, were examined for comparison with the young internode of *E. giganteum*. In all of them the differentiation of the metaxylem, although subject to occasional irregularities, as EAMES has pointed out, was in the great majority of cases clearly centrifugal.

It seems difficult to doubt the essential homology of the characteristic lateral groups of metaxylem throughout the genus *Equisetum*. The question, therefore, arises whether the primitive order of development of the metaxylem was centripetal or centrifugal. It is undeniable that the metaxylem, both nodal and internodal, is better developed in *E. giganteum* than in any of the other species the anatomy of which has been studied. Moreover, in a genus showing an obviously reduced stelar structure the species with the largest amount of xylem would naturally seem to be the most primitive. On the other hand, *E. giganteum* is exceptional, so far as we know unique, in the centripetal development of its metaxylem. It is even possible that such centripetal development might be confined to the base of the internodes, although on general grounds and in view of the constantly much

smaller size of the peripheral tracheids this does not seem to be likely. Perhaps the strongest argument against the primitiveness of the centripetal development of the lateral metaxylem lies in the position of this tissue outside the endarch protoxylem, which makes it difficult to regard it as a vestige of the central centripetal xylem of a protostele. For the present, therefore, it seems premature to draw any definite conclusions from the exarchy of the internodal metaxylem in the axis of *E. giganteum*.

GWYNNE-VAUGHAN also stated that in *E. hiemale*, and better still in *E. giganteum*, the lateral internodal strands of xylem could be traced, after joining on to the nodal wood, as externally projecting ridges into the internode above.[8] He claimed that these lateral strands diverged while passing through the node, so that in the internode above they were situated on adjacent sides of separate bundles; that at the next node these strands again approached one another, and that in the internode above this node the strands were again included in the same bundle. In fact, according to this author, the course of the metaxylem strands at the node is exactly that described in this paper as characteristic of the protoxylem of *E. giganteum* in this region. In this species the lateral strands of metaxylem are composed of numerous elements, and if they persisted through the node they would be very conspicuous. From the serial sections of the nodes at my disposal, however, it was clear that the identity of the lateral strands was completely lost in the relatively large tracts of nodal xylem. As we approach the nodes the tracheids of the lateral strands gradually assume the appearance of typical nodal tracheids. QUÉVA has noted this gradual passing of the lateral metaxylem into the nodal wood in *E. maximum*. In this species, in which according to him the lateral metaxylem consists of tracheids with spiral or annular thickening, elements with a type of ornamentation intermediate between these and reticulate thickenings occur, he says, below the nodal wood and in continuity with the internodal metaxylem. Elements with this internodal type of thickening

[8] I regret that in a recent paper (5) I alluded to GWYNNE-VAUGHAN's claim of the persistence of the lateral strands "inside and over" the ring of reticulate tracheids. The passage should read "*outside* and over the ring of reticulate tracheids."

of course do not occur below the node of *E. giganteum*, since, as GWYNNE-VAUGHAN himself has shown, the internodal metaxylem, as well as the nodal xylem of this species, consists of reticulately thickened elements. Fig. 7 shows diagrammatically the distribution of the xylem at the nodes and internodes of *E. giganteum*. The spirally thickened protoxylem is there shown by broken vertical lines, while the reticulately thickened tracheids, nodal wood, and internodal lateral strands alike are shown by a dotted surface. For the sake of convenience the internodes have been drawn as much shorter than they would be in a mature specimen.

In *E. hiemale* the lateral strands are smaller than in *E. giganteum*, but it seemed clear from serial sections that they too completely lose their identity in the nodal wood. Indeed, this occurs relatively quickly, for the crossbar of the tracheids, which as we approach the node forms a bridge between the two lateral strands (fig. 1, stage 3 of *E. giganteum*), is here usually two, three, or more cells in depth, and therefore nearly as deep as the lateral strands. Thus the latter hardly project at all outward, and are early merged in an almost straight, oblong band of metaxylem lying outside and parallel to the radially narrow carinal canal. GWYNNE-VAUGHAN also claimed that in *E. maximum* no metaxylem departed from the axis to the leaf trace, although he made no statement on this point for *E. giganteum*. EAMES, however, claims that the metaxylem unquestionably takes part in the formation of the leaf trace, and figures such a case for *E. hiemale*, in which species the metaxylem is well developed. In nodes from three stems of *E. hiemale* examined the departing protoxylem carried with it metaxylem tracheids from the periphery of the bundle. At least in my specimens, however, these were less numerous than in *E. giganteum*, and died out before the trace with its endodermis was completely free from the axial bundle. In serial sections of nodes from two fertile stems of *E. maximum* and from a fertile stem of *E. sylvaticum* examined the metaxylem did not, in most of the bundles, contribute to the formation of the leaf trace, although occasionally in *E. maximum* and not infrequently in *E. sylvaticum* two or three reticulate tracheids, at the level of the departure of the trace, did bend out into the cortex, where, however, they seemed to die out.

Summary

1. The direction of differentiation of the metaxylem in the internode of *E. giganteum* is subject to slight irregularities, but is mainly centripetal. The outer elements are the smallest, and the tracheids, usually about 10–15 in number, become wider in passing inward.

2. These lateral, internodal strands of xylem join on to the nodal wood, at which level they lose their identity. There is no indication in *E. giganteum* of their persisting as strands external to the nodal wood.

3. The protoxylem is continuous through the node of *E. giganteum*. By the departure of the medianly situated tracheids to the trace the protoxylem is divided into two small groups of elements. These two groups diverge and enter neighboring but separate bundles of the internode above. Each of these small strands of protoxylem, diverging still farther from its sister strand, now situated in another bundle, fuses in the median region of the new bundle with an equivalent strand of protoxylem derived from the adjacent bundle of the internode below. The fusion is effected partly by a sudden increase in number of the protoxylem elements.

4. The nodal wood of *E. giganteum* attains a considerable height and radial depth. Wide, reticulate, typically nodal tracheids appear considerably below and persist for some distance above the departure of the traces.

5. The protoxylem elements of *E. giganteum* are situated at the interior of the xylem, and pass through the inner part of the metaxylem of the bundle in a kind of parenchymatous sheath, two to four cells in thickness. A considerable number of small metaxylem tracheids pass out into the trace, the metaxylem in the trace being usually greater in amount than the protoxylem.

6. In young branches of *E. giganteum* which had not yet broken through the leaf sheath of the parent axis, two of the ribs of the ochreola contained a small vascular bundle.

7. It is concluded that the continuity of the protoxylem of the internodes through the nodes, which, although not characteristic of all species, is not confined to *E. giganteum*, is a primitive character within the genus. The question as to whether the lateral

metaxylem of the genus was primitively centripetal, as in *E. giganteum*, or primitively centrifugal, as in the other species the detailed anatomy of which is known to us, is left open.

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OVERWINTERING OF TOMATO MOSAIC[1]

MAX W. GARDNER AND JAMES B. KENDRICK

(WITH PLATE XVII)

The annual recurrence of the mosaic disease in epiphytotic form in the canning tomato crop of Indiana has made it highly important to ascertain the mode of overwintering of the causal virus. It seemed within the realm of possibility that the virus might be perpetuated over winter in hothouse tomato crops, in tomato seed, in related perennial weed hosts, and by insects. The agency of insects in this connection has not been studied. The work of MCCLINTOCK and SMITH (9) on aphids as carriers of spinach blight would indicate that such insects might perpetuate other mosaic viruses, but MCCLINTOCK (10) has been unable to find this true for tomato mosaic. DOOLITTLE'S (7) work on cucumber mosaic has failed to incriminate any of the insects studied in connection with that disease. The present work has to do mainly with the second and third possibilities just mentioned.

Hothouse tomatoes as carriers

The mosaic disease has been found very commonly in hothouse tomato crops, and in the immediate neighborhood of hothouses it is possible that the disease may be carried from the late hothouse crop to the field crop plant-beds. This danger is very great in cases in which the plants for the field crop are started in hothouses or coldframes adjacent thereto. In one case noted in June 1920 at Kokomo, a severe early infestation of mosaic was present in a field, the plants for which had been grown in part of a hothouse occupied by a tomato crop. In fact, mosaic was found on many of the tomato plants left in the plant-bed. Hothouse tomatoes, however, are grown only in a relatively few localities in the state, and are usually near the towns and cities. The canning tomato crop, on the other hand, is contracted primarily among general farmers

[1] Contribution from the Botanical Department of Purdue University Agricultural Experiment Station, Lafayette, Indiana.

rather than truck gardeners, so that the fields are widely scattered through the country, and as a rule are not in the neighborhood of hothouses. Hothouses, therefore, can play only a very minor rôle as reservoirs of mosaic infection for the canning tomato crop.

Transmission with tomato seed

Miss WESTERDIJK (12), in her work with tomato mosaic, concluded that the disease was transmitted through the seed, but her evidence appears to be based on a field test with only ninety-six plants, and these unprotected from insects. ALLARD (3), in extensive tests involving about a thousand plants grown from seed from mosaic tomato plants, obtained no evidence whatever that the disease was transmitted through the seed. The same investigator (2) found that the related tobacco mosaic is not seed-borne.

The general occurrence of mosaic in fields used as a source of seed for the canning tomato crop of Indiana made it necessary to test thoroughly the possibility of seed carriage of the virus. A quantity of tomato seed was saved from mosaic tomato plants in the fall of 1920, in cooperation with I. C. HOFFMAN and H. D. BROWN of the department of horticulture, and was planted in a greenhouse December 2, 1920. Because of the season the plants grew rather slowly. In a careful examination made on January 20, 1921, no mosaic was found in a total of 13,573 of these plants. The crop was thinned at this date, and on February 26 no mosaic was found among the 2823 plants which remained. On February 3, 1921 seed saved from two mosaic tomato plants in 1920 was planted in soil flats in the greenhouse, and in the 135 plants present on May 9 no mosaic had appeared. In the summer of 1921 another test of tomato seed collected from mosaic plants in 1920 was made in the greenhouse and under a cloth cage in the field. The seed was planted on June 23, and on August 10 no mosaic was found in a total of 5091 plants in the greenhouse and 218 under the field cage. Thus, in a total of 19,017 plants grown from seed from mosaic tomato plants, no mosaic appeared. Similar tests in the greenhouse in 1921 with two-year-old tomato seed from mosaic plants also yielded negative results. In a total of 3927 plants grown from such seed no mosaic occurred.

The possibility of the presence of the mosaic virus dried on the exterior of the seed coat was also taken into consideration. About four ounces of tomato seed collected from mosaic plants four months previously was washed in sterile water, and eight tomato plants were inoculated by wounding the stem near the growing tip with a needle, and rubbing the wounded area with cotton soaked in this wash water. No mosaic developed in these plants. In the light of this evidence there appears to be no indication that tomato mosaic is transmitted through the seed.

Mosaic in perennial Solanaceous weeds

Historical

The susceptibility of certain perennial weeds to tobacco and tomato mosaic is highly significant in connection with the overwintering of the virus. Allard (1) transmitted mosaic from tobacco to the perennial *Solanum carolinense*, and points out the possibility of the mosaic virus persisting over winter in the rootstocks of this weed. He states, however, that he had noted only one case of mosaic occurring naturally in *S. carolinense*, but recognizes the difficulty of detecting the disease in this weed because the symptoms may be very inconspicuous. He also found the mosaic which occurs commonly on the perennial *Phytolacca decandra* to be distinct from and unrelated to the tobacco mosaic.

Nishimura (11) transmitted mosaic from tobacco to *Physalis alkekengi*. In his tests the *Physalis* plants developed no mosaic symptoms, but the juice expressed from the inoculated plants proved infectious to tobacco. This exotic species of *Physalis* is recorded as a perennial which is not hardy in the northern states. Nishimura also proved that a mosaic disease found on the perennial *Solanum aculeatissimum* in Florida by R. A. Harper was transmissible to tobacco.

Recently Crawford (6) in Iowa has reported successful cross inoculations from mosaic tomatoes to *Physalis longifolia*, a common weed of that region. He also found mosaic occurring in the field on that weed, and with the virus from the rootstocks made successful inoculations of tomato plants. He points out the probability of the mosaic virus overwintering in the rootstocks of *Physalis longifolia*. This species has not been found in Indiana.

An example of the persistence of a mosaic virus in a perennial herbaceous host is afforded by the pokeweed mosaic studied by ALLARD (4). The work of CARSNER (5) on the weed hosts of the virus of the curly-top disease of sugar beets in California has been very suggestive in connection with the problem of overwintering of mosaic viruses. He has pointed out the probability that the curly-top virus may persist over winter in *Erodium cicutarium*, a winter annual. Recent work by DOOLITTLE on the relation of *Micrampelis lobata* (7) and *Asclepias syriaca* (8) to cucurbit mosaic also has been suggestive.

PERENNIAL SOLANACEOUS WEEDS IN INDIANA[2]

The following Solanaceous perennials occur in Indiana: *Lycium halimifolium* Mill., *Solanum dulcamara* L., *S. carolinense*, *Physalis lanceolata* Michx., *P. heterophylla* Nees., *P. subglabrata* Mack. and Bush, and *P. virginiana* Mill. *S. carolinense* and the three species of *Physalis*, *P. heterophylla*, *P. subglabrata*, and *P. virginiana*, are weeds of common occurrence in and about cultivated fields. Of these, *P. subglabrata* and *P. virginiana* have been found to be by far the most abundant in the tomato regions, and most of the observations have been made upon these species. These two species are not easily differentiated, and no consistent attempt has been made in this work to separate them. The larger leaved *P. subglabrata* has appeared to be the more abundant of the two in central Indiana. Unless otherwise qualified, the term *Physalis* as used herein should be understood to refer to these two very similar species.

CROSS INOCULATION TESTS

Mosaic has been found occurring naturally in the field on *Solanum carolinense*, *Physalis heterophylla*, *P. subglabrata* (pl. XVII), and *P. virginiana*. On July 5, 1921, ten potted tomato plants in the greenhouse were inoculated by wounding the stem and rubbing the wounded area with cotton soaked in the juice from mosaic *S. carolinense* plants collected at Vincennes. By July 29 all had developed mosaic. None of the ten control plants, similarly treated except that distilled water was substituted for the juice

[2] CHARLES C. DEAM, state forester, very kindly furnished authoritative records concerning the Solanaceous flora of Indiana.

from the mosaic plants, developed the disease. On July 28, 1921, sixteen potted tomato plants in the greenhouse were similarly inoculated with the juice from mosaic *P. heterophylla* plants, and by August 18 nine had developed mosaic. Four uninoculated plants held as controls remained healthy. In a tomato field, on August 21, 1919, thirteen plants of *P. subglabrata* were inoculated with the juice from crushed leaves of mosaic tomato plants. Twelve days later eight had developed mosaic. Nearby uninoculated plants observed as controls did not develop the disease. Late in August 1919, seventeen potted tomato plants in the greenhouse were inoculated with the juice from mosaic *P. subglabrata* plants, and fourteen developed the disease; the seventeen uninoculated control plants remaining free from mosaic. On May 25, 1921, four potted tomato plants in the greenhouse were inoculated by wounding the stem with a needle and rubbing the wounded region with cotton soaked in the juice of crushed leaves of mosaic *P. subglabrata* collected at Frankfort, and fifteen days later all had developed mosaic. The two control plants, similarly treated except that distilled water was substituted for the mosaic virus, remained healthy. On July 15, 1921, forty-seven tomato seedlings grown under a cloth cage in the field were inoculated with the virus from mosaic *P. virginiana*. Ten days later twenty had developed mosaic. None of the numerous uninoculated seedlings in the cage developed the disease. The identity of this *Physalis* species was verified by PAUL C. STANDLEY of the United States National Museum. The results of these cross inoculations show that the mosaic disease found on these weeds in the field is transmissible to tomatoes.

OBSERVATIONS ON PHYSALIS MOSAIC IN 1919 AND 1920

The attention of the writers was directed to the importance of *Physalis* as a carrier of tomato mosaic in the summer of 1919. Large numbers of *P. subglabrata* occurred in an experimental field of tomatoes near Frankfort, Indiana. Mosaic became epiphytotic on the tomatoes during the latter part of the season, and also appeared on many of the *Physalis* plants, especially in a low-lying section of the field where the weeds were most abundant. In this

part of the field about 5 per cent of the *Physalis* plants showed mosaic. The reciprocal cross inoculations proved that the causal viruses were identical. *Physalis* was generally distributed in this vicinity, and a survey showed that mosaic did not occur to any extent on the plants at a distance from the tomato field. In a corn field about 40 rods distant, several hundred *Physalis* plants were examined and only two showed mosaic. Horse nettle was present in the tomato field, but showed no mosaic symptoms.

The location of a number of the mosaic *Physalis* plants in the tomato field was carefully noted. The following year this field was planted in corn and no tomatoes were grown in the neighborhood. On July 15, 1920, an inspection of the field showed the *Physalis* plants again abundant, and in the same part of the field where mosaic was noted in 1919 the disease was now conspicuous on a much higher percentage of the plants than had been observed the preceding fall. In a corn field adjacent to the west side of the experimental field, mosaic was found among the *Physalis* plants along the edge, but not over 100 feet distant from the fence. In another corn field near the east side of the experimental field, mosaic was also found on many of the *Physalis* plants. Since no tomatoes were being grown in the vicinity this season, the prevalence of mosaic on *Physalis* at this early date indicated that the disease must have persisted in the weeds over winter. The greater prevalence of the disease as compared with the preceding September may possibly be explained by the fact that many of the weeds had not shown definite mosaic symptoms in the fall, whereas the young shoots of the following spring showed conspicuous symptoms. It has been noted that mosaic symptoms on old plants in the fall may become very inconspicuous.

Overwintering of virus in rootstocks

Physalis subglabrata is perennial by means of a thick rootstock 12–18 inches below the surface of the soil, deep enough to escape harm from ordinary cultivation practices. In the fall of 1919 some of these rootstocks of mosaic plants were dug, and an unsuccessful attempt was made to carry them over winter in pots of soil. The test was repeated the next year. Late in August

1920 a number of rootstocks of mosaic *P. subglabrata* plants were dug in the Frankfort field and planted in a small plot surrounded by a wooden frame sunk in the soil in a garden at Lafayette. These rootstocks established themselves and produced shoots in the fall of 1920. The rootstocks remained alive over winter, and in the spring of 1921 sent up shoots showing mosaic. Six shoots had appeared by May 13, thirteen by May 23, and on June 3 fifteen plants were present. These mosaic *Physalis* shoots appeared well before the date that tomatoes are transplanted to the field, and all showed definite mosaic symptoms as soon as the leaves unfolded.

A number of aphids were found on these *Physalis* plants early in the season. On May 23 about twenty-five of these aphids were collected and caged on three small healthy tomato plants in the greenhouse. Fourteen days later one of these tomato plants showed mosaic. None of the six control plants developed the disease. The aphids soon disappeared from the *Physalis* plants in the field, but this test indicates that mosaic might be transmitted from *Physalis* to tomatoes by these insects.

Artificial inoculation of tomatoes with the virus obtained by crushing some of the leaves from three of these mosaic *Physalis* shoots also was successful. Ten small tomato plants were inoculated on June 24 by wounding the stem with a needle and rubbing the wounded area with cotton soaked in the *Physalis* virus. Eleven days later all had developed mosaic. Ten control tomato plants were similarly treated except that sterile water was substituted for the mosaic virus, and nine of these remained free from mosaic. These tests show that the mosaic virus persists over winter in the rootstocks of *P. subglabrata*, that the young shoots come up diseased at an earlier date than tomatoes are set out in the field, and that the disease is readily transmissible from these shoots to tomatoes.

Mosaic Physalis in fields previously in tomatoes

To determine how generally the mosaic disease was carrying over winter in the *Physalis* plants (including both *P. subglabrata* and *P. virginiana*), an examination was made in and near fields

where tomato mosaic had occurred in previous years. On May 24, 1921, an examination was made in and near the experimental field at Frankfort which had been in tomatoes in 1919, and in which mosaic was found on the *Physalis* plants in 1920. In the old tomato field, 147 out of 203 *Physalis* plants examined (or 74 per cent) showed mosaic. The disease occurred more generally distributed throughout the field, and on a much higher percentage of the plants than in 1919 and 1920. In the manner characteristic of the perennial species of *Physalis*, many of the plants occurred in clumps, and as a rule the plants in each clump were all healthy or all mosaic.

In the field west of the old tomato field, 11 out of 179, or 6 per cent of the *Physalis* plants examined in a strip about 50 feet wide along the fence showed mosaic. In the fields east of the old tomato field, 11 out of 39, or 29 per cent of the *Physalis* plants examined showed mosaic. In the field to the north, no mosaic was found on the 66 plants examined, but most of these were at a considerable distance from the tomato field. From these mosaic *Physalis* plants the disease was transmitted to tomato plants in the greenhouse, as noted in a previous paragraph. In this area, therefore, the mosaic disease persisted in *Physalis* plants two years after the tomatoes, and even became more prevalent on the weeds.

On May 23, 1921, mosaic *Physalis* plants were found in a small plot and in a field near Lafayette, in both of which tomato mosaic had occurred in 1920. On May 25, 1921, a study was made of the *Physalis* plants in a three-acre field near Indianapolis in which tomato mosaic had been especially severe in 1920. On one side of the field, 6 out of 209 *Physalis* plants examined showed mosaic, and on the other side, 67 out of 159 showed mosaic. Thus a total of 73 out of 368, or 20 per cent of the *Physalis* plants were affected with mosaic. No mosaic had been noted on the *Physalis* plants among the tomatoes in this field on September 14 of the preceding fall. In an adjacent portion of this field which had been in corn in 1920, 104 *Physalis* plants were examined and none showed mosaic.

These observations showed that the mosaic *Physalis* shoots were appearing rather generally in fields which had been in tomatoes in

previous years, well before the date when the tomato plants for the current season would be transplanted to the field. It is evident that the *Physalis* plants once infected constitute a perennial reservoir of mosaic infection, which remains a constant danger to any future crops of tomatoes (or tobacco) in the vicinity.

Further observations were made later in the season upon the prevalence of mosaic on the *Physalis* plants in fields previously planted to tomatoes. In the field near Lafayette in which tomato mosaic had occurred in 1920, 43 out of 77, or 55 per cent of the *Physalis* plants examined the third week in July showed mosaic. Many volunteer tomato plants had come up in this field, but among the 186 examined, no mosaic was found at this time, although later in the season a few developed the disease.

TABLE I

Physalis MOSAIC IN OLD TOMATO FIELDS

Field	Crop			*Physalis* plants, July 13, 1921		
	1919	1920	1921	No. examined	No. mosaic	Percentage mosaic
1.....	Tomatoes	Corn	Corn	79	43	54
2.....	Tomatoes	Oats	Clover	34	7	20
3.....	Tomatoes	Corn	Oats	61	16	26
4.....	.....	Tomatoes	Oats	27	17	63
5.....	.....	Tomatoes	Weeds	40	27	67
6.....	.....	Tomatoes	Wheat	107	55	51
7.....	Not tomatoes		Tomatoes	138	34	25
8.....	Not tomatoes		Wheat	543	4	0.7

A study of the mosaic prevalence among the *Physalis* plants was made July 13 on a large farm near Indianapolis, of which a considerable acreage was devoted to tomatoes in 1918, 1919, 1920, and 1921. The 1919 tomato crop, comprising about 100 acres, was practically 100 per cent mosaic in September. No observations were made on the 1920 crop on this farm, but it is safe to assume that mosaic was prevalent that year. Mosaic was already prevalent in the 1921 crop. The results of the survey of eight fields on this farm and the relation between previous tomato crops and *Physalis* mosaic are presented in table I. From these data it is evident how prevalent mosaic may be on *Physalis* one and two

years after tomatoes have been grown. The mosaic in field no. 7 probably was due to the tomatoes in the field at the time, since many of these also showed the disease. The scarcity of the disease in field no. 8, which had never been in tomatoes, indicates that the high incidence of mosaic in field nos. 1 to 6 was due to the previous tomato crops.

Distance mosaic may spread

Field no. 8 had never been in tomatoes before, and the nearest tomato crop, that of 1919 in field no. 1, was 400 feet distant. The few cases of *Physalis* mosaic in field no. 8 were found along the edge nearest to field no. 1, and are probably attributable to long distance transmission of mosaic from that crop. In a wheat stubble field which had never been in tomatoes, mosaic was found on *Physalis* along the edge adjacent to one of the 1919 tomato fields (field no. 3) in a strip 150–200 feet wide. No mosaic *Physalis* plants were noted at a distance of 250 feet from the edge of the field. The occurrence of mosaic *Physalis* plants along the edges of fields adjacent to the Frankfort experimental field has previously been noted. The occurrence of two mosaic *Physalis* plants 40 rods distant from this field, if attributable to spread from the tomatoes, would represent an exceptionally long distance of mosaic transport.

Surveys of numerous wheat stubble and corn fields have revealed that mosaic very rarely occurs on *Physalis* plants except in the vicinity of tomato crops, past or present. In only two instances have apparently spontaneous cases of mosaic on *Physalis* been found. One mosaic plant was found in a wheat stubble near Knightstown, and another in a corn field near Monticello. In the light of such observations it is unsafe to assume that mosaic is indigenous in these wild hosts. It seems evident, however, that once the disease is introduced by means of tomatoes, it may become enphytotic in the *Physalis* flora of the immediate vicinity.

Prevalence and correlation of Physalis and mosaic in tomato fields

To ascertain the general prevalence of *Physalis* and *Physalis* mosaic in Indiana tomato fields, and the correlation between these factors and mosaic in the tomato crop, a number of tomato fields

in six localities were examined in the summer of 1921. Of necessity much of this survey work was rather hastily performed. Included in this survey are 2 fields in Washington County visited June 29; 13 fields in Johnson County, June 30; 21 fields in Howard and Tipton Counties, July 2; 4 fields on a large farm near Indianapolis, July 13; 11 fields in Hancock County, July 20; 25 fields in Marion County, July 21; and 5 fields in Grant County, September 17. The results may be summarized as follows:

Tomato fields examined	81
Fields in which *Physalis* was found	65
Fields in which mosaic on *Physalis* was found	35
Fields in which mosaic on tomatoes was found	60
Fields in which *Physalis* and mosaic on tomatoes were found	48
Fields in which mosaic on both *Physalis* and tomatoes was found	29

The wide occurrence of *Physalis* is evidenced by its presence in 65 out of 81, or 80 per cent of the tomato fields examined. The prevalence of mosaic on *Physalis* is shown by its presence in 35 out of 65, or 54 per cent of the fields in which the weeds were noted. The prevalence of tomato mosaic is shown by its occurrence in 60 out of 81, or 74 per cent of the fields examined. Some correlation between tomato mosaic and the presence of *Physalis* is indicated by the fact that 48 out of 65, or 74 per cent of the fields containing *Physalis* showed tomato mosaic, and the fact that 48 out of 60, or 80 per cent of the fields showing tomato mosaic contained *Physalis* plants. Some degree of correlation between the occurrence of mosaic on both *Physalis* and tomatoes is indicated by the presence of mosaic on tomatoes in 29 out of 35, or 83 per cent of the fields in which *Physalis* mosaic was found, and by the presence of mosaic *Physalis* plants in 29 out of 60, or 48 per cent of the fields in which tomato mosaic was found.

Plant-bed origin of mosaic

There were convincing indications in many of the fields examined that mosaic was transported to the field with the tomato transplants. In many of the fields in Johnson and Hancock counties, originally set out with tomato transplants imported from southern states, the heavy losses in stand due to the presence of *Fusarium*

wilt in these imported transplants necessitated the use of large numbers of locally grown replants to fill the blank spaces. Mosaic was distinctly more prevalent on these locally grown replants.

An examination was made of the plant-beds in three localities which served as sources of these replants, and *Physalis* plants were found in or near these beds in all cases. No mosaic, however, was noted on these *Physalis* plants. The replants from one of these localities had been very generally diseased in every field in which they were used, and on July 20 mosaic was found very general on the tomato plants remaining in the outdoor plant-beds from which these replants had been taken. These plant-beds were grown up to weeds at this time, and thirty *Physalis* plants were found, but none showed mosaic. In fact, *Physalis* was a particularly abundant weed in this neighborhood, and was also noted in the coldframes of another grower.

The occurrence of *Physalis* plants in and about coldframes and plant-beds is considered of especial significance, because here tomato plants are grown year after year, and once mosaic gains a foothold in these weeds, all succeeding crops of tomato plants will be exposed to infection before they are transplanted to the fields. This source of infection is considered especially dangerous, because from the plant-beds the disease may be introduced into numerous fields, and because mosaic reduces the yield much more severely on plants infected when very young. It has been shown that the mosaic disease, once introduced into a locality, may persist year after year in the perennial weed relatives of the tomato. Since, under Indiana conditions, canning tomatoes are grown in rotation with other crops, and many new fields are being used each year for tomato production, the mosaic disease will undoubtedly be thus introduced into the perennial weed flora of new fields and localities each season. This will inevitably result, it would seem, in the disease becoming more and more widespread in the weed flora each year, and consequently in an alarming annual increase in the reservoir of mosaic infection for future tomato crops unless the vicious cycle is broken.

Mosaic transmission

The means by which the mosaic disease may be transmitted from *Physalis* to tomato have not been thoroughly studied, although from analogy with other mosaic diseases it has seemed safe to assume that insects are the responsible agents. Certain it is that insects are responsible for much of the spread of mosaic among tomatoes, because, by the use of cages to exclude insects, the occurrence of mosaic has been uniformly prevented. Plants thus caged remain free from mosaic in badly diseased fields.

The occurrence of aphids on mosaic *Physalis* plants early in the season, and the successful transmission of the disease to a tomato plant by these insects has been mentioned. Flea-beetles (*Epitrix cucumeris*) are abundant on *Physalis* plants throughout the season, and these insects also attack young tomato plants. A preliminary test indicates that they may carry the disease. On July 16, 1921, a number of flea-beetles collected on mosaic *Physalis* plants were placed in a large cloth cage containing young tomato plants. On August 17 six of the 338 plants in this cage showed mosaic, while no mosaic was found in the 218 control plants in a similar cage in which no flea-beetles had been placed.

Mosaic on Physalis heterophylla and Solanum carolinense

Although not as abundant as the two *Physalis* species previously discussed, *Physalis heterophylla* and *Solanum carolinense* are of common occurrence in cultivated fields in Indiana, the former usually in sandy soils. Mosaic in a conspicuous form was found in abundance on both of these species in a peach orchard near Vincennes, June 28, 1921. A clump of five mosaic *P. heterophylla* plants was found on July 27 along the edge of a field near Lafayette in which tomato mosaic occurred the previous year. Mosaic was noted on *S. carolinense* near a canning factory at Indianapolis, September 7. Successful cross inoculations of mosaic from both of these species to tomatoes have previously been described. Both species are commonly attacked by flea-beetles.

Among the eighty-one tomato fields visited in the survey, *P. heterophylla* was noted in seven fields and *S. carolinense* in thirteen

fields. In three fields, all in Marion County, mosaic was noted on *P. heterophylla* and also occurred on the tomatoes. In one of these (field no. 7) ten *P. heterophylla* plants were noted and one showed mosaic. Mosaic was noted on *S. carolinense* in only one field, a garden near Kokomo in which mosaic also occurred on *P. subglabrata* and on the tomatoes. It is evident that *P. heterophylla* and *S. carolinense* may function as reservoirs of mosaic infection. Both species are perennial by deep rootstocks and difficult to eradicate or control by cultivation.

Mosaic in annual Solanaceous weeds

ALLARD (1) transmitted mosaic from tobacco to two garden species of *Physalis* (probably annuals) and to the annual *Solanum nigrum* and *Datura stramonium*. In Indiana mosaic has frequently been noted on these weeds. Attempts to cross inoculate from *D. stramonium* to tomato and vice versa have yielded negative results. In preliminary tests mosaic has been transmitted successfully from tomato to *S. nigrum* and to *S. integrifolium* and *Lycopersicum pimpinnellifolium*. Mosaic has been noted on cultivated *Physalis pubescens*. The disease, of course, is common on tobacco, and has been transmitted to tomatoes by artificial inoculation. Mosaic has been noted on tobacco plants occurring as weeds in hothouses. While annual hosts cannot carry the mosaic disease over winter, they may serve as sources of infection during the growing season, and aid in the annual spread of the disease. Annual Solanaceous weeds are undesirable in tomato fields and plant-beds and in hothouses.

Mosaic control suggestions

The danger involved in growing plants for the field tomato crop in hothouses used for tomatoes should clearly be understood.

Tomato growers should recognize in the perennial ground cherries and horse nettle a distinct danger to their crop. Drastic measures should be taken to eradicate these weeds in the vicinity of tomato seed-beds and plant-beds. Furthermore, during the early part of the season these weeds should be destroyed or at least kept down in and around the tomato field by frequent cultivation and hand

pulling. This is especially important during the first part of the season, since early mosaic infection results in the greatest loss.

These perennial species present extreme difficulties in the way of control because of the deep rootstocks and the prompt reappearance of new shoots after the old ones are destroyed.

The annual Solanaceous weeds, such as nightshade and certain ground cherries, should be destroyed in and near tomato fields and plant-beds.

Hothouses to be used for tomatoes should be kept free from Solanaceous weeds.

Tomato plant-beds should be cleared of all weeds and remaining tomato plants as soon as no more transplants are needed.

Transplants from plant-beds in which mosaic is present should not be used.

Theoretically these weed relationships are equally important in connection with the control of mosaic in tobacco.

Summary

1. Tomato mosaic may be carried over winter in hothouse tomato crops, but this does not account for the great bulk of mosaic infection in the canning crop.

2. In a total of 22,944 tomato plants grown from seed from mosaic plants, no evidence of seed transmission of the disease was obtained.

3. The mosaic disease has been found occurring in the field on the following perennial weed relatives of the tomato in Indiana: *Physalis subglabrata*, *P. virginiana*, *P. heterophylla*, and *Solanum carolinense*. Mosaic has been transmitted to tomatoes from each of these species.

4. It has been proved that the mosaic virus persists over winter in the rootstocks of *P. subglabrata*. The young mosaic shoots appear in the spring before tomatoes are transplanted to the field. From these shoots the disease has been transmitted to tomatoes.

5. *Physalis subglabrata*, with some admixture of the very similar *P. virginiana*, is a very prevalent weed in Indiana tomato fields.

6. Examination of these weeds in fields previously in tomatoes shows that a considerable percentage of the *Physalis* plants come

up showing mosaic the next year, and likewise the second year after the tomatoes. The disease persists among these weeds year after year, and such weeds serve as a perennial reservoir of mosaic infection for future tomato crops.

7. Mosaic has not been found to any extent occurring spontaneously in *Physalis*, and is present in the weeds only in and near fields once used for tomatoes. As more and more new fields are used for tomatoes, however, the reservoir of mosaic infection in the perennial weed flora will increase each year.

8. Evidence of spread of the disease to *Physalis* plants 200 to 400 feet from tomato fields has been adduced.

9. In a field survey *Physalis* was observed in 65 out of 81 tomato fields, and mosaic was noted on *Physalis* in 35 of these fields, and on both *Physalis* and tomatoes in 29 fields. Tomato mosaic was noted in 60 fields, and in 48 of these *Physalis* was found.

10. In many fields the tomato mosaic was undoubtedly of plant-bed origin. Mosaic was found on tomatoes in plant-beds. *Physalis* is often present in and near plant-beds.

11. Aphids and flea-beetles may play a part in the transmission of mosaic between *Physalis* and tomatoes.

12. *Physalis heterophylla* was found in 7 of the 81 tomato fields examined, and in 3 fields showed mosaic.

13. *Solanum carolinense* was found in 13 of the 81 tomato fields examined, and in one field showed mosaic.

14. The eradication of perennial Solanaceous weeds in and near tomato fields, and particularly the plant-beds early in the season, is recommended as a mosaic control measure.

The writers wish to acknowledge their indebtedness to Professor H. S. JACKSON for helpful suggestions and criticism.

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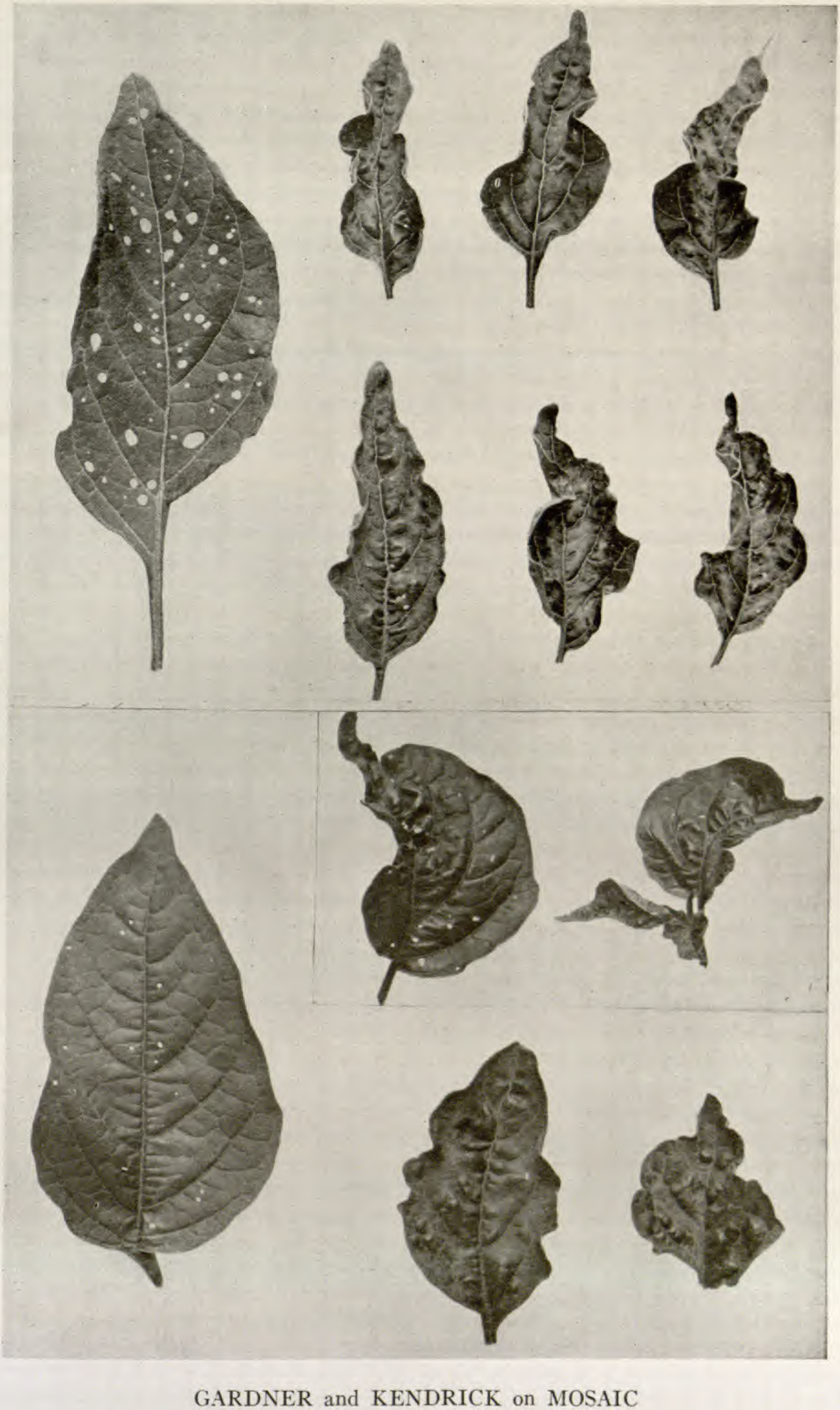

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11. NISHIMURA, MAKOTO, A carrier of the mosaic disease. Bull. Torr. Bot. Club 45:219–233. 1918.
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EXPLANATION OF PLATE XVII

Symptoms of mosaic in *Physalis subglabrata:* at left two leaves from normal plant; at right ten leaves from mosaic plants; small holes in leaves eaten out by flea-beetles.

STROMA AND FORMATION OF PERITHECIA IN HYPOXYLON

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 296

PATSY LUPO

(WITH PLATE XVIII AND SEVEN FIGURES)

Hypoxylon and its allies have been left more or less uninvestigated on account of the coriaceous structure of the stroma and the difficulty of cutting satisfactory thin sections. DE BARY gave a general summary of FUISTING'S researches on *Hypoxylon* and other members of the family, and said that it agreed with *Xylaria polymorpha* in the structure of stroma and the development of asci. He also stated that there appeared in the young coil that is the primordium of the perithecium "a row of broad cells irregularly rolled up and full of protoplasm," called by FUISTING Woronin hyphae. As the perithecium grows, these disappear by gelatinization, and the ascogenous hyphae, the periphyses, and the paraphyses grow out from a subhymenial layer of 6–8 cells that line the perithecium. The whole ascocarp, according to DE BARY, is filled with a mass of paraphyses before the ascogenous hyphae appear at their base and grow up between them.

In recent years only two of the family Xylariaceae have been studied, *Poronia punctata* by DAWSON,[1] and *Xylaria* by BROWN.[2] In that part of his study related to the development of the perithecium, BROWN says that in the center of a tangle of hyphae smaller than the others there are broad cells shorter and richer in protoplasm. These he identifies as Woronin hyphae, and states that they enlarge and probably divide, and then round off to form the large multinucleate ascogonia which usually fall to the bottom of the perithecium and there bud out the ascogenous hyphae. He further

[1] DAWSON, MARIA, On the biology of *Poronia punctata*. Ann. Botany 14: 245–260. 1900.

[2] BROWN, H. B., Studies in the development of *Xylaria*. Ann. Mycologici 11: 1–13. 1913.

states that paraphyses arise by an increased growth from the cells of the inner perithecial wall. The nuclear program, although not clear, is thought to involve an increase in the number of nuclei from the first uninucleate Woronin hyphae, and probably also further division in the ascogenous hyphae. From the comparative size of the nuclei he inferred fusion in the ascus, and this was the only fusion occurring in the life history.

Material and methods

The material used was collected in September from dead beech bark at Sullivan, Ohio, by Professor CHAMBERLAIN, who noticed that it seemed soft when all the other stromata around it were characteristically hard and mature. Specimens were sent to Mrs. FLORA PATTERSON, mycologist at the Bureau of Plant Industry in Washington, and she identified the form as *Hypoxylon coccineum*. It was fixed in chromoacetic acid and stained in haemotoxylin. Some of the material, which had not been satisfactory in safranin, was destained and then run into haemotoxylin; and some of the very young material was counterstained in gold orange. Both of these latter methods gave good results, because they differentiated the fungus cellulose and outlined the hyphae sharply, yet left the nuclei clear black and sharply marked. The sections were cut 2, 3, 4, 5, 8, and 10 μ thick, the younger stages being cut very thin. Whether or not the late fall development of this stroma is usual is not known to the writer.

Description of stroma

In longitudinal section (fig. 1) the stroma of *Hypoxylon* shows a differentiation into four distinct regions that can be seen in thin sections even with the naked eye. These are (1) an innermost central region, round in shape, which under the microscope is marked by a loose arrangement of hyphae emerging from the substratum; (2) a compacted zone above this of large, parallel, mostly empty hyphae that form a dome over the central region and up the main body of the stroma; (3) the perithecial layer of loosely woven hyphae with much interhyphal space and perithecia scattered throughout the region; and (4) the superficial layer which is further differentiated into a line marking off the fruiting zone by

intertwining hyphae running parallel to the stroma and taking a very dark stain, a space occupied by loose hyphae with dense protoplasmic contents, and a bounding surface of close hyphae staining black and doubtless containing the remnants of conidiophores.

In the central region three types of hyphae are distinguishable. The most conspicuous of these (fig. 2) is the one of large long cells

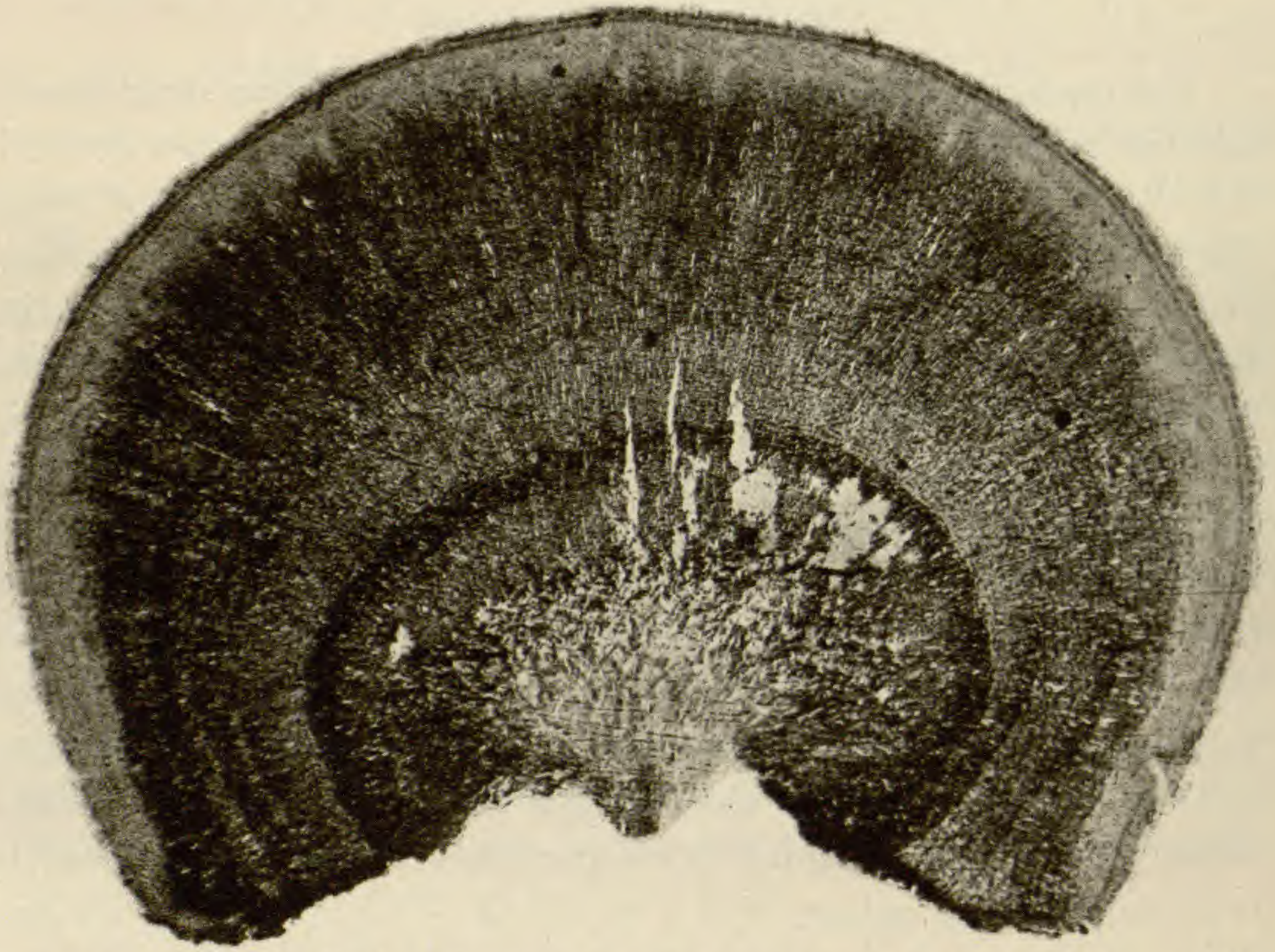
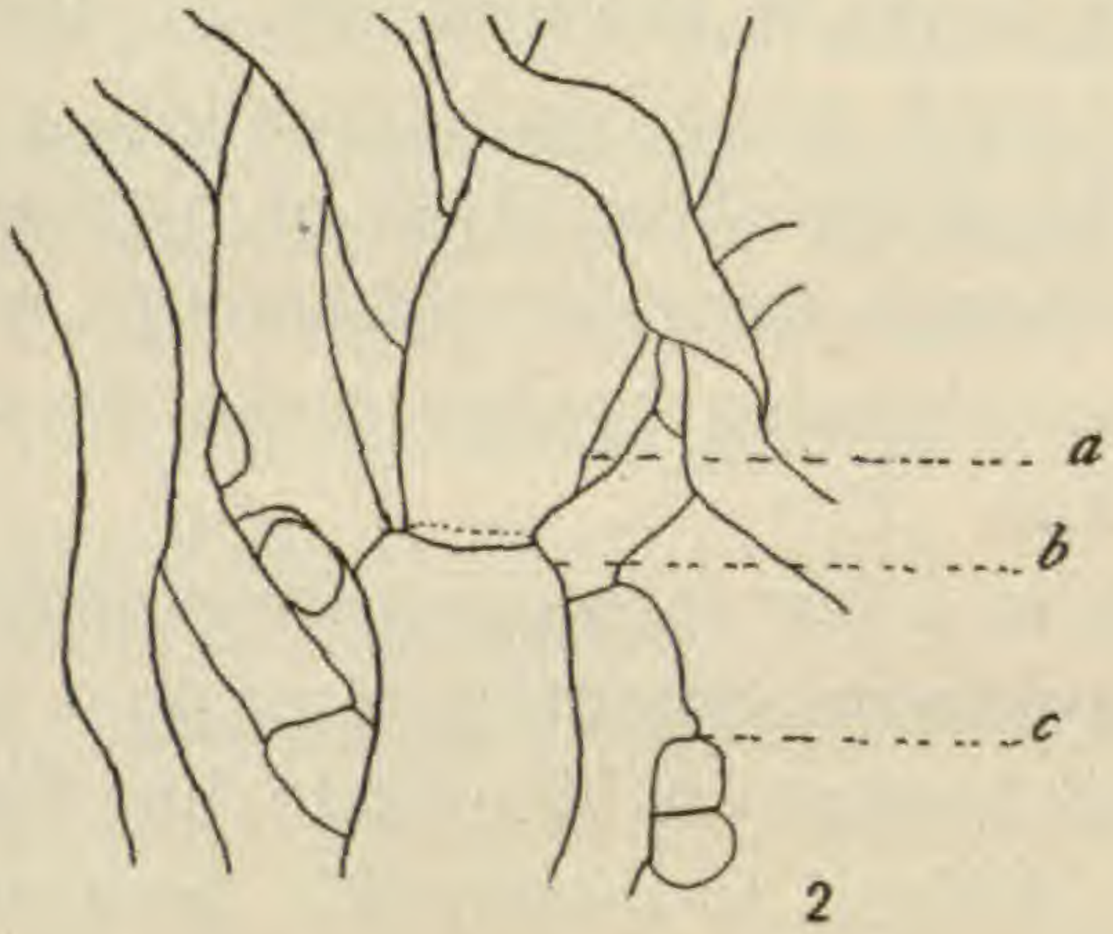


FIG. 1.—Longitudinal section showing borders of the four zones of stroma and the additional markings seen in compact layer of stroma; perithecia visible in fruiting zone; ×26.

staining brown, between which are small hyphae with dense protoplasm, and still smaller ones not staining so densely. These are apparently differentiated from the time of emerging from the substratum into stroma-forming hyphae, into hyphae which form the perithecium, and into those which form the conidiophores and superficial layers. The supporting structure is formed entirely by the largest filaments by means of various devices for interlocking the whole mass and giving to the stroma sufficient firmness, and the same devices are used also in the zone above this. One of the

most frequent devices is that of specially adapted articulation surfaces near the cross-walls (fig. 2*b*), where the cell of another



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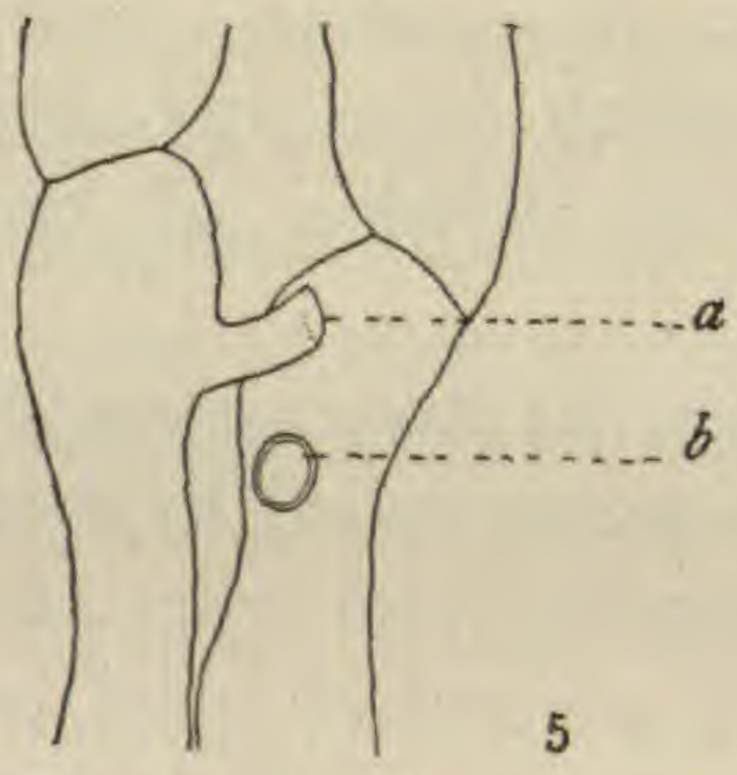

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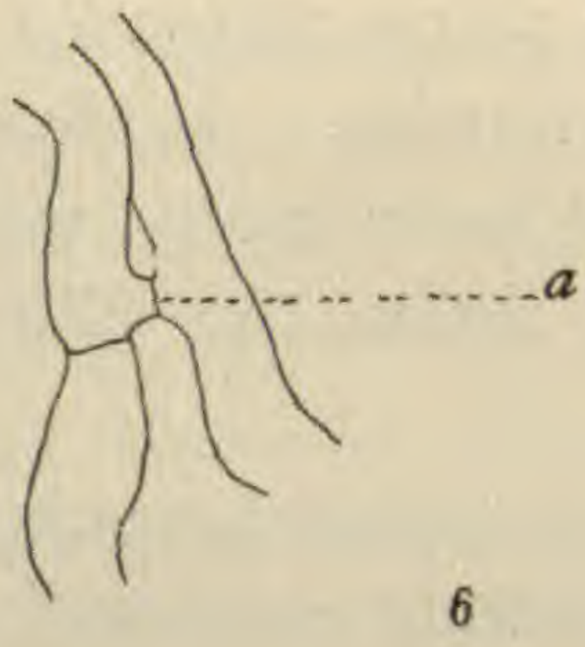

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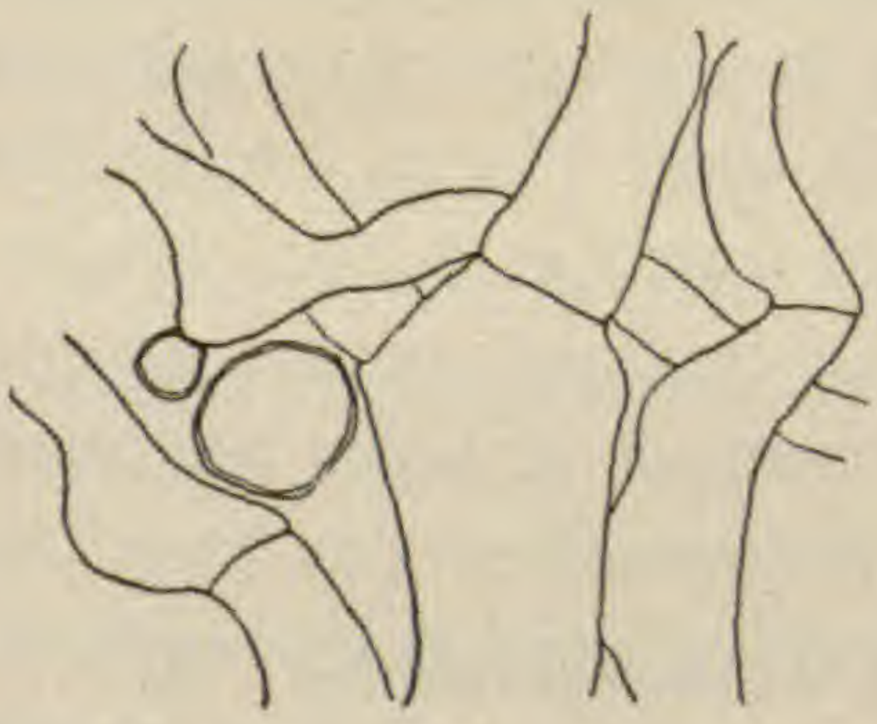

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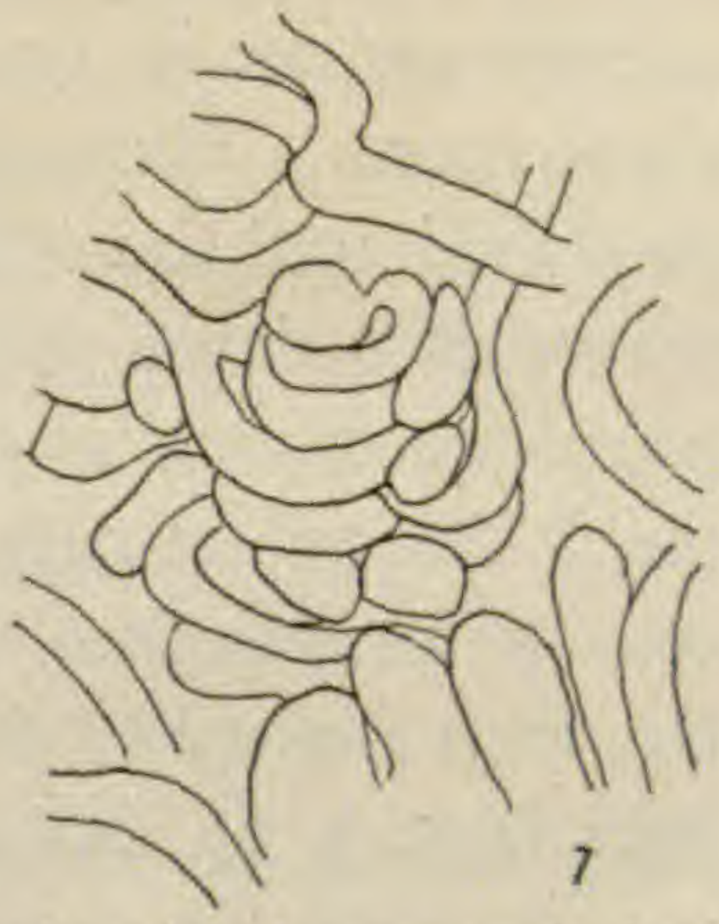

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FIGS. 2–7.—Fig. 2, portion of hyphae from central region showing two principal variations in size and methods of mechanical support; *a*, pit through wall; *b*, articulation surface at septum; *c*, bulbous swellings and depressions fitting together; ×1250; figs. 3, 4, hyphae from same zone showing support gained by twisting and branching; ×1250; figs. 5, 6, interhyphal connections: *a*, bordered hole from sectioning protuberance; ×1250; fig. 7, surface view of early perithecial coil; ×1250.

thread rests against a flat supporting surface for a brace. Another method of support (figs. *2c*, 3) is the interlocking of adjoining hyphae

by bulbous swellings from one cell fitting into corresponding depressions of the next. Sometimes the fungus thread branches dichotomously and forms a rest for another hypha in the fork (fig. 4), and again the two often intertwine and bend around each other (fig. 3). Contrary to expectation, the walls of the three types of hyphae are uniform in thickness, and although irregular thickenings of cellulose in the walls of the stroma-forming hyphae make them uneven, they are not really thicker. Pits (fig. 1*a*) through the walls are abundant, and although in a few rare cases these seem to be open, in the great majority a definite separating membrane is very clear. The cells of the smaller hyphae are binucleate, but the nuclear condition in the larger is difficult to distinguish, because in the few places where the contents are visible they contain dark-staining granules. As definitely as could be distinguished, however, they have many multinucleate cells which have probably developed from the binucleate condition by division of the nuclei.

In the compact zone above the central region the stroma appears to be uniformly composed of the same large empty-celled hyphae already mentioned, which radiate out in parallel rows from the center to the periphery; but if the filaments are spread apart or a cross-section is examined the same differentiation of the three sizes is seen. The walls show the same thickenings and pits already described, and the cells fit into each other in the same way, making a very firm dense pseudoparenchyma. In some places, beside the incurving of cells to each other, they hold together in the corners, much like xylem cells. One peculiarity of this part of the stroma seen in longitudinal section is the large number of holes bordered by walls (fig. 5*b*). These are cut ends of protuberances from the cells (figs. 5, 6), and are one of the methods of mechanical support not found in the previous zone. They are not the technical clamp connections of DE BARY'S description, because they occur not only near the septa, but from any place along the cell to another in the same hypha, and because they cannot be so named until their method of development has been established. Here they are blind tubes that reach from one cell to fit flat against the wall of an adjacent hypha and clamp the two together.

Other peculiarities of this part are the brown mottled appearance of the whole region, due probably to irregular depositing of some substance in the walls, and the further zonation of this portion of the stroma (fig. 1). This last character is one of the peculiar taxonomic features of *Daldinia*, and in *Hypoxylon* is not evident except through the microscope. These parallel markings are due to irregularities in growth that result in a region of short cells bordering a region of long cells, and to the hyphae intertwining more at this point.

On the outer edge of this portion of the stroma the large hyphae end at varying levels, sometimes with a club-shaped enlargement, and do not give a definite line of demarcation. From between these the two types of hyphae rich in protoplasm pass out to form the upper layers, and of these the larger do not pass much beyond the lower part of the perithecial zone, where some of them form the fruiting bodies, and the smaller continue on beyond this to form the three superficial boundaries. It is this situation which indicates that the sizes of these hyphae were differentiations according to function and not accidental variations. In this region of scattered hyphae that form the perithecial layer, and also in the three outer regions, the cells are typically binucleate, although a very few with several nuclei are seen; and this is true also for the hyphae forming the perithecial wall. On the outer surface of the stroma are spherical excrescences like bubbles, and these are related to the interhyphal spaces. They are probably the excretion of some oily substance through the stroma, and it is owing to this that the young stromata feel smooth and slightly greasy to touch.

Formation of perithecia and ascogonia

In the formation of perithecia in *Hypoxylon* the first evidence of their origin is the coiling of hyphal ends or of branches. As already stated, it is the larger of the two protoplasm-filled hyphae differentiated from the substratum that do this, and apparently they do not show any increase in size before or immediately after this stage. Other hyphae of the same size surround these initial coils and form a small circular knot (figs. 7–9), which was the earliest stage BROWN recognized in the development of the perithecia in

Xylaria. From the evidence there is no basis for believing in any earlier differentiation of Woronin hyphae as initiating the coiling process; but they do become differentiated in the center of the coil by an increase in size very soon after the perithecial primordia are well started (fig. 10). Growth in the size of the perithecium is accomplished by increase in the length of the wall hyphae, and also by the addition of other hyphae around the outside. With this increase in size the wall layers become thinner and compact, and some of the inner hyphae decrease in size and become absorbed, probably furnishing nourishment for the fertile branches (figs. 10, 11*d*). A large number of perithecia start but few mature, and these, logically in relation to their food supply, are mostly toward the inner line of the perithecial zone. The others remain intact and are scattered throughout the fruiting region, apparently inhibited from further growth at any stage in their development, and remaining without change at that stage. As an exception to this, some of the larger perithecia that have reached the point where they contain ascogonia and then become abortive show signs of deliquescing and disintegrating. As a rule the older perithecia are toward the top of the stroma and the younger stages are down toward the substratum.

Within the perithecium the filament in the center develops into the Woronin hypha (fig. 10). It increases in length, and in size and number of cells; and after this some of the cells round out, increase in size, and eventually separate from each other to form the ascogonia. The enlargement is not uniform as to the size and shape attained, for these ascogonia (fig. 11*b*) are many of them uneven and contorted in outline, and many of them retain narrow stalklike connections with cells from which they have not become completely separated.

The nuclear condition in the Woronin hyphae and ascogonia is the critical point, and is hard to determine because of the extreme variations in the size of the nuclei and the difficulty of determining successive stages. As said before, the cells of the Woronin hyphae are binucleate (fig. 9), and as they enlarge (fig. 10) they show a steady and marked increase in the size of the nuclei from the time the knot is well formed. There are one or two divisions, after

which they increase their size, and at the time of the rounding out of the ascogonia there are four, sometimes more, large nuclei five or six times the size of the originals. As the ascogonia increase to the enormous odd shapes found in the more mature perithecia, the nuclei show great variation in size and number (fig. 11). During this stage they undergo rapid division without maintaining their size, and in the ascogonia which show evidences of budding out the ascogenous hyphae they are small and number sixteen or more in a section. Evidence for this interpretation of the nuclear program is gained also from the perithecial wall, because as it grows more mature in character these changes in size and number are unmistakable. There is no evidence of fusion in any stage of the development of the ascogonia. No mitotic figures were visible, but in many cases the position of the two nuclei was such as to suggest late division.

The ascogonia do not drop to the bottom of the perithecium before germinating. Instead the increase in size of the wall is toward the periphery of the stroma, and the ascogonia, although in the same actual position, seem to have dropped because of the expansion of the perithecial wall toward the surface.

Ascogenous hyphae

The material used was just at the stage when the ascogenous hyphae were beginning to bud out, and no detail about the formation of these can be given. Great care was necessary not to confuse some of the stalk connections where separation was incomplete with ascogenous hyphae. These could be recognized by their direction and evident connection with some part of the filament traced through serial sections. Legitimate cases of buds (figs. 12, 13) just formed were found, however, and although of course the nuclear situation, separation, and branching could not be determined, apparently the procedure would be the same as described for other forms where the nuclei migrate into the buds from the ascogonia, and further division takes place there.

A supposition lacking evidence to support it is that the paraphyses described by De Bary and Brown might be formed from these early ascogenous hyphae or branches of them instead of

from a subhymenial layer. This seems reasonable, since at this stage the compact wall of the perithecium is an unlikely place for new growth, and there is no evidence of new hyphae being found to account for the paraphyses in other forms. It seems very probable that this is their origin here.

Summary

1. The stroma is differentiated into four regions, and in one of these further zonation is evident.

2. The firm structure of the stroma is gained by various mechanical devices for support, such as tubular extensions from cells, branching and intertwining of hyphae, and special articulation surfaces.

3. The hyphae are differentiated into three types from the time of their emergence from the substratum: those that form the major part of the stroma, those that form the perithecia and Woronin hyphae, and those that form the superficial layers and probably the conidiophores.

4. The cells of the hyphae are originally binucleate, but may become multinucleate.

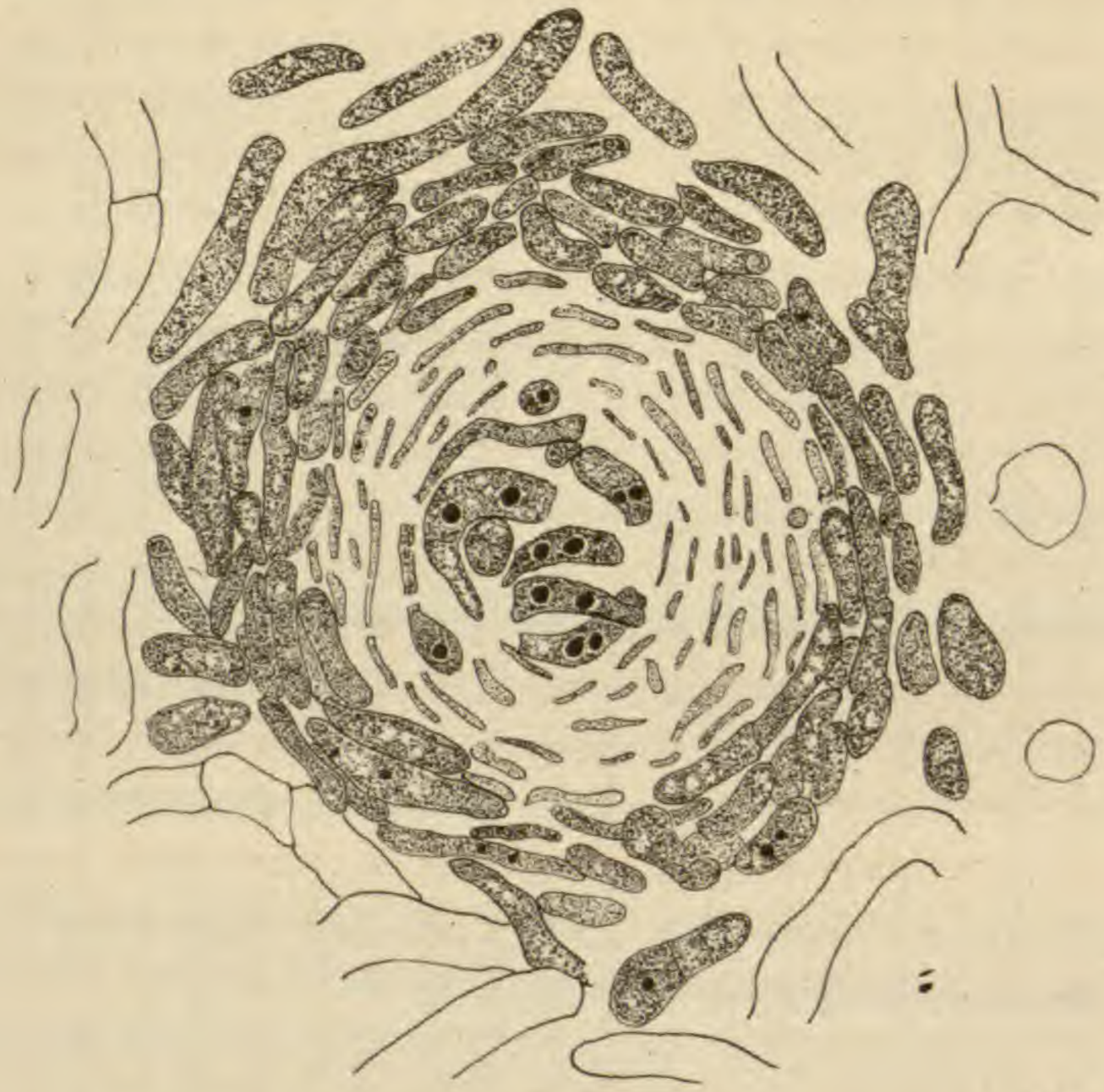
5. The formation of the perithecia is initiated by the massing of the hyphae into a circular knot, within the center of which the Woronin hyphae differentiate.

6. The ascogonia develop from the cells of the Woronin hyphae by rounding out, partially separating from each other, and increasing in size.

7. The ascogonia do not drop to the bottom of the perithecium in the older stages, but come to lie comparatively closer to the bottom by an expansion of the perithecial wall toward the periphery of the stroma.

8. The nuclear program within the ascogonia is one of few divisions and great increase in size, up to the stage where the ascogonia are well rounded out, and then of rapid division without the maintenance of size.

9. The ascogonium buds out protuberances that are the beginnings of the ascogenous hyphae.



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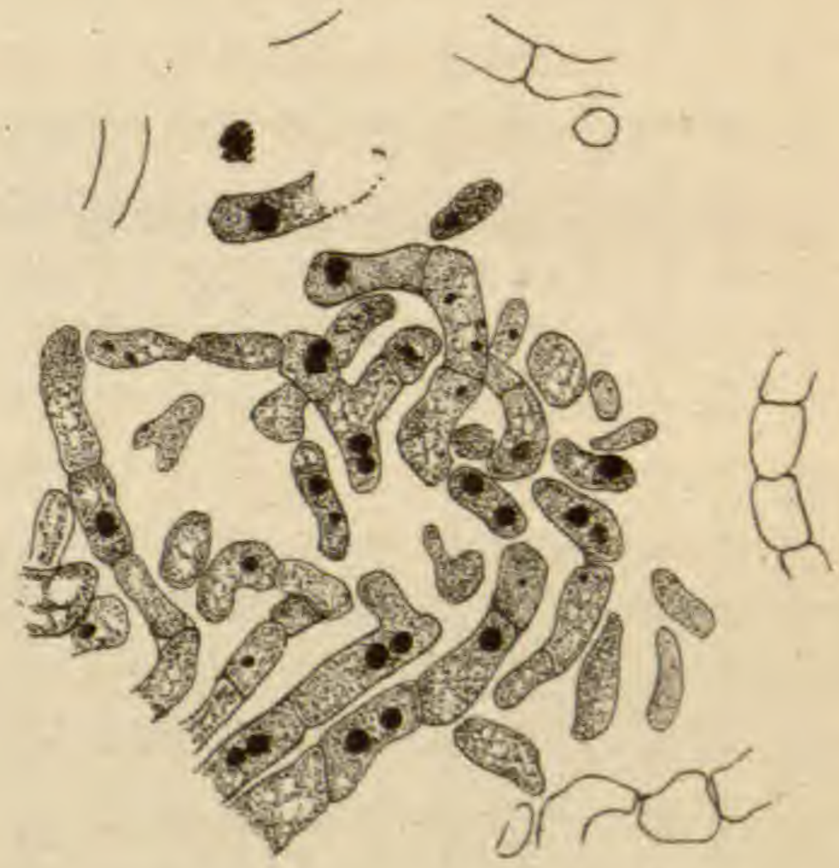

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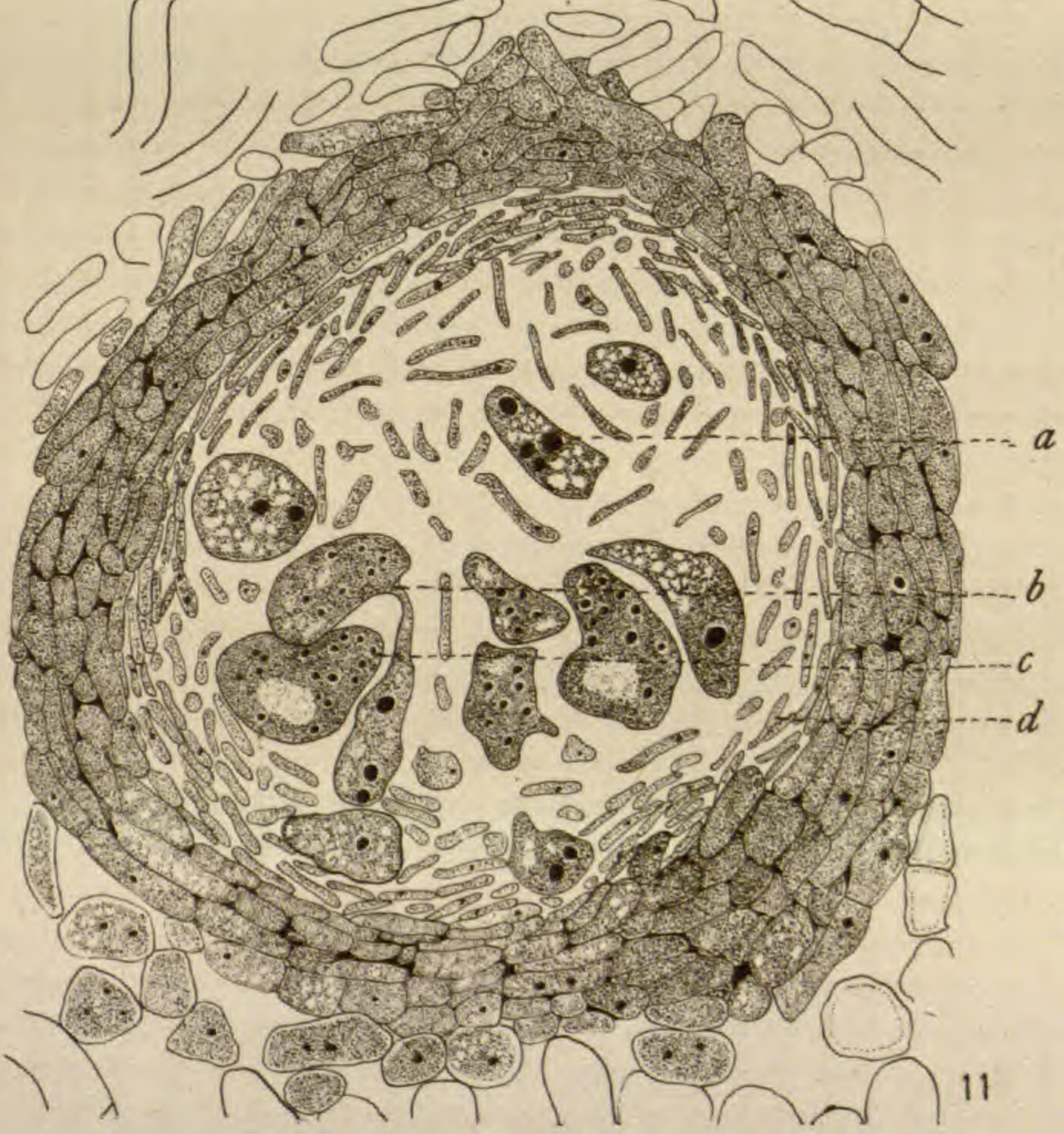

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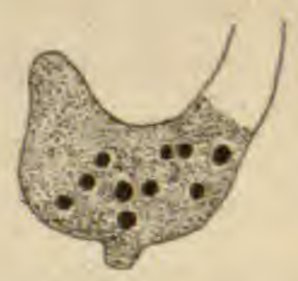

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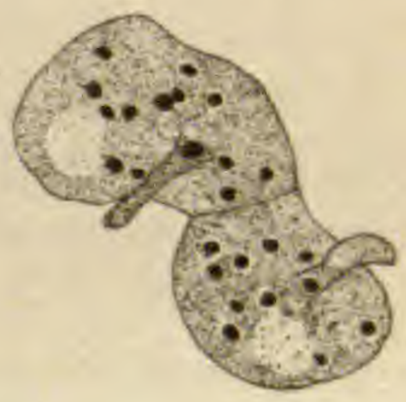

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LUPO on HYPOXYLON

The writer wishes to express appreciation and thanks to Professor C. J. CHAMBERLAIN, under whose direction the study was made; and to Mrs. FLORA PATTERSON for identifying the material.

ROCKFORD COLLEGE
ROCKFORD, ILL.

EXPLANATION OF PLATE XVIII

FIG. 8.—Early stage of coil in section, showing nuclei already increased in size; ×1375.

FIG. 9.—Another view of approximately same stage, including several cells with nuclei of original size; ×1562.

FIG. 10.—Later stage of perithecium with wall yet loosely formed: large nucleated Woronin hyphae in center of perithecium; ×1562.

FIG. 11.—Still later stage with compacted wall and growth toward periphery started so that ascogonia lie relatively nearer bottom: *a*, large nucleated ascogonium; *b*, ascogonia of irregular shape and stalklike connection left with what was original coil; *c*, multinucleate mature ascogonium; *d*, hyphae decreasing in size and disintegrating; ×1562.

FIGS. 12*a*, 13.—Beginning stages in budding of ascogonia to form ascogenous hyphae; ×1562.

CURRENT LITERATURE

NOTES FOR STUDENTS

Quantitative relations of regeneration.—REED[1] has contributed a new quantitative study of growth in pruned trees as compared with unpruned, with particular reference to such questions as whether the growth response is merely the restoration of lost parts, in what manner the amount of regeneration is correlated with length, and number of buds of the mother shoot; also what light these relations afford in the problem of the dominance of the shoot apex. He finds no correlation between the length of mother shoots and the amount of new growth produced by them, whether pruned or unpruned. On the other hand, shoots pruned the previous winter produced about 65 per cent more total growth than comparable unpruned shoots; the degree of heading back had little effect on the amount. Hence, regeneration following pruning is not a tendency to restore lost parts, but in terms of growth rate of the shoot is taken to indicate increased activity of the growth catalyst. In connection with the characteristically greater growth of laterals at the distal end of a pruned shoot, the conclusion is reached that this greater activity results from the elimination of the growth-inhibiting chalones produced in the apical part of the shoot which normally keep the lateral buds in dormancy. The alternative hypothesis that pruning reduces the number of potential growing points, hence permits of greater development of the remaining buds, is rejected because it was found that the more heavily pruned mother shoots, that is, the shoots in which the number of remaining buds was least, failed to make more total lateral growth than the longer mother shoots. It is worthy of note that according to the author's hypothesis of basipetal migration of a growth-inhibiting substance, the greatest concentration, and hence the most pronounced inhibiting effect, is at the base of the shoot. When a mother shoot is pruned to a few basal buds, however, the laterals developing from such buds become longer than laterals of less severely pruned shoots. This would seem to be evidence indicating some effect from reduction of competition between the several buds.

LOEB[2] reaches a somewhat different conclusion from experiments on regeneration in *Bryophyllum*, in which the weight of shoots produced from the

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[1] REED, H. S., Correlation and growth in the branches of young pear trees. Jour. Agric. Res. **21**:849–876. *pl. 1*. 1921.

[2] LOEB, JACQUES, The quantitative basis of the polar character or regeneration in *Bryophyllum*. Science **54**:521–522. 1921.

apical buds of an intact defoliated stem was compared with the total weight of shoots produced from similar stems divided into portions of one node each. From the approximate equivalence of these quantities he concluded that "the polar character of the regeneration of shoots is due to the fact that all the material available for growth reaches the apical and none of the other nodes of a long piece of stem." The relation of this conclusion to LOEB's well known earlier work on regeneration in *Bryophyllum* is not quite clear to the reviewer, since nothing appears here concerning the effect of a growth-inhibiting hormone by which is to be explained the dominance of the apical and the inhibition of the lateral buds.

Although the reviewer is aware that the two cases are not strictly comparable, it would seem that if inhibition of lateral buds is the effect of a basipetally migrating growth-inhibiting substance, the maximum effect of "pruning" would result from complete isolation of each bud from the deleterious effects of its neighbors, as LOEB was able to do in *Bryophyllum*, and as was impossible in REED'S experiments with the pear. Yet the experiment with *Bryophyllum* resulted in the production of a greater amount of new growth from the intact than from the divided stem.

The arguments in support of the several theories to account for the dominance of the apical portion of a shoot have been reviewed recently by CHILD,[3] and several objections to the hypothesis of growth-inhibiting hormones pointed out. It would appear that further progress toward the solution of this problem must wait on more adequate information concerning the anatomical relations of apical and lateral buds, and of tissue changes in regeneration. For instance, the transport efficiency of the vascular supply to the apical as compared with lateral buds does not appear to have been adequately investigated. The relative age of the terminal as compared with other buds is important in this connection. In shoots with the indeterminate growth habit the apical bud may establish its dominant rôle in the axial gradient, which it thereafter retains, in the judgment of CHILD, by virtue of its priority. In shoots of limited apical growth different relations may obtain. Again, if inhibition of a growing tip is a transmission through protoplasmic connections rather than the physical transportation of a substance through the vascular tracts, as CHILD holds on the basis of the behavior of simple animals and non-vascular plants, evidence of cytoplasmic cell connections should be sought in such active portions of the shoot. The effect of callus deposition in the sieve tubes and of changes in the phloem (both sieve tubes and parenchyma) toward lignification should be determined in relation to a possible gradient in transport efficiency from apex to base. The coordination of histological and physiological studies in this problem is greatly to be desired.—FREEMAN WEISS.

The rapprochement in ecology.—A notable feature in the development of ecology has been the marked divergence between the American and Continental

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[3] Amer. Jour. Bot. 8: 286–295. 1921.

European schools. Continental ecology, developing naturally and by degrees from the older study of floristic plant geography, has retained much of the taxonomic standpoint, and methods for distinguishing, classifying, and arranging vegetational units according to their physiognomy have been very highly developed. In America, on the other hand, and latterly also in the British commonwealths, the tendency has been much more to emphasize the dynamic and genetic aspects, especially under the leadership of COWLES, in the development of the successional idea. The quadrat method of POUND and CLEMENTS, to be sure, was a notable contribution to physiognomic ecology, but CLEMENTS became an early convert to the successional doctrine, and has been one of its most voluminous exponents. Thus the Anglo-Americans and the rest of the world have come to present united and opposing fronts, and the situation has become sufficiently tense to induce the remark from ROMELL,[4] "Entre divers partis des phytogéographes la divergence grandit, on pourrait déjà dire la lutte s'enflamme." The first part of his statement is true enough, and the latter part could easily have become so, if the one side had been as willing to accept battle as the other was to offer it. It goes without saying that such a situation is a most regrettable one for the science of ecology. It is encouraging therefore to note, in a group of recent Continental papers in ecology,[5] a markedly increased toleration of the successional-dynamic point of view, as well as indications that physiognomic methods may yet be developed that will not seem too metaphysical in conception and too unwieldy in practice for the American temperament.

RÜBEL'S three papers are especially valuable as a comprehensive and highly condensed summary of the progress of ecology. He traces the early growth of the science, from the vague first beginnings with THEOPHRASTUS and the more or less disconnected comment of early modern taxonomic botanists, through its evolution as a subspecies of phytogeography, to its present and independent development. The history of the numerous efforts to arrive

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[4] ROMELL, LARS-GUNNAR, Physionomistique et écologie raisonnée. Svensk Botanisk Tidskrift **14**:136–146. 1920.

[5] RÜBEL, EDUARD, Anfänge und Ziele der Geobotanik. Vierteljahrsschr. Naturf. Gesells. Zürich **62**:629–650. 1917.

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BRAUN-BLANQUET, J., Prinzipien einer Systematik der Pflanzengesellschaften auf floristischer Grundlage. Jahrb. St. Gallischen Naturw. Gesells. **57**:305–351. 1921.

FITTING, HANS, Aufgaben und Ziele einer vergleichenden Physiologie auf geographischer Grundlage. pp. 42. Jena. 1922.

PALMGREN, ALVAR, Die Entfernung als pflanzengeographischer Faktor. Acta Soc. Fauna et Flora Fennica. **49**:no. 1. 1921.

at some satisfactory method for quantitative estimation of vegetational values is of particular interest. The methods of the earlier workers were heterogenous and often confused. Later methods gained in the matter of clarity of ideas, but were highly complicated and exceedingly laborious. Present day effort is toward simplification, and in this the work of RAUNKIAER and BRAUN-BLANQUET is perhaps most promising. The latter's paper, in addition to the effort toward clarification and simplicity, just mentioned, is notable for its proposal to classify his plants also according to their dynamic value, that is, in American terminology their successional value. He proposes a series of five valuations to be applied to the species in any given association, together with symbols for their convenient designation. His class names, with approximate English equivalents, are aufbauend (constructive), erhaltend (maintaining), festigend (consolidating), neutral (neutral), abbauend, zerstörend (disruptive). The idea of progressive and regressive succession is thus clearly postulated.

The ecological implications in FITTING'S contribution are all the more valid in that they are not directly intended. The author is a physiologist, and his primary concern is the avoidance of the artificial and abnormal conditions imposed on his material by the greenhouse methods of ordinary laboratory practice. He contends that the physiology of plants should be studied where the plants naturally occur, and insists on the study of climatic, edaphic, and biotic factors as they affect the space actually occupied by the plant in the field. This, of course, coincides with the activities of the younger American ecologists who are carrying physiology out of doors.

PALMGREN'S study is one of migration, and thus implies the successional viewpoint throughout; it is the more noteworthy in that it was conducted on the Aland archipelago, almost within sight of Uppsala. These islands, he estimates, have not been emerged for more than 3500 years; moreover, they are high-boreal in position, so that an ecologist with the successional viewpoint can extract much aid and comfort from his conclusions.

All this is gratifying to Americans, but as yet there is not much indication of reciprocity on our part. FULLER and BAKKE anticipated the German publication of RAUNKIAER in making his ideas accessible to the English-reading public, but little or nothing has been done in this country with his methods. Probably most American ecologists feel that still further simplification is needed. RAUNKIAER himself admits that his methods involve a good deal of labor. Then there is also the element of time; it generally takes about two graduate "generations" to establish a new idea.—FRANK THONE.

Stomatal regulation.—Using LLOYD'S methods of studying stomata, which he thinks have been criticized without sufficient reason, LOFTFIELD[6] has made

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[6] LOFTFIELD, J. V. G., The behavior of stomata. Carnegie Publ. no. 314. pp. 104. 1921.

an extensive study of diurnal stomatal changes, the influence of physical factors on the opening and closing of stomata, and the effects of these stomatal movements on transpiration. The main observations were made on alfalfa, potato, sugar beet, onions, and cereals, but some sixty species in all have been examined. He finds three types of stomatal behavior: the cereal type, typified by barley; the thin-leaved mesophyte type, typified by alfalfa; and the fleshy-leaved type (not confined however to plants with fleshy leaves), typified by such plants as the potato, cow beet, and onion; each of these has a different closure reaction to extreme conditions. The cereals show no opening of stomata at night, no matter how slight the opening by day has been. The thin-leaved mesophytes have the stomata usually open by day and closed at night, but under extreme conditions show a closure during the middle of the day, correlated with an opening at night. The thick-leaved plants behave much as marsh plants do, having their stomata open day and night when water content is high and evaporation low, and showing a tendency to close only when the evaporating capacity of the air is high.

Many details regarding the effects of environmental changes, such as light intensity, temperature, evaporation, wind flow, water content of soil, leaf turgor, and habits of growth on stomatal behavior are presented, from which one may draw the general conclusion that stomata are sensitive to environmental conditions, particularly to light, and to factors that reduce the water content of the leaf, and that they open and close as conditions necessitate. The evaporation studies indicate that atmometers and potometers do not measure accurately the total effect of evaporation factors upon plants, a result that is not surprising.

The final section on the effect of stomatal movement upon transpiration throws much light on the mooted question as to whether stomata exert a regulatory function in transpiration. LLOYD had concluded in 1908 that stomata have no regulatory function, a conclusion which was rendered doubtful by several investigators, including ILJIN, who studied the transpiration of mesophytes and xerophytes in ravines and on the Russian steppes, and who found marked evidences of regulation of transpiration. This work by LOFTFIELD seems to settle the question definitely in favor of stomatal regulation, particularly when the apertures are nearly closed. As long as the apertures are more than 50 per cent open, the transpirational water loss is controlled by evaporation factors alone, but with closure almost complete, the stomata regulate very closely the water loss from the plant. The paper is beautifully illustrated with plates showing photomicrographs of stomata.—C. A. SHULL.

Anthocyan pigments.—NOACK[7] has found rhamnose-free flavonol diglucosides to be much more abundant in green leaves than has generally been supposed. In such leaves as he studied he was able to establish the existence

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[7] NOACK, K., Zeitschr. Botanik **14**:1–74. 1922.

of flavonol-anthocyan in couples of similar glucosidal nature, but secured no evidence of aromatic nucleus composition in such cases. He suggests that these couples function as a reduction mechanism during photosynthesis.

WHELDALE[8] had already indicated reasons for believing anthocyan formation associated with photosynthetic defect. NOACK's work strengthens this view. He finds fluorescent media in the presence of light capable of oxidizing anthocyanins to flavonols, and a general inverse relation between chloroplast integrity and anthocyan content. Especially has he shown that absence of carbon dioxide will cause anthocyanin formation in sunlight, and that engorgement with sugar probably acts indirectly (he thinks by disturbing the photosynthetic mechanism) in producing anthocyanins. That there are serious gaps in his work he has been the first to admit. He has, however, given added dignity to a point of view which comes surprisingly close to harmony with much of our knowledge. The action of narcotics, low temperatures, high insolation, and ultraviolet light, nitrogen and phosphorus starvation, as well as extremes of youth and senescence in photosynthetic organs, certainly fall in line with such a conception in a most disarming way.

The formation of anthocyanins by reduction from flavonols, as well as WILLSTÄTTER'S[9] general scheme for color change, are elucidated by EVEREST and HALL[10] in a trenchant reply to the paper of SHIBATA, SHIBATA, and KASIWAGI.[11] It will be recalled that the latter workers attribute color conditions to metal organic or complex compounds of reduced flavonol glucosides, rather than to alkaline phenolic salts, free stages, and red oxonium salts of anthocyanins. In the present paper strong evidence is adduced that this contention is based upon work with supposedly pure substances which were actually mixtures, and the resultant analyses are pronounced of no value. It is interesting to note that EVEREST and HALL now believe blue flower pigments to be of two general types, the alkali phenolic salt of anthocyan, which polymerizes to colorless on standing, and the iron double salt, stable in dilute solution. They have also demonstrated the existence of flavone substances in a number of very young buds previous to anthocyan formation, but have failed to find anthocyans preceding flavone formation in flavone-holding organs.

There can be little doubt that botanists are now ready to look with the keenest interest toward precise chemical comparisons of flavone and anthocyan pigments wherever the two are found associated, whether simultaneously or in sequence.—PAUL B. SEARS.

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[8] WHELDALE, M., The anthocyan pigments of plants. 1916 (p. 81).

[9] WILLSTÄTTER, R., and EVEREST, A. E., Liebig's Ann. Chem. **401**:189–232. 1913.

[10] EVEREST, A. E., and HALL, A. J., Proc. Roy Soc. B **92**:150–162. 1921.

[11] SHIBATA, SHIBATA, and KASIWAGI, Jour. Amer. Chem. Soc. **41**:208. 1919.

Ecology of heather.—One of the most commendable features of modern ecology is seen in the present tendency to focus attention on some particular plant which is examined as to its responses and limitations. A notable example of this type of investigation is seen in Miss RAYNER'S studies of *Calluna vulgaris*. In a preliminary paper[12] she pointed out that while the plant has been regarded as a typical calciphobe, it occurs in sharply defined communities on the chalk downs in Wiltshire and Berkshire, and appears able to compete with the vegetation characteristic of the downs. According to this investigation the heather communities appeared limited to a heavy rich loamy soil relatively high in magnesium content and neutral in reaction. Beyond these communities there was a poor chalk soil with 40 per cent of calcium carbonate.

An examination of the germination and seedling habits of *Calluna*[13] showed that the seedlings developed normally upon the soil from the heather areas and abnormally upon the chalk soil. Upon the latter the germination was reduced and retarded, the development of the root and shoot arrested, and the leaves were small in size and red in color. The seedlings were found to be infected with a mycorhizal fungus shortly after germination, the mycelium coming from the seed coat which seems to have been infected while still in the ovary. Seed can be sterilized with no effect upon germination, but in the absence of infection complete arrest of root formation occurs, showing the relation of *Calluna* to the root fungus to be obligate.

A further examination of this symbiosis,[14] already noted in this journal,[15] proved even more conclusively its obligate character and the absence of root development in sterile cultures. The fungus was found to be present in all parts of the plant infecting the testa, but not the embryo and endosperm of the seed. The fungus was isolated, grown in pure cultures, and sterile seedlings were inoculated with resulting normal development.

In the most recent contribution Miss RAYNER[16] has succeeded in demonstrating by experimental cultures that *Calluna vulgaris* will not grow on calcareous soils because of an inimical factor, chemical in nature, present in such soils. The exact chemical character of this factor is as yet unknown, but it seems probable that it is effective by altering the infectibility of the root cells of the seedlings and their relations with the mycelium after infection. It does not seem to affect the fungus when growing outside the plant.—GEO. D. FULLER.

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[12] RAYNER, M. C., and JONES, W. N., Preliminary observations on the ecology of *Calluna vulgaris* on the Wiltshire and Berkshire Downs. New Phytol. **10**:227–240. *figs. 2.* 1911.

[13] ———, The ecology of *Calluna vulgaris*. New Phytol. **12**:59–76. *pl. 1. figs. 2.* 1913.

[14] ———, Obligate symbiosis in *Calluna vulgaris*. Ann. Botany **29**:97–153. *pl. 6. figs. 4.* 1915.

[15] BOT. GAZ. **60**:166. 1915.

[16] RAYNER, M. C., The ecology of *Calluna vulgaris*. II. The calcifuge habit. Jour. Ecol. **9**:60–74. *pl. 1.* 1921.

Cytology of Neottia Nidus-avis.—A paper by MODILEVSKI,[17] dated 1918, has just reached this country from Kiev, Russia. The paper is in Russian, but has a rather complete summary and an explanation of figures in English. The cytological part deals principally with the behavior of the chromatin during the two reduction divisions in oogenesis. Particular attention was given to the character of the spirem thread, and the conclusion was reached that no true double structure is present either before synapsis, during synapsis, or immediately after, although in rare cases a parallel orientation could be seen in the late spirem stage. Before the diakinesis stage is reached, a double character is easily observed, and eighteen bivalent chromosomes are formed, some of which are larger than others. One of the bivalents is much longer than the rest, and is conspicuous during the subsequent stages of division. MODILEVSKI believes that in structure the chromosomes are masses of threads, and that there is no vacuolization, like that described by GREGOIRE and many others. Besides the long chromosome, there are three others which are morphologically different from the remaining fourteen chromosomes; however, he does not seem to think that this situation has any serious value for theoretical considerations. Reduction was also studied in the pollen mother cell.

Some attention is given to the nucleolus, which he thinks consists of two distinct morphological and chemical constituents. One element is the permanent nucleolus, which stains with iron-haematoxylin, and is identical with the nucleolus of somatic nuclei. The second has the shape of a sickle and rests upon the other like a cap. It stains like chromatin. These two kinds of nucleoli always appear during late synapsis in *Neottia Nidus-avis*. During the two reduction divisions of the megaspore mother cell no walls are formed, and all four megaspores take part in the development of the embryo sac. The two antipodal nuclei do not divide again, but the other two enlarge and divide, so that there are four nuclei at the micropylar end of the sac. They develop a typical egg apparatus and a polar nucleus. One of the male nuclei fuses with the egg nucleus and the other with the micropylar polar nucleus. As the young embryo develops, four free nuclei are found in the embryo sac, one of them a synergid nucleus, the two antipodal megaspore nuclei, and the nucleus formed by the fusion of a sperm with the micropylar polar nucleus. There is no free nuclear division or any formation of endosperm.—C. J. CHAMBERLAIN.

Sporidial infection in Puccinia graminis.—A recent contribution to the series of studies in the physiology of parasitism emanating from the Imperial College of Science and Technology (London) is by WATERHOUSE,[18] describing

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[17] MODILEVSKI, J., Cytological and embryological studies on *Neottia Nidus-avis*. pp. 55. *pls. 1, 2.* 1918.

[18] WATERHOUSE, W. L., Studies in the physiology of parasitism. VII. Infection of *Berberis vulgaris* by sporidia of *Puccinia graminis*. Ann. Botany **35**:557–564. *figs. 19.* 1921.

host penetration by the sporidial germ tube of the cereal stem rust fungus. Although ERIKSSON studied sporidial infection in the mallow rust, concluding that penetration is directly through the epidermal cell wall and never through stomata, no careful study of the mechanism of entry of the sporidial germ tube has previously been made. In the present account penetration is shown to result from mechanical action alone, the structures concerned in the process being a mucilaginous investment of the germ tube and a fine style-like infection hypha, originating either from the germ tube or the sporidium directly. The entry of the parasite at first causes no visible alteration of the host cell contents. This manner of parasitic entry, that is, in the absence of visible chemical softening processes of the cuticle, is similar to that previously reported for the infection hypha of *Botrytis* and *Colletotrichum*, and for the zoospore of *Synchytrium* (CURTIS). A new interest is thereby given to studies of disease resistant or disease escaping plants directed toward the mechanical properties of the cuticle and cell wall. Evidence that resistance to infection of potato by *Pythium debaryanum* is of this type has already been presented (HAWKINS and HARVEY); similarly for resistance in the tomato to infection by *Macrosporium tomato* (SANDO and ROSENBAUM).

The question is pertinent whether the resistance or immunity shown by different species of *Berberis* to infection by *Puccinia graminis* is due to mechanical exclusion of the germ tube by a heavy cuticle. There is some evidence that this may be true for the evergreen thick leaved species of *Berberis* generally referred to *Mahonia* or *Odostemon*. Greenhouse inoculations with several forms of *Puccinia graminis* have resulted in infection of very young leaves of *Berberis trifoliolata*, *B. Fremontii*, and the tall form of *B. Aquifolium=Odostemon Nutkanus* (DC) Rydb., although on the last named host only abortive pycnia and no aecia developed. Some other factor appears to be concerned in the immunity of *Berberis Thunbergii* to cereal stem rust, since this plant has soft, thin leaves which lack a well developed cuticle.—FREEMAN WEISS.

Further studies on Tmesipteris.—The life history of the Psilotales is becoming as well known as that of more accessible lycopods through the continued researches of HOLLOWAY,[19] who has published a second paper on the prothallus of *Tmesipteris*, containing additional observations made possible by the finding of more than 200 additional prothallia. *Tmesipteris* and *Psilotum* both have sporelings which resemble their gametophytes. This similarity is not considered by HOLLOWAY as being sufficient evidence for the primitiveness of the Psilotaceae; but he points out that this close correspondence is not found in the life history of other modern Pteridophytes. This resemblance between the two generations, the superficial position of the sex organs, the persistent single apical cell of the prothallus, the dichotomous

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[19] HOLLOWAY, J. E., Further studies on the prothallus, embryo, and young sporophyte of *Tmesipteris*. Trans. New Zealand Inst. **53**:386–422. 1921.

branching, the absence of a primary tubercle, and the lack of differentiated tissue "may all be urged as more or less primitive features." The absence of a suspensor the author thinks may be compensated for by the haustorial protuberances of the foot. The same foot structure, however, also occurs in certain species of *Lycopodium* which do develop a suspensor. The absence of the suspensor is also counted as a primitive feature.

In discussing the significance of its embryogeny, which is "the simplest among existing pteridophytes," the author states: "While not suggesting that *Tmesipteris* has actually been derived from the *Anthoceros* cycle of affinity, it is clear that the absence from the former of any such organs as root or cotyledon suggests that they approximate in so far as they both represent primitive lines of development. That the simplicity of *Tmesipteris* is not due to reduction is a belief which has greatly been strengthened by the discovery of the rootless and leafless Rhyniaceae. The embryogeny of *Tmesipteris* as described in the present paper makes more clear-cut the theory of the origin of the sporophyte of the Pteridophyta from an *Anthoceros*-like sporangium. . . . . The only new feature to be postulated here is the extension in length of the shoot from an apical meristem instead of, as in *Anthoceros*, from an indefinite basal meristem, and the initial cause of the shoot-elongations might be set down as being the adoption of a subterranean mode of life by the gametophyte."—E. A. SPESSARD.

Life cycles of bacteria.—LÖHNIS[20] has published a comprehensive survey of the literature dealing with cell forms of bacteria and their significance in relation to the life history of these organisms. The discussion is amply illustrated with over 40 plates containing nearly 400 figures.

The first section of the monograph contains a discussion of cell forms. The author attempts to refute the monomorphistic doctrine of COHN, KOCH, and their followers. It is shown that many bacteria, possibly all, are pleomorphic, and that the varying cell forms often referred to as "involution" or "degeneration" forms are really different stages in the life cycles of bacteria. It is admitted, however, that our knowledge concerning the relationships of these forms is all too meager.

In the second section reproductive organs are discussed. These are gonidia, regenerative bodies, exospores and endospores, arthrospores, and microcysts. Of these the gonidia and regenerative bodies appear to take the most active part in reproduction, while the other organs may represent resting forms. It is claimed that gonidia are common to all bacteria. The fact that they have not always been observed may be due to their small size and high motility. Regenerative bodies may be of any shape, and are produced either by the vegetative cell or the "symplasm."

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[20] LÖHNIS, F., Studies upon the life cycles of the bacteria. Part I. Review of the literature, 1838–1918. Mem. Nat. Acad. Sci. **16**: Second memoir. pp. 252. *pls. A–S* and *1–23*. 1921.

The third section of the monograph contains a discussion of this symplastic or amorphous state, in which it is claimed that all bacteria may live and from which new cells may form. The monograph concludes with brief discussions of "conjunction" and of methods of study. While LÖHNIS' discussion of pleomorphism is excellent, it must be admitted that the sections dealing with reproductive organs and with symplasm are not entirely convincing. It is at times difficult to follow his interpretations of the illustrations. At the same time, the monograph is very suggestive of lines of work which ought to be followed in the study of the life cycles of the lower organisms. Such investigations would be well worth while.—J. F. NORTON.

Mycorhiza of forest trees.—The conclusions of MCDOUGALL[21] that "the tree is not benefited by association with the fungus, and that the ectotrophic mycorhizas are not symbiotic associations, but are instances of the parasitism of fungi on the roots of trees," have caused some doubt of the importance ascribed to root fungi by FRANK and other earlier workers. A recent preliminary paper by MELIN,[22] however, indicates that in all probability MCDOUGALL was unwarranted in rendering so general a verdict, and while mycorhizas may be quite unimportant for many American trees, they nevertheless assist in the nutritive processes of certain species, and may be an absolute necessity for some, as recently shown by RAYNER[23] in the case of *Calluna vulgaris*.

In the present investigation MELIN has found that the mycorhizas of *Pinus silvestris* and *Picea Abies* cause a limited development of rootlets. In the former the dichotomous branching is often modified by the development of nodules as large as peas, composed of many densely crowded short branches. Three mycorhizal fungi have been isolated from the *Pinus* by this worker, and one from the *Picea*. They have been preliminarily called *Mycelium radicis silvestris* and *M. radicis abietis*. Their systematic position and internal relations are for the present left open. They are aerobic organisms growing more vigorously in an acid substratum, are exceedingly specialized, and develop slowly. No fixation of nitrogen takes place in pure cultures of the fungi, although there is evidence that the mycorhizas of *Pinus silvestris* fix the nitrogen of the air. Seeds of both these trees germinate without the fungi, and there is no dissemination of the fungi by the seed. The fungi from pure cultures infect sterile seedlings through root hairs, and the young plants then develop more vigorously. At first the hyphae grow principally in the interior of cortical cells, where they form a pseudoparenchyma of the same appearance as in the fungus mantle of the completely developed mycorhiza. Later the "Hartig tissue" and the fungus mantle are formed.—GEO. D. FULLER.

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[21] MCDOUGALL, W. B., On the mycorhizas of forest trees. Amer. Jour. Bot. **1**:51–74. *pls. 4. fig. 1.* 1914.

[22] MELIN, ELIAS, On the mycorhizas of *Pinus silvestris* L. and *Picea Abies* Karst. A preliminary note. Jour. Ecol. **9**:254–257. 1922.

[23] RAYNER, M. C., Obligate symbiosis in *Calluna vulgaris*. Ann. Botany **29**:97–153. 1915.

Pigment development in Cyanophyceae.—BORESCH[24] finds that as cultures of *Phormidium Ritzii* var. *nigroviolacea* age the color gradually changes from the normal olive green, olive, or sepia brown, through violet, red violet, brown red, red brown, or even yellow brown. The addition of a small amount of iron salts leads to the return of the original color in a few days in diffuse light. These changes can be repeated in a given culture at pleasure. The author believes this is the first case of iron chlorosis reported for algae, although a number of cases have been reported in higher plants under practical growth conditions. In the young cultures there is much of a red violet water soluble protein pigment with a Venetian brown fluorescence along with much chlorophyll and carotin. As the color changes with iron deficiency, the carotin remains undiminished, but the other two pigments largely disappear.

The author states that it was known already that aside from species characters, the main conditions that have interested investigators in pigment development in the blue green algae are N-chlorosis, which is very likely to occur in ordinary cultures with aging, and the effects of intensity and quality of light. In monochromatic light there is a change in the quantity of phycocyanin, and in full sunlight there is a great diminution in the amount of both chlorophyll and phycocyanin.—WM. CROCKER.

Use of nutrient salts of low solubility.—The value of certain relatively insoluble salts as sources of necessary ions for the growth of seed plants has been tested by DUGGAR[25] in a variety of combinations, but by no means covering the entire range of possibility. It is argued that in certain types of work many advantages may accrue from the use of combinations of insoluble salts, because of the tendency to maintain a constant concentration of the various ions furnished, and also because no renewal of the solution (except as to addition of NO_3) is required from day to day. In each of three cultures in which wheat or wheat and corn were used, one or more of the combinations containing two or more insoluble salts exceeded the growth in the best control culture employed. Soluble ferric phosphate, and in certain cases ferric citrate, proved very valuable. The reason for the marked beneficial action of these is not yet determined. In most cases in these experiments the P_H lay between 5.6 and 8.0, and with growth the P_H shifted somewhat toward alkalinity.—WM. CROCKER.

Life history of a Pezizella.—SHEAR and DODGE[26] have uncovered an interesting life history of an Ascomycete, and have illustrated the present

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[24] BORESCH, K., Ein neuer die Cyanophyceenfarbe bestimmender Faktor. Ber. Bot. Gesells. **38**:286–287. 1920.

[25] DUGGAR, B. M., The use of "insoluble" salts in balanced solutions for seed plants. Ann. Mo. Bot. Gard. **7**:307–327. 1920.

[26] SHEAR, C. L., and DODGE, B. O., The life history and identity of "*Patellina Fragariae*," "*Leptothyrium macrothecium*," and "*Peziza oenotherae*." Mycologia **13**: 135–170. *pls. 8–10.* 1921.

chaos in the taxonomy and morphology of this group, and the imperative need of a more stable system of nomenclature. A study of the life history of the Ascomycete to which they assign the name *Pezizella lythri* reveals the following facts. The life cycle includes three stages. The conidial stage has received at least seven generic and ten specific names; the pycnidial stage has been referred to at least four genera, and has had at least twelve specific names; while the ascogenous stage has been described but once so far as known. In one or another of its stages, this fungus has been found on about fifty different host plants, widely distributed throughout North America and Europe, and it also occurs in South America. With such a range of forms and hosts and geographical occurrence, it is not surprising that names multiplied, but intensive studies of life histories will bring some order out of such confusion.—J.M.C.

Rhus poisoning.—The nature of the poisonous principle in *Rhus* and the method of its transmission from plant to person has excited much controversy. There have been two main theories: (1) that the poison is volatile, and therefore infection can take place without contact with the plant, and (2) that the poison is non-volatile, contact with the plant being necessary for infection. McNAIR[27] reports the results of experiments which lead him to conclude that the poisonous principle is non-volatile. Poisoning without contact with the plant can occur only by contact with something, such as clothing, shoes, etc., which has the poison on it, or from the smoke of the burning plants, the soot of which seems to carry the poison. He finds that the poisonous principle is confined exclusively to the resinous sap of the resin canals. The literature of the subject is well summarized, the work of PFAFF, who concludes that the poison is a non-volatile skin irritant, being especially emphasized. PFAFF applies the name toxicondendrol oil to the poison.—S. V. EATON.

Inhibition by metabolic products.—CHAMBERS[28] finds that the hydrogen ion concentration of the culture medium is very important in cultures of *Bacillus coli*. There is a slight checking of growth at P_H 5.5, and an increasing intensity to lethal concentration between P_H 5.1 and 4.9. Inhibition begins on the alkaline end from P_H 7.0 and 7.6, depending upon age of culture and other factors. P_H 7.6 is comparable in inhibitory action with P_H 5.1. In an asparagin-$CaCO_3$ bouillon P_H 9.5 is not fatal. In cultures with the hydrogen ion concentration controlled, the maximum count was 3,750,000,000 bacteria to the cubic centimeter, contrasting with 281,000,000 in dextrose bouillon with the hydrogen ion uncontrolled. "The inhibitory action of the metabolic products of dextrose other than the hydrogen ions is only evident near the critical acid concentration."—WM. CROCKER.

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[27] MCNAIR, JAMES B., The transmission of *Rhus* poison from plant to person. Amer. Jour. Bot. **8**:238–250. 1921.

[28] CHAMBERS, W. H., Studies in the physiology of the fungi. XI. Bacterial inhibition by metabolic products. Ann. Mo. Bot. Gard. **7**: 249–289. 1920.

GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties, are printed in **bold-face** type; synonyms in *italic*.

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