STUDIES ON THE INITIATION OF SPORANGIA IN FERNS

Thesis by

Luiz Gouvêa Labouriau

In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1958

ACKNOWLEDGEMENTS

ABSTRACT

A survey of available data in the literature shows that
initiation of sporangia in ferns is not strictly localized,
but is possible in many areas of the fern organism, such as
the gametophytes, the first leaves and several areas of the
leaf of the adult plant, which do not have to be contiguous.

In the usual sites of initiation, sporangia show cor-
relations with the leaf veins and they may be replaced by a
variety of alternative differentiations, such as cell pro-
liferations, vegetative buds and aposporous prothalli.

Initiation and differentiation of sporangia may be
arrested in several stages, both in natural conditions and
experimentally. Some of the experimental procedures that
produce this effect use changes in environmental factors.

Experimental results recorded in the literature indi-
cate a day-neutral behavior in several species and a quali-
tative short-day behavior in one species (Salvinia natans).

The results of experiments reported in this thesis show
that Asplenium bulbiferum is a quantitative long-day plant
with a critical night of 23 hours at 20°C. In this species
adventitious buds are capable of producing sporangia on
their leaves, while attached to the adult leaf, but this
capacity is lost upon isolation of the buds.

In Osmunda claytoniana determination of the sporophylls
was found to be caused by processes that are independent of
those that determine the cataphylls and to be enhanced by

long days and high temperatures.

Salvinia rotundifolia was found to behave as a short-day plant at temperatures above 20°C and as a long-day plant at 17°C. Initiation of sporangia in this species responds also to daily thermoperiodicity.

Regnellidium diphyllum was found to develop sporocarps as a response to seasonal and to daily thermoperiodicity. This response is quantitatively modified by photoperiodism.

These results are discussed in relation to the available data on the differentiation of vascular tissues, on heteroblastic leaf development, photoperiodism and thermoperiodicity.

TABLE OF CONTENTS

PART I

INTRODUCTION

The general interest of the study of the physiology of
the initiation of sporangia in ferns lies in the connections
between this problem and two general questions of morpho-
genesis of vascular plants, namely the physiology of develop-
ment of reproductive structures and the physiological deter-
mination of the alternation of generations. Therefore it
is necessary to outline here some of the general features
of these fields in order to place the studies on initiation
of sporangia in ferns in their proper perspective.

1. Initiation of sporangia and the physiology of develop-
ment of reproductive structures in vascular plants.

Studies of reproductive structures in vascular plants
were developed first by observations and experiments through
which the function of reproduction was identified, located
and described in Angiosperms (Camerarius, 1694; Kölreuter,
1761; Amici, 1824-1847) and extended to Gymnosperms,
Pteridophytes and Bryophytes (Hofmeister, 1849) (125).

This period was followed by one in which interest
shifted from function to structure and led to the accumula-
tion of data on the anatomy and cytology of reproductive
organs. As this descriptive information was gradually clas-
sified, a few general principles emerged, establishing a
correspondence between the life-cycles of the different groups
of vascular plants.

The third step, which we are now witnessing, is characterized by interest on the physiology of development. Although this trend was clearly present in the work of Sachs, Gassner, Bonnier and other precursors (141), systematic research in this field started in 1920, with the publication of the experimental results of Garner and Allard (80).

Research in each of these periods required the introduction of new types of technique: microscopy, for identification of function; microtechnique, for anatomical and cytological analysis and culture of plants under controlled conditions, for studies on developmental physiology. The utilization of these methods has systematically disclosed the existence of homologies between the reproductive structures of vascular plants.

Morphological analysis has clearly established such a correspondence between mature structures, such as the pollen sacs and ovules of Angiosperms, the pollen sacs and ovules of Gymnosperms, the micro- and macrosporangia of heterosporous Pteridophytes and the sporangia of homosporous Pteridophytes.

The correspondence holds also for their ontogenetic sequences. As this correspondence was expanded, it became increasingly improbable that such reproductive structures could be constructed, in different plant groups, by entirely different mechanisms.

In Angiosperms, the study of climatic requirements for flower initiation has shown a recurrence of the same patterns

of reaction towards environmental stimuli in different plant groups. Moreover, direct, although limited, evidence for physiological homology of flower initiation is given by grafting experiments, in which it has been possible to transmit flower induction from induced donors to non-induced acceptors belonging to different genera of the same family and having different environmental requirements (117). Long-day and short-day requirements were even found together in the flowering behavior of the same plant (154, 155, 158).

All these facts justify the postulate that, <u>in spite of the differences of physiological organization in different species, there is a common basic mechanism involved in the physiology of initiation of reproductive structures in vascular plants</u>.

As the link between the initiation of sporangia in Angiosperms and in Pteridophytes is represented only by morphological data, it is of interest to investigate if there is any similarity in the types of environmental requirements for the initiation of these homologous organs. From this point of view, the study of initiation of sporangia in ferns represents an extension of the studies on flower initiation

2. <u>Initiation of sporangia and the determination of the alternation of generations in ferns.</u>

Following Strasburger's studies in last century it was believed that the alternation of sporophytic and gametophytic generations was coincident with the alternation of a diplo-

phase and a haplophase. However, later on, it was realized
that it is not necessarily so, for, in the same species,
there may be sporophytes that are haploid (65, 195) and
gametophytes that are diploid (13, 14, 127). Regeneration
studies have shown that primary leaves (of diploid sporo-
phytes) regenerate diploid gametophytes very easily in many
species of ferns (64, 119), as well as adult leaves (64).
Gametophytes can regenerate gametophytes (2), or produce
sporophytes as apogamous buds (171). These facts show that
each generation is potentially able to produce the other with-
out going through the chromosome cycle in which haplophase
and diplophase alternate. This is also true on the Bryophyte
level (128) and becomes particularly striking in some Rhodo-
phyta, the Ceramiaceae, where in 70 species a single genera-
tion, the carposporophyte, is both a gametophyte and a
sporophyte (63). Although this complete superposition of
gametophyte and sporophyte is not critically established
in any fern, there are known cases in which a single plant
part shows contiguous gametophytic and sporophytic tissues
(42, 119, 194), the most remarkable being the "peculiar"
mutant of Phyllitis scolopendrium, in which the homozygous
recessive plant shows sporophytic tissue in the leaf veins
and gametophytic tissues in the mid-vein areas (14).

The differentiation of tracheids was so firmly be-
lieved to be a sporophytic property in Pteridophytes that
the occurrence of isolated patches of tracheids in prothalli
was considered a dependable indication of apogamy (170). It

was found, however, that tracheids occur normally and regularly, in discontinuous strands, in the gametophytes of Psilotum (97) and of Tmesipteris (96), without any indication of apogamy. Callus tissue cultures obtained by growth of spores of Osmunda cinnamomea on a mineral medium supplemented by vitamins differentiate tracheids and were verified to have the haploid set of the species and no apogamic development (139). Likewise, although stomata are considered to be a typical differentiation of the sporophyte, they have also been found in aposporous gametophytes (194). It seems, therefore, that the most dependable organs for definition of sporophytes and gametophytes, both structurally and functionally, are the sporangia and the gametangia. Consequently, a better understanding of the physiological mechanism that determines the alternation of generations requires the study of the causes that determine the initiation and development of gametangia and of sporangia.

3. Plan of the work.

The variety of features of sporangia is so richly displayed among ferns that sporangial characters are extensively used by taxonomists of this group for the definition of families and of many genera. Therefore, if we are to avoid studying particular cases, attention will have to be concentrated on the basic features of this organ. For this purpose we may use Bower's definition: "Wherever there is found an isolated spore-mother cell, or a connected group of them, or their products, this, together with the tissues

that protect it, constitutes the essential feature of an individual sporangium" (39).

Studies on the ontogenesis of the sporangium in many plants demonstrate that this organ arises from the differentiation of a mass meristem. It is, therefore, natural to begin the study of this organ by trying to understand where and when does this meristem arise in the life-cycle of the plant.

Next we may ask ourselves if this meristem has only a definite pathway of differentiation (leading to the mature sporangium) or if there are other possible paths of differentiation.

It becomes also of interest to investigate whether the occurrence of such meristems and their subsequent development involves a correlation with other organs of the plant and whether initiation of sporangia is affected, at least in some species, by such environmental factors as photoperiod and temperature, which are known to affect flower initiation in many angiosperms.

The answers to these questions represent preliminary information, which is indispensable for the quantitative study of the physiological processes involved in the initiation of the sporangia. We hope that the answers presented in this work will indicate that the study of the initiation of sporangia in ferns can provide: a) information for building a broader picture of the morphogenesis of reproduc-

tive structures in vascular plants; b) biological objects which are favorable for the investigation of some aspects of the basic mechanisms involved in the morphogenesis of these reproductive structures.

PART II

INCIDENCE OF THE INITIATION OF SPORANGIA
IN THE LIFE-CYCLE OF FERNS

In ferns the initiation of sporangia usually takes place in the leaves of the mature plant. There are, however, the deviations from this normal incidence and it is useful to consider such deviations for the purpose of clarifying the topographical requirements of the process.

1. <u>Occurrence of sporangia in gametophytes.</u>

There have been several reports of this occurrence. In Scolopendrium vulgare var. ramulosissimum and in Nephrodium dilatatum var. cristatum gracile, sporangia were found that were initiated (and sometimes developed up to viable spores) directly on the walls of archegonia, and in association with organs considered of sporophytic description, such as tracheids, ramenta, vegetative buds, "cylindrical processes," which may or may not bear roots (Lang, 114).

Similarly Pace (144) found sporangia, with differentiation of stalk, epidermis, tapetum and a central cell mass (which, in one observed case, differentiated spores), in gametophytes of Dryopteris spinulosa var. intermedia.

In 1929 Lang described another case (116) in Scolopendrium, in which sporangia were initiated from epidermal cells of old gametophytes that were prevented

from undergoing fertilization by avoiding the presence of water collections at the surface of the soil.

Subsequently Lawton (119) observed sporangia in gametophytes of Aspidium marginale, obtained from regeneration of excised primary leaves. These sporangia were found to reach an advanced stage of differentiation, with capsule, epidermis, tapetum and a core of 16 cells.

In the case reported by Lawton the gametophytes were found to be diploid; the other cases were not cariologically studied, but in the first case reported by Lang the presence of binucleated cells below the sporangia is remarked upon (and documented in one picture). Occurrence of isolated diploid cells in haploid gametophytes of ferns has been reported by Farmer and Digby (68) as resulting from nuclear fusions of neighboring cells, but this observation has never been critically established and was subsequently attributed to an error (62, 127), although the existence of binucleate cells has been confirmed as occurring sporadically in fern gametophytes (62). In the absence of cytological evidence we cannot decide whether these prothallial sporangia are all produced by diploid gametophyte cells or whether they are produced as early differentiations of an apogamous embryo (which could be haploid or diploid). Whatever the case will be found to be, occurrence of sporangia in gameto-phytes clearly shows that the initiation and differentiation of sporangia may precede the differentiation of other sporophytic organs, such as the stem and the leaves.

This shortening of the sporophytic generation, which is exceptional in living Pteridophytes, is the natural sequence of events in some lower vascular plants, such as the living liverworts Cyathodium (49, 115), Geothallus and Riellia (45), in which the sporophyte is practically reduced to a sporangium only, and some devonian Psilophytales, such as Rhynia (45).

2. Occurrence of sporangia in primary leaves.

This is rarely the normal occurrence (38, 41, 151), and has also been observed abnormally (90).

Both the initiation of sporangia in gametophytes and in primary leaves, if it can be reproduced experimentally, will open the possibility of a test for treatments considered inductive of the initiation of sporangia.

3. Occurrence of sporangia in adult leaves.

a) Individuality of sporangia.

Sporangia may occur, in adult leaves, as single units ("monangial condition"), or in groups (sori). Further degrees of association are the sporocarps of the Marsiliaceae and the synangia (in which their individuality holds only for the tapetum and the sporogenous tissue). In another type of organization ("acrostichoid condition") the sporangia are found over the whole abaxial leaf area.

b) Time patterns of initiation and development.

Comparative studies led Bower (39) to classify ferns in 3 groups (Simple, Gradate and Mixed types) according to the

chronological patterns of initiation: <u>simultaneous</u> <u>initiation</u> of all sporangia of a sorus: simple type; <u>initiation in a basipetal gradient</u> along an elongated or laterally compressed receptacle: gradate type; <u>intermittent</u> <u>initiation</u> on a flat or conical receptacle: mixed type.

c) <u>Number of initials.</u>

Young sporangia are mass meristems, which upon onto-genetic study, were found to originate either from <u>several</u> <u>initials</u> (Eusporangiate ferns), or from a <u>single initial</u> (Leptosporangiate ferns), an intermediate situation being found in Osmundaceae (where the number of cell initials may vary even among sporangia of the same plant (39, 44, 86). These patterns of initiation do not represent an isolated feature of sporangia, but they were found to be general norms of organogenesis of meristems in these groups of plants (39, 41, 184).

d) <u>Normal spatial patterns of initiation.</u>

Usually sporangia are initiated in some leaves (sporo-phylls), being absent from others (trophophylls). These two types of leaf may differ only in this respect, or they may show other morphological (84) and physiological (196, 197) differences (heterophylly, dimorphism). Dimorphism of leaves: a) is not general in ferns; b) ranges from very small differences to extreme diversifications (84); c) is found in at least 55 genera scattered among most of the families (60); d) does not show any correlation with the habitat (60), although it has been considered as an adaptive feature (43, 57).

For developmental physiology dimorphism serves as a morphological marker that allows the detection of incipient or residual differentiation of a leaf or leaf part towards the sporophyll pattern, as well as to circumscribe the normal place of initiation of sporangia, and thus, permit the detection of abnormal localisations of their initiation.

If we refer the place of initiation of sporangia to the leaf margin it will be seen that in some genera they are marginal; in others, superficial. Comparative and developmental studies (39) allowed Bower to establish the fact that the original position is always marginal; the shift to a superficial position is caused by further marginal growth of the leaf. Thus it can be seen that the marginal position will be retained when there is little expansion of the leaf blade or when the expansion precedes the initiation of sporangia. As the position of sori relatively to the margin is remarkably constant, this indicates that initiation of sporangia and leaf expansion are two events of relatively constant timing.

The most striking correlation displayed by sporangia in the leaf is with the veins.

e) Correlations between initiation of sporangia and the vascular system of the leaf.

This correlation is substantiated by comparative and topographic anatomy, by the consideration of mutants of Phyllitis scolopendrium and by results of some experiments with Asplenium bulbiferum.

Evidence from comparative and topographic anatomy of the
sporophylls.

1) Isolated sporangia have individual vascular supplies:
this has been established for Botrychium (Bruchmann, in
(40)), for Helminthostachys (40), for Mohria (61); in the
case of some fossil Coenopterids the vascular supply goes
through the sporangial pedicels and stops just below the
capsule, as in Stauropteris oldhamia (Scott, in (40)).

2) When sporangia are aggregated in sori each sorus
develops close to a vein end. For details see (40, 41),
where there may be found clear evidences in practically
every picture and in every description.

3) In the sporocarps, sori have individual veinlets.
This was found to be the case for Pilularia (Luersen, in
(40)), for Marsilea (103) and for Regnellidium (54, 104).

4) In synangia: Ophioglossum shows a vascular branchlet
in each septum separating fused sporangia (40) and in the
synangia of Marattiaceae there is a vascular supply for
every synangium, which develops as a radiate-uniseriate
sorus (40).

5) In acrostichoid sporophylls spreading of the
sporangia over the whole leaf area coincides with very close
open veins (Elaphoglossum), with open veins in very narrow
pinnae (Polybotrya, Stenosemia), with reticulate venation
(Anetium, Chrysodium, Acrostichum, most of the species of
Leptochilus), with reticulate diplodesmic venation (Cheiro-
pleuria, Platycerium, Christopteris tricuspis, Leptochilus

tricuspis and L. varians, Hymenolepis spicata), with vein
plexuses and diplodesmic tracheidal plates (Pleopeltis,
Neocheiropteris). (41).

6) In genera of close affinity differences in the
organization of the veins coincide with parallel differences
in the organization of the sorus:

In the Davallioids, absence of vein fusion coincides
with absence of soral fusion (Davallia, Humata); occasional
vein fusion by commissural veins coincides with occasional
soral fusions, forming coenosori (Nephrolepis, Diellia);
frequent vein fusions coincide with frequent soral
fusions (Tapeinidium, Lindsaya, Odontosoria); constant vein
fusions coincide with constant coenosorial condition
(Dictyoxiphium). (41).

In the Blechnoids the presence of commissural veins
parallel to the midrib coincides with the presence of
coenosori below the commissures (Blechnum) and the coeno-
sorus stops where the commissural vein stops. In Blechnum
occidentale only the fertile pinnae have commissural veins
(to which the coenosorus is adjacent), whereas the sterile
pinnae are devoid of these commissures. Arching of the
commissural veins coincides with arching of the coenosorus
(Blechnum punctulatum var. Krebsii). Occasional opening of
the commissures coincides with occasional disruption of the
coenosori (Blechnum cartilagineum, B. punctulatum var.
Krebsii); absence of commissures coincides with the absence
of the coenosorus (Woodwardia, Doodia, Phyllitis,

Camptosorus).

Evidence from the consideration of mutants of Phyllitis
scolopendrium.

In these mutants there are changes in leaf structure
which result in several modifications of the distribution
of sporangia in the leaf, such as: separation of twin sori,
initiation of sporangia in the leaf margin, pairing of
non-twin sori and initiation of sporangia in the upper
surface of the leaf. In all these cases the position of
the sorus in the leaf is profoundly altered, but the position
relatively to the veins is maintained. (10, 11).

Evidence from observations and experiments with Asplenium
bulbiferum.

In this species adventitious buds develop at the leaf
blade, usually on the adaxial surface (94, 106, 137), where
they start as little callus masses and gradually become
organized into buds, forming scales, leaves, roots and,
internally, a vascular strand (see figs. 1, 2, 3). By the
time the buds are organized as small adventitious plants
there is a complete vascular connection between the plantlet
and the vein of the adult leaf (fig. 3). Old leaves may
display a very large number of such adventitious plantlets.

We have observed a very large number of plants of this
species, which is grown in large scale by some nurseries
in Los Angeles County. Both in these and in the plants we
have cultivated for three years in the Earhart Laboratory,
adventitious plantlets developed on fertile leaves, if they

Fig. 1. Asplenium bulbiferum. Habit
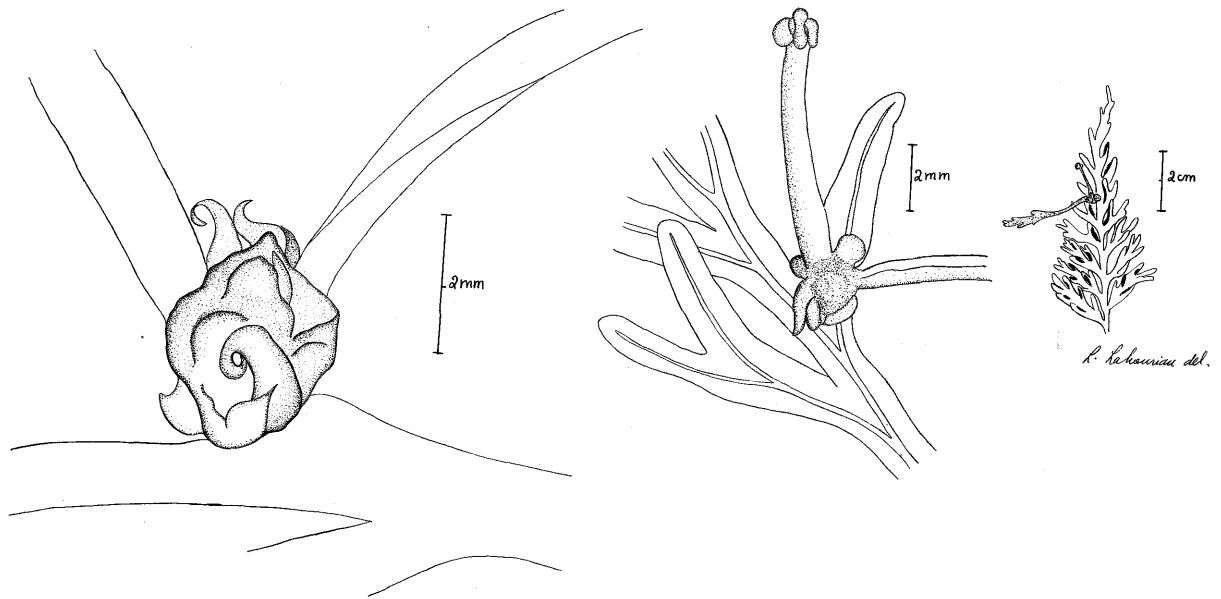
Fig. 2. Asplenium bulbiferum. Adventitious plantlets. Right: Fertile pinna of a leaf of adult plant, with adventitious plantlet (adaxial view). Middle: Detailed view of the adventitious plant. Left: Bulbill from which grows an adventitious plant.
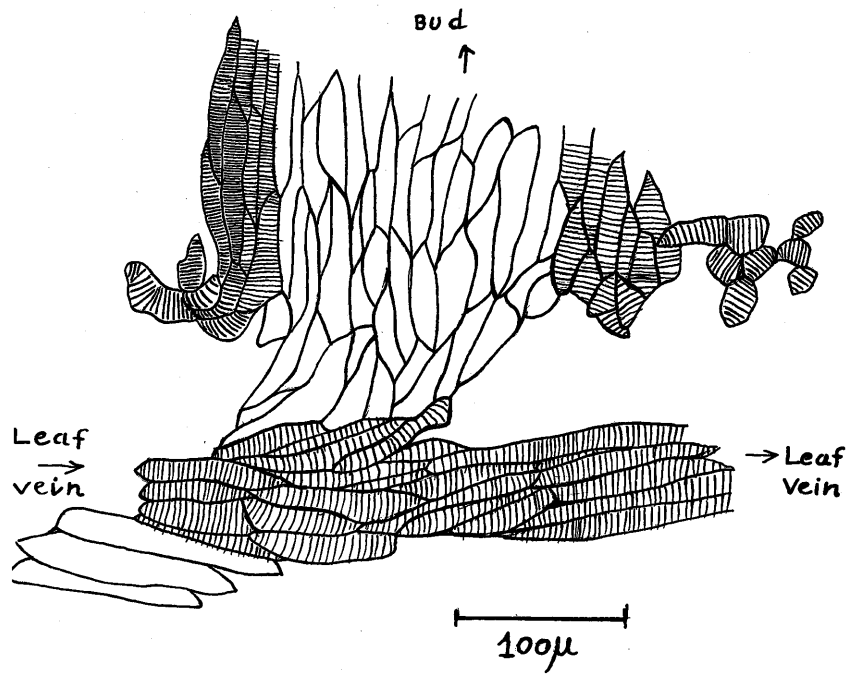
Fig. 3. Asplenium bulbiferum. Vascular connection between adventitious bud and vein of mature leaf segment.

are left attached to the adult plant, initiate and develop
sporangia on their own leaves; adventitious plantlets
developed on sterile leaves do not initiate sporangia on
their leaves, unless these leaves are exposed for long
periods to long-day conditions. In this species fertile
leaves differ from sterile leaves also by a typical shape,
with very dissected pinnae (see figs. 4, 5) and the fertile
leaves of adventitious plants conform to this rule (see
fig. 6). It seems, therefore, clear that the fertile veins
of the adult leaves not only are adjacent to the sori of
the adult leaf, but are also connected to the fertile leaves
of the adventitious plantlets.

Once this was found, we have tried to observe what
would happen to the leaves of adventitious plantlets if
such plantlets were severed from the adult leaf and allowed
to develop as separate individuals. The experiments done
with this purpose are described in detail in the section
referring to the behavior of Asplenium bulbiferum. The
results show that when the vascular connection between the
adventitious plantlet and the adult leaf is broken, the
plantlet loses the capacity to initiate sporangia, even in
favorable photoperiodic conditions, till it reaches
maturity. This fact, in contrast with the continued
initiation of sporangia in the leaves of plantlets left
attached to the adult plants, indicates that the initiation
of sporangia in attached plantlets is due to translocation
of materials from the adult plant to the adventitious
plantlets.
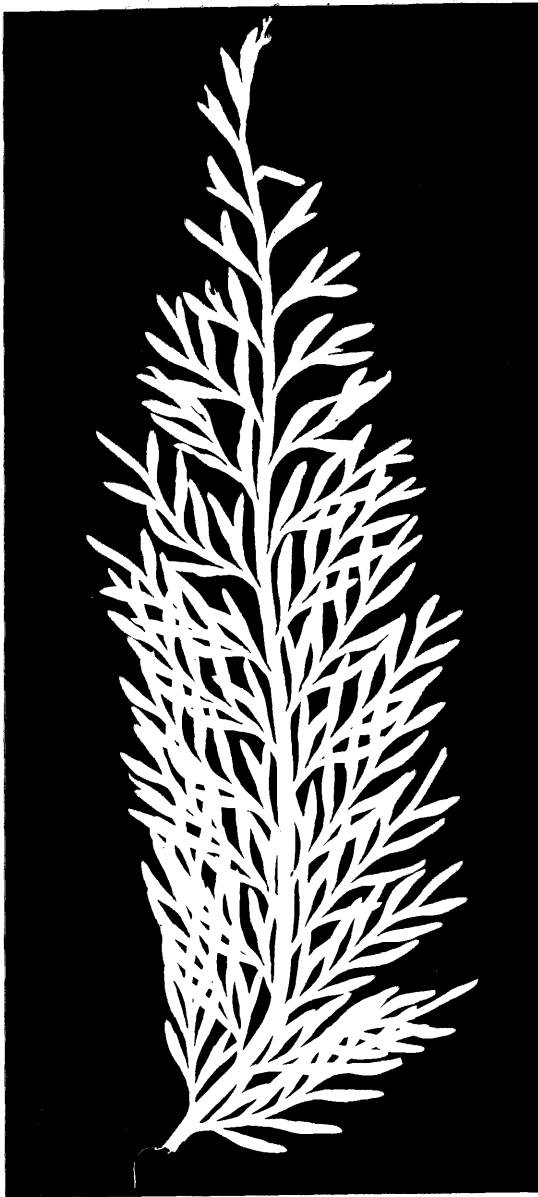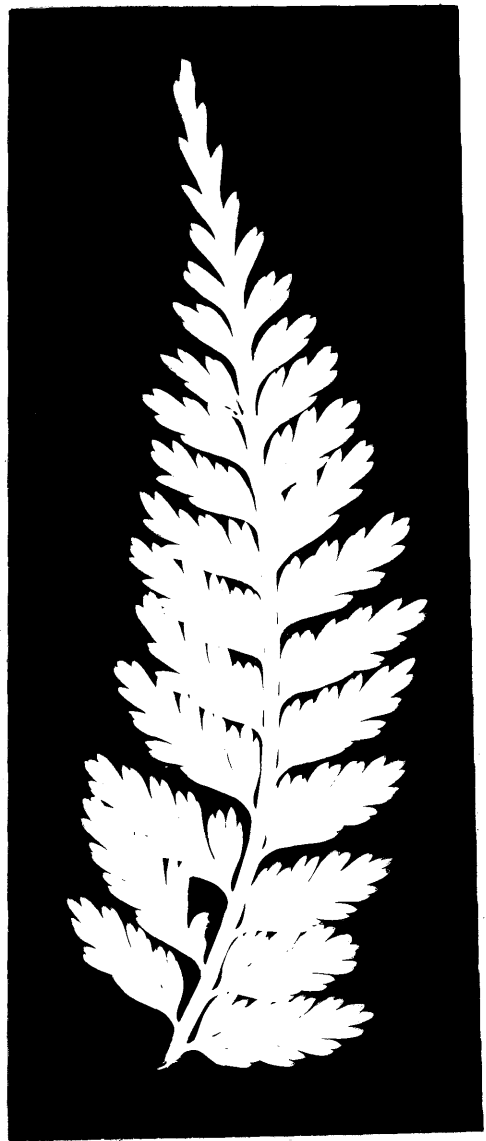
Fig. 4   Fig. 5

Shadowgraphs of sterile (fig. 4) and of fertile
(fig. 5) pinnae of Asplenium bulbiferum.

Fig. 6. Asplenium bulbiferum. Fertile pinna of adult
plant with fertile adventitious plantlet (shadowgraph).

Fig. 7. Asplenium bulbiferum. Sterile pinna of
adult plant with sterile adventitious plantlet.

f) Abnormal localization of sporangia in adult leaves.

In the species in which initiation of sporangia is restricted to some leaves or to some well defined leaf areas it is possible to observe the occasional initiation of sporangia outside of these normally fertile areas. The observations reported in the literature, as well as our own, fall under three main categories: extension of the fertile area; unilateral initiation of sporangia in bilaterally symmetrical leaves and discontinuous areas of initiation.

Extension of the fertile area.

In most species of Botrychium sporangia are initiated only in the basal segment of the leaf. Occurrence of sporangia in supplementary fertile areas in the first basal pinnae has been reported (52, 53, 73, 88, 107), as well as Helminthostachys (40). A similar condition is found in Anemia (Euanemia), where normally only the two basal pinnae bear sporangia. Fronds with one or more supplementary fertile pinnae have been reported in several species (84, 107, 108, 109, 110, 151).

Unilateral initiation of sporangia in bilaterally symmetrical leaves.

This has been reported for Dryopteris thelipteris (30), for Blechnum occidentale (129) and for Anemia (110). In Onoclea sensibilis and in Pteris cretica Dickason (60) tried to explain these cases as a possibility arising from the fact that these leaves have two parallel leaf traces

(see fig. 8). This interpretation does not hold for
Anemia where there is only a single trace per leaf (110).
Discontinuous areas of initiation of sporangia.

This fact was observed in Botrychium (120, 165), in
Anemia (108) and, among Equisetineae, in Equisetum maximum
(156). In Osmunda claytoniana this condition was occasionally
displayed in one of our experiments (see fig. 65). Extreme
cases are found in Platycerium, where sporangia occasionally
occur in the nest leaves (175) and in several species of
ferns which were found to produce, abnormally, sporangia
on the adaxial surface of the leaf (88, 179).

4. Conclusions.

1) The most common localization of sporangia in leaves
of adult plants suggests that their initiation may depend
on correlations carried by vascular tissues.

2) The fact that sporangia may be found in such varied
places as gametophytes, primary leaves and usually in
fertile areas of leaves of adult plants shows that their
initiation is potentially possible in many areas of the
fern organism, i.e., that the causes involved in their
initiation can recur independently in restricted areas.
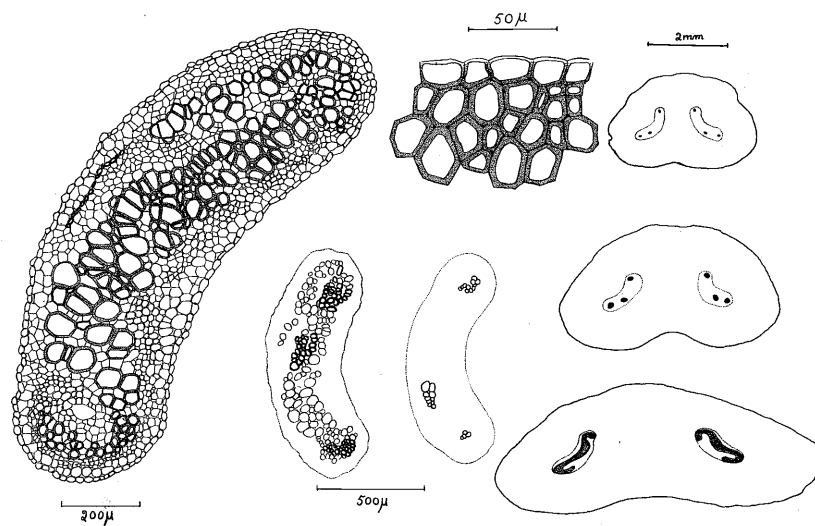
Fig. 8. Leaf trace of sporophyll of Onoclea
sensibilis. Right: Cross sections of the petiole at
three different levels, showing the 2 leaf traces.
Middle: Detail of the vascular bundle showing 2 stages
with 3 protoxylem groups. Left: One of the leaf traces
of an adult leaf, limited by the endodermis. Metaxylem
arches connecting the 3 protoxylem groups. Above: Detail
of the liquified hypodermis.

PART III

INHIBITIONS AND DEVIATIONS IN THE DIFFERENTIATION
OF SPOROPHYLLS

If we concentrate our attention in the organ where
sporangia are ordinarily initiated and developed--the sporo-
phyll of the adult plant--it will become evident that the
differentiation of this type of leaf may suffer inhibitions
and deviations, which can provide additional information on
the initiation of sporangia.

Sporangia may be partially or totally replaced by other
structures, such as cell proliferations, paraphyses, vegeta-
tive buds and aposporous gametophytes.

1. Cell proliferations replacing the sorus.

Since this is not an uncommon phenomenon we will limit
ourselves to the consideration of some dimorphic ferns,
where the study is facilitated by secondary sporophyll
characters, that identify the position of the arrested sori.
Onoclea sensibilis is one of such plants. Figs. 9 and 10
show the habitus and the sterile pinna. Figs. 11 and 12
show two stages in the development of the fertile leaf. As
is easily observed, the sterile pinna has a reticulate
venation, abundant abaxial stomata and an expanded blade,
whereas the fertile pinna has open venation, no stomata and
a contracted and abaxially folded blade.

Figs. 13 to 25 show abnormal fertile pinnae, in which
it is possible to follow a series of types, ranging from a

Fig. 9.   Onoclea sensibilis, Habit
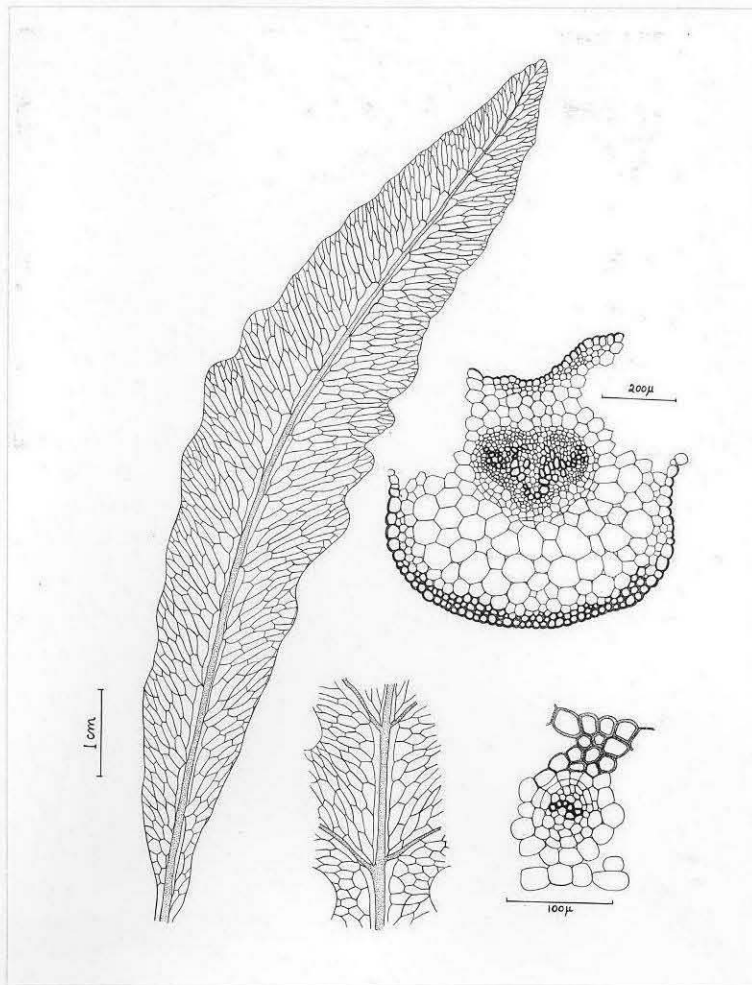
Fig. 10.   Onoclea sensibilis. Sterile pinna; venation,
cross section through the midrib (up, right); cross section
through a secondary vein (right, down).
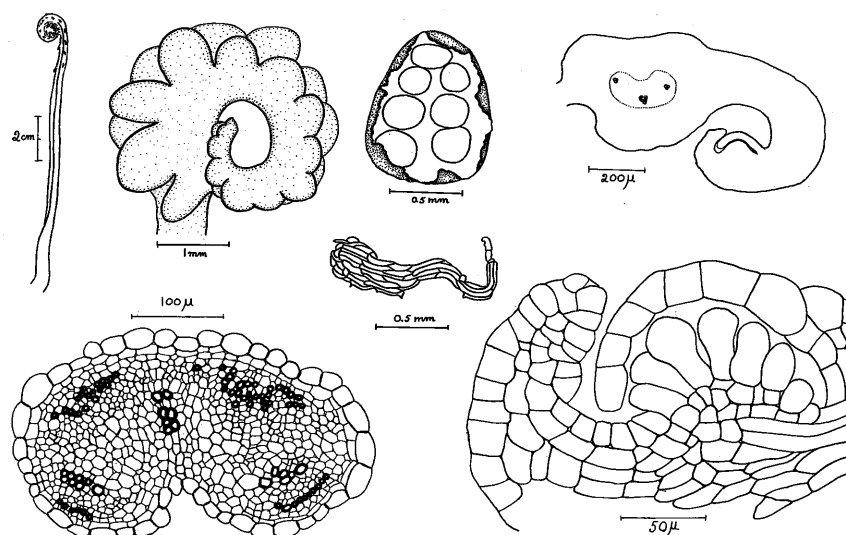
Fig. 11. Onoclea sensibilis. Young fertile frond.
Upper row: frond, first order pinna, second order pinna
in abaxial view, cross section through a first order pinna.
Lower row: detail of vascular bundle of first order pinna,
scale, detail of the cross section of the second order pinna,
showing receptacle and young sporangia, below the indusium.
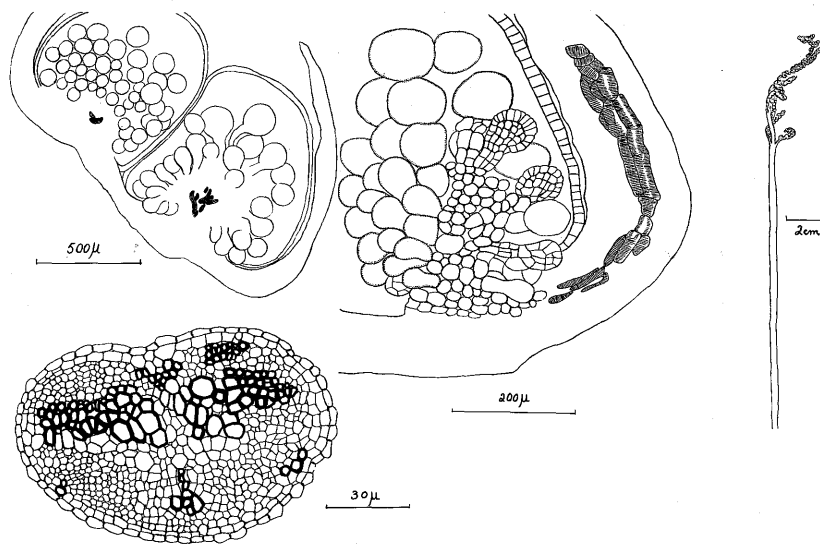
Fig. 12. Onoclea sensibilis. An older fertile frond (right); cross sections through the region of the receptacle showing the gradate development of the sorus. Left, down cross section of the vascular bundle of second order pinna.
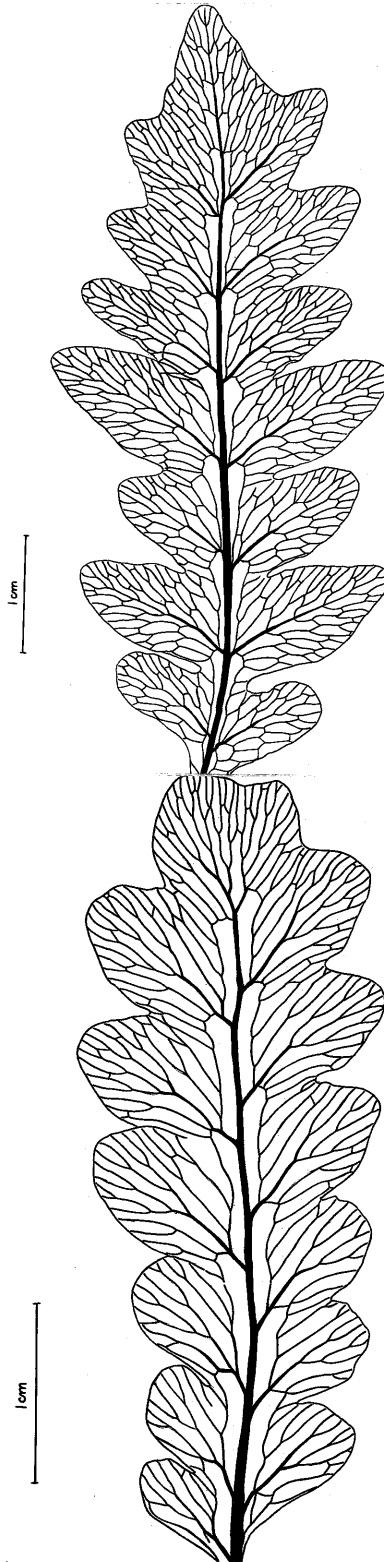
Figs. 13 and 14.   Onoclea sensibilis.   Pinnae of arrested fertile fronds.
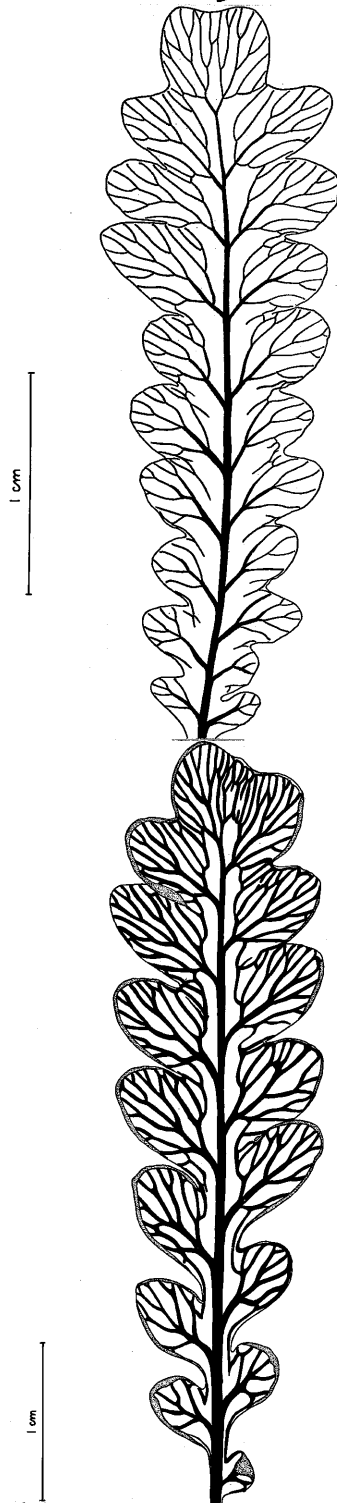
Figs. 15 and 16. Onoclea sensibilis. Pinnae of arrested fertile fronds.
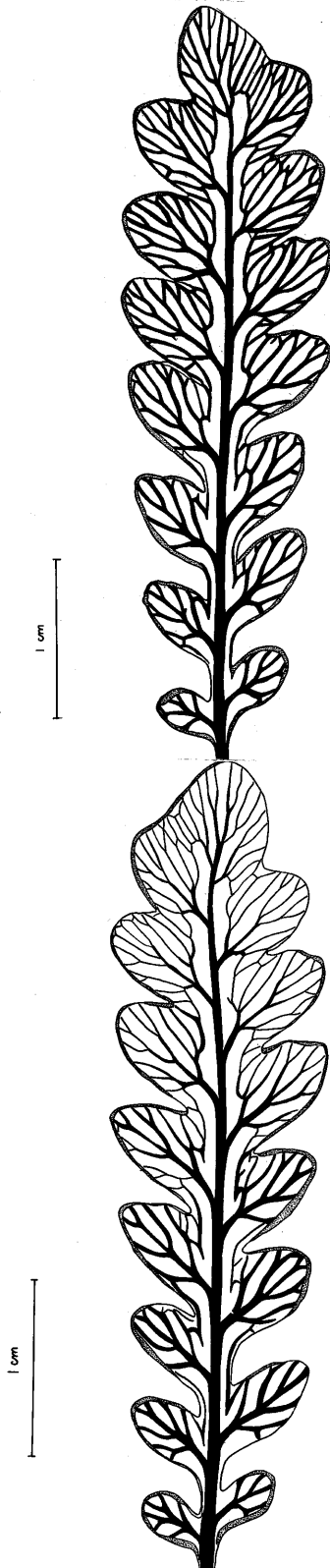
Figs. 17 and 18. Onoclea sensibilis. Pinnae of
arrested fertile fronds.
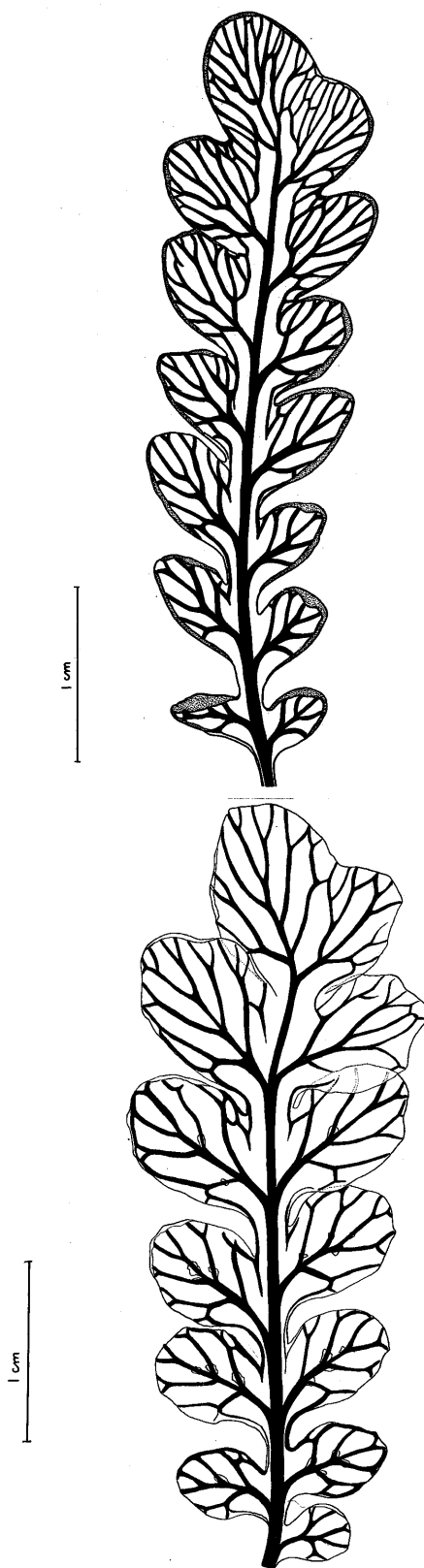
Figs. 19 and 20. Onoclea sensibilis. Pinnae of arrested fertile fronds.
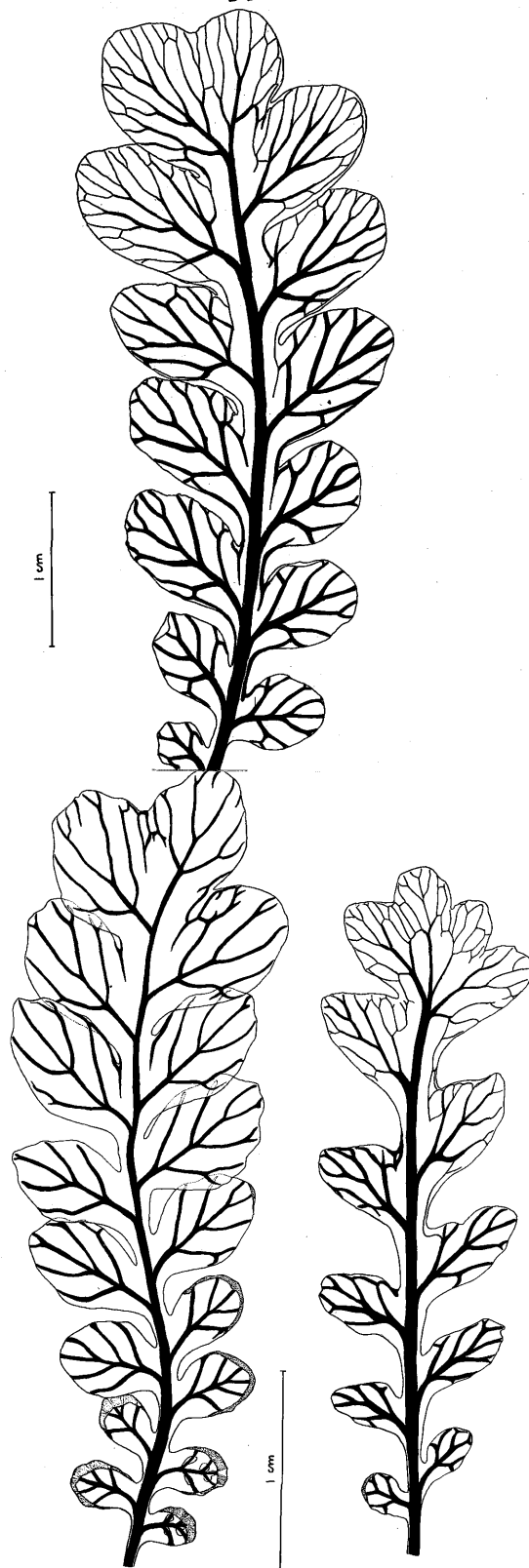
Figs. 21 and 22. Onoclea sensibilis. Pinnae of arrested fertile fronds.
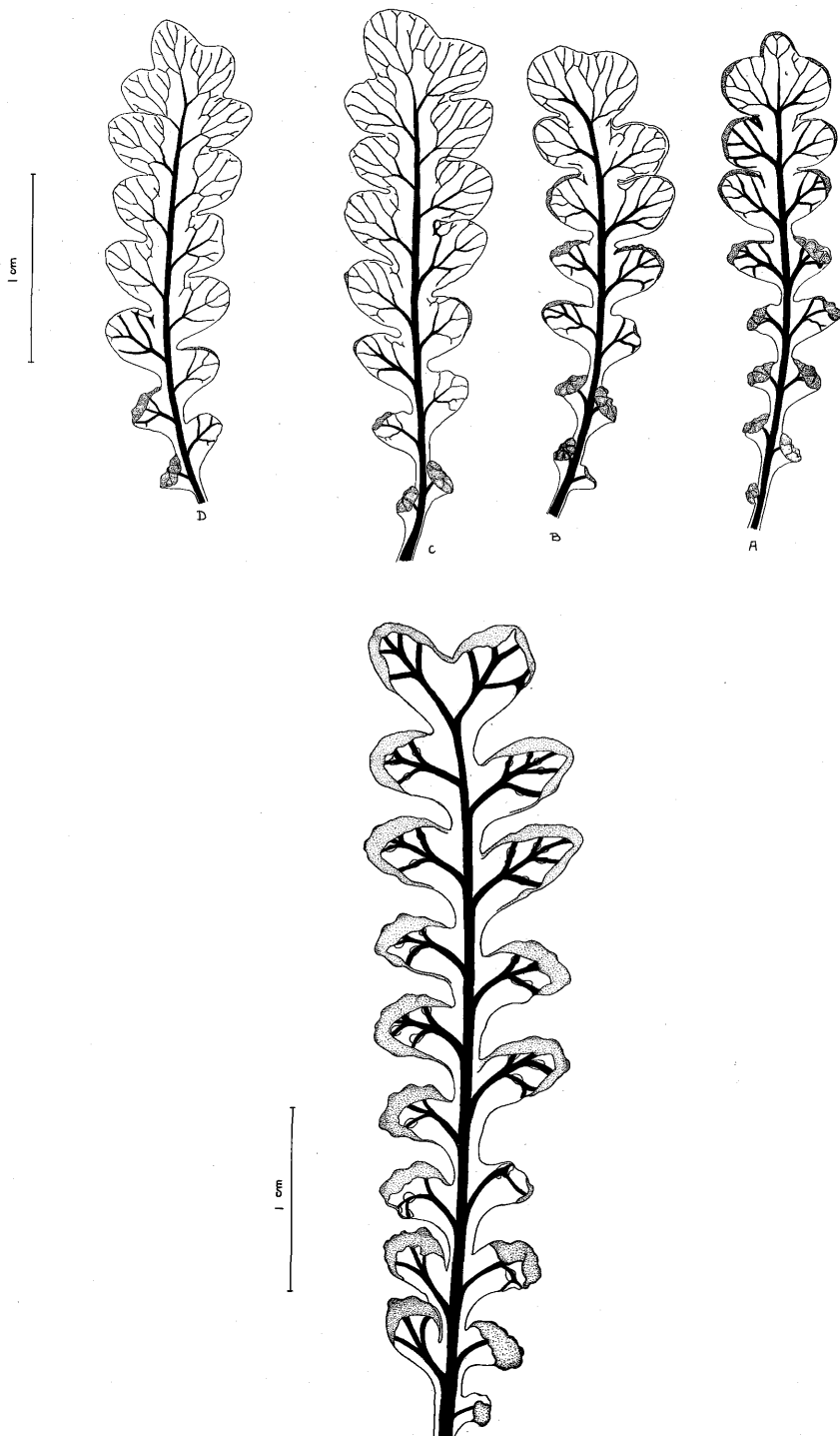
Figs. 23 and 24. Onoclea sensibilis. Pinnae of arrested fertile fronds.
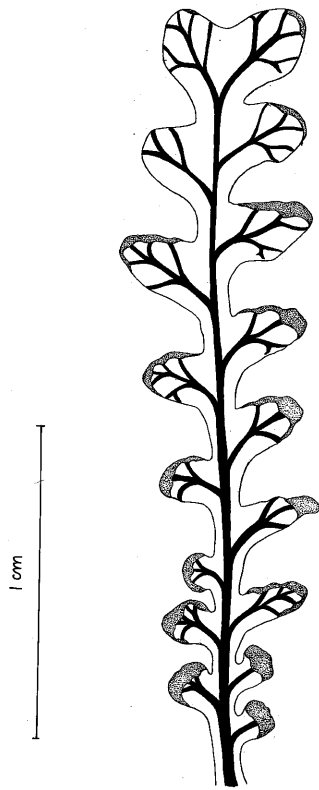
Fig. 25. Onoclea sensibilis. Pinnae of
arrested fertile fronds.
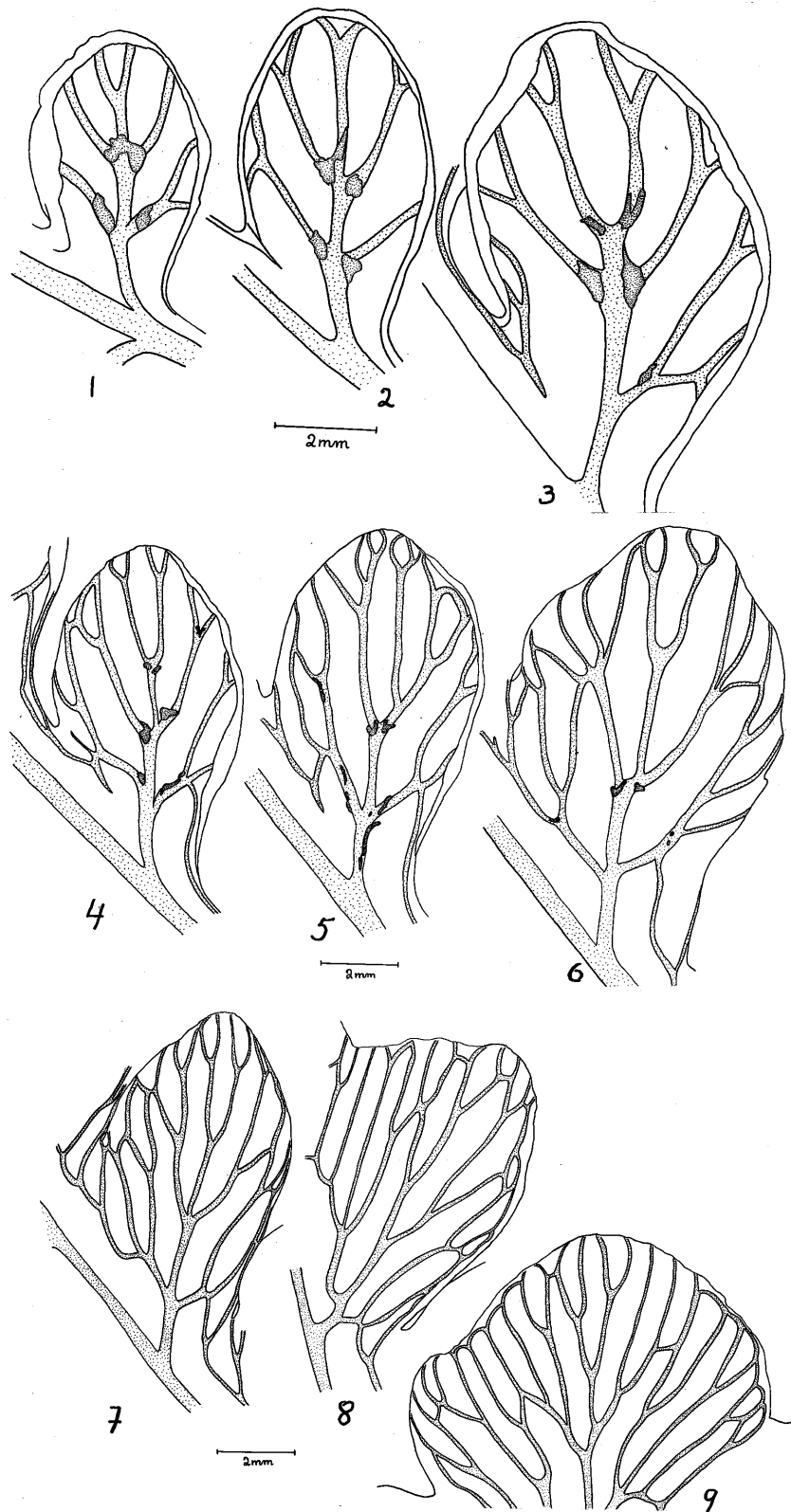
Figs. 26, 27 and 28. Onoclea sensibilis. Successive pinnae of an arrested sporophyll, showing a longitudinal transition from sporophyll features to trophophyll characteristics, in the same frond. The numbers indicate the successive pinnae at one side of the rachis in acropetal order.

type very similar to the normal trophophyll, to one with many sporophyll features and even a few sporangia in the sorus. These leaf forms have been referred to as Onoclea sensibilis forma obtusilobata (59, 136, 143,153, 167, 177, 185). A similar form has been admitted for Pteretis nodulosa (72).[*] For developmental physiology the interest of these abnormal leaves lies in the fact that they display a series in which the development of the sporophyll is arrested at different stages and deviated to the trophophyll pattern. This is shown by comparison of the vein system, of the cutting of the margin, of the involution of the margin and of the residual indusia. In some of these leaves the incompletely developed indusium faces a region of the leaf surface where there is a "hillock" of cells, that is, a local cell proliferation (Fig. 29), similar in shape and in position to the receptacle of a young sorus (compare with Fig. 11). Several degrees of cell proliferations of this description may be observed (Fig. 30). The incompletely developed indusium may also range from a large flap to a very small one (Fig. 31).

In Osmunda Claytoniana abnormal fertile pinnae are also very common. Fig. 32 shows the habitus of the plant and fig. 33 shows a closer view of the fertile frond, with its

---

[*]It may be incidentally remarked that such taxonomic designations should be dropped, since these forms are long known to be abnormalities of individual leaves, which can be produced experimentally by defoliation (19, 21, 22, 23, 24, 87).
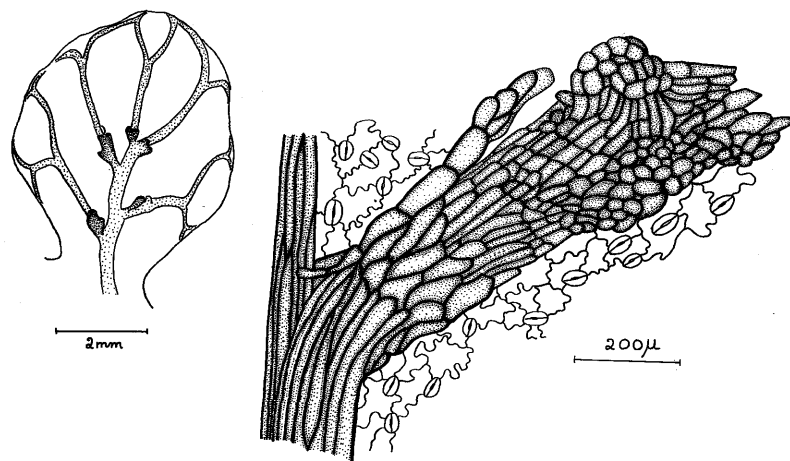
Fig. 29. Onoclea sensibilis. Right: cell prolifera-
tion replacing a sorus; Left: an abnormal pinna where these
proliferations are found.
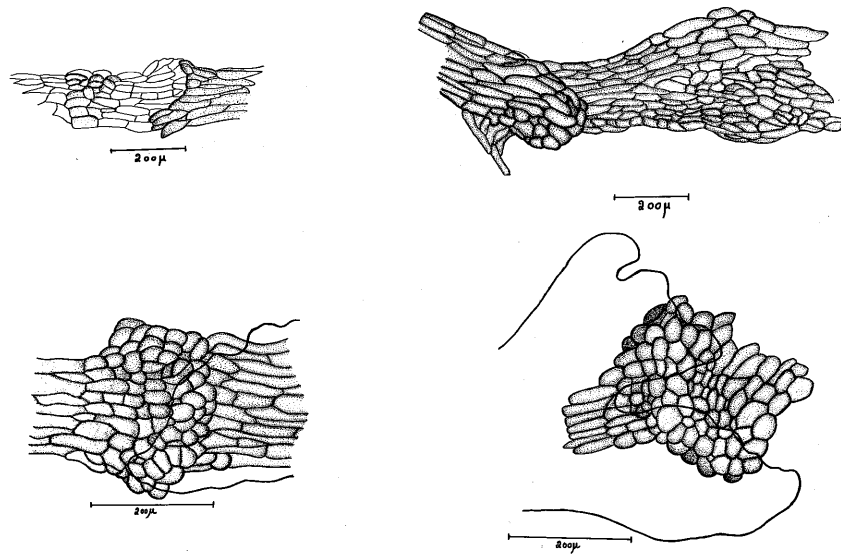
Fig. 30. Onoclea sensibilis. Several types of cell proliferations found in arrested sporophylls, in the position of the sori.
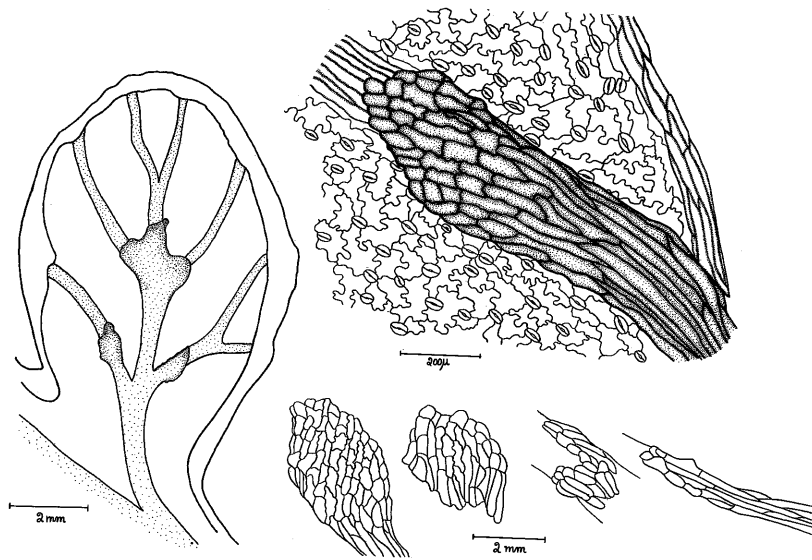
Fig. 31. Onoclea sensibilis. Several types of residual indusia found in arrested fertile pinnae. Left: a pinna of an arrested sporophyll, showing the open venation and the residual indusia.

Fig. 32.   Osmunda Claytoniana.   Habit
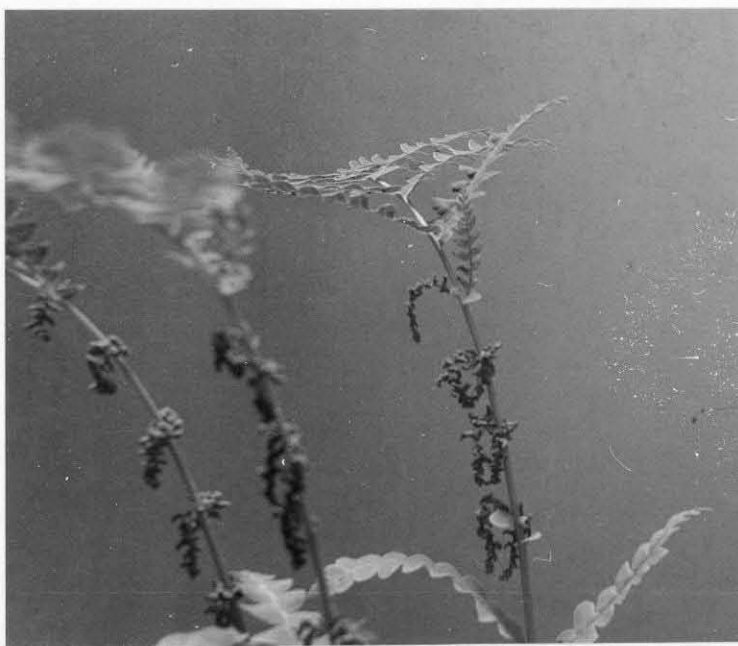
Fig. 33.   Osmunda Claytoniana.   Sporophylls with middle
set of fertile pinnae.

middle set of fertile pinnae. These have a very narrow blade
(fig. 35), contrasting with the sterile pinna. Figs. 36 to
41 show a normal sterile and several abnormal fertile pinnae,
with cell proliferations and scattered sporangia on the
veins. Fig. 42 shows some details of these cell prolifera-
tions. These abnormalities were observed by us in natural
populations of the species, at Michigan, and had been also
observed in both of the areas of distribution of the plants
(51, 56, 92, 93, 138, 150).

In 1953-54 we recorded the presence or absence of these
abnormalities in 330 fronds of plants growing in natural
conditions in Ann Arbor, Michigan. Table I presents these
data for 11 groups of 30 fronds each. The average number
of abnormal fronds per group of 30 fronds is: 17 ± 2 (stand-
ard deviation). Therefore, in these conditions, the number
of sporophylls with abnormalities ranges from 50% to 63% of
the number of sporophylls of the sample.

It is worthy of note that in this species the abnormali-
ties were never found to occur in the pinnae of the middle
of the fertile set of the frond, but always in the lower
and upper pinnae of this set. From the data of table I, it
is clear that the organization of the sporophyll in which
there is a mixed pattern of development (partially fertile)
in the pinnae of the extremes of the fertile region of the
sporophyll is at least as frequent as the organization with
a sudden transition from one type of pinna to the other.
This fact can be understood in part when we consider the
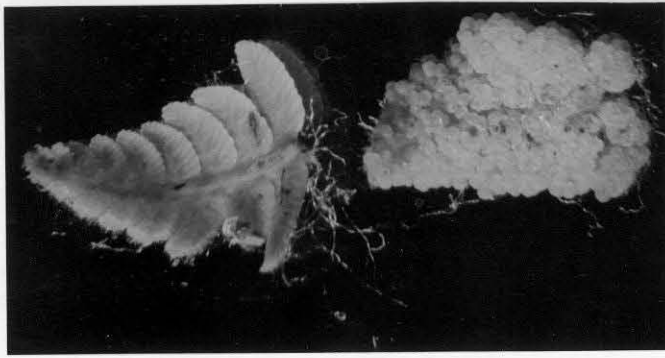
Fig. 34. Osmunda Claytoniana. Left: sterile pinna;
Right: fertile pinna of the same frond, with sporangia.
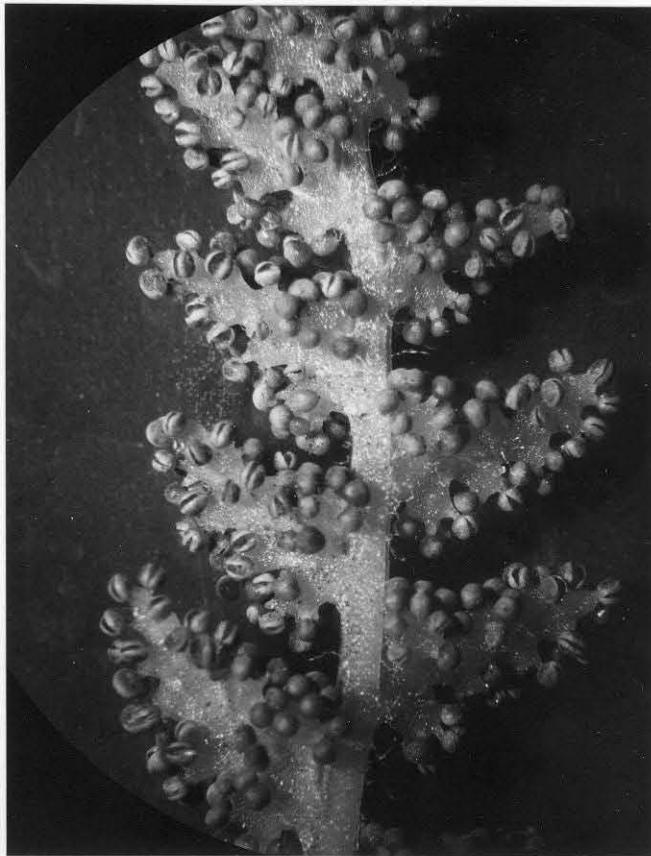
Fig. 35. Osmunda Claytoniana. Detail of fertile pinna
showing sporangia.
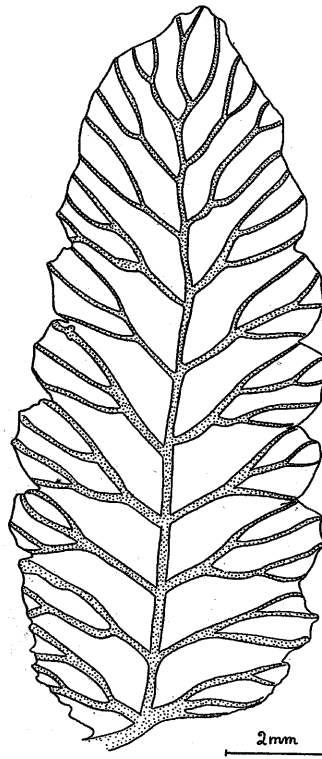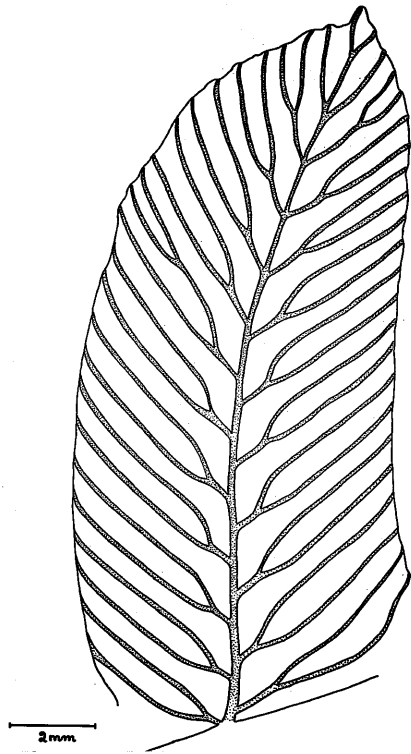
Figs. 36 and 37. Osmunda Claytoniana. Pinnae of arrested sporophylls. Fig. 36: normal sterile pinna.
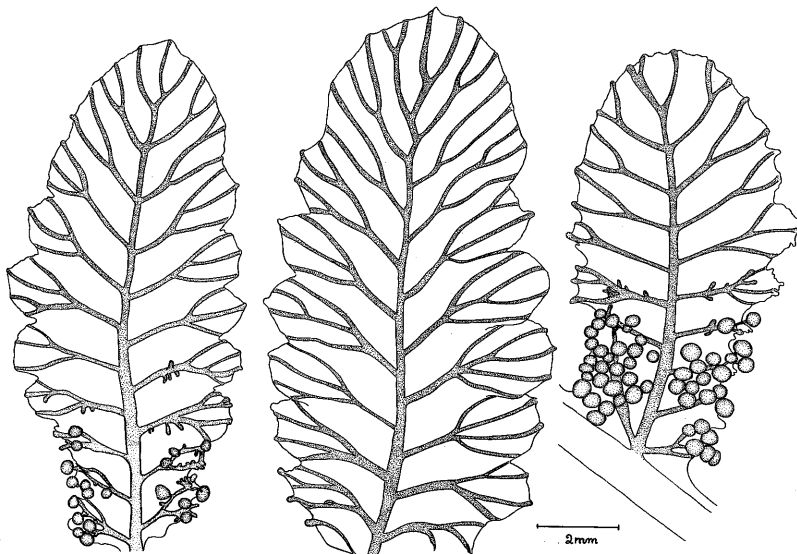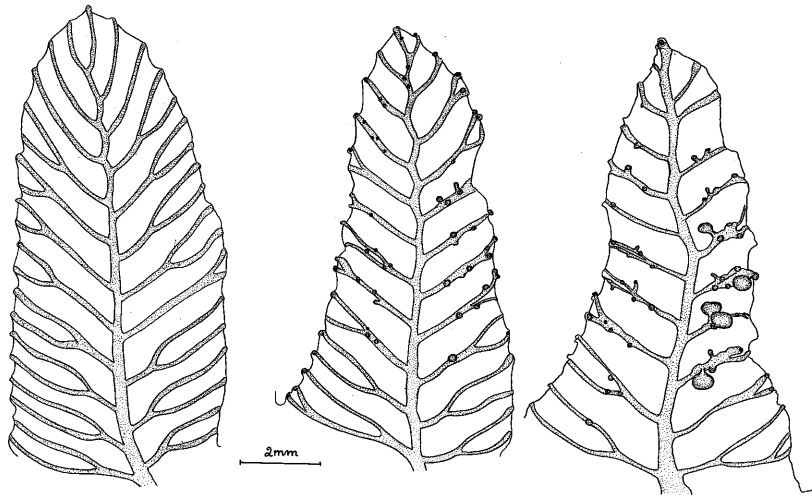
Figs. 38 and 39. Osmunda Claytoniana. Pinnae of arrested sporophylls.
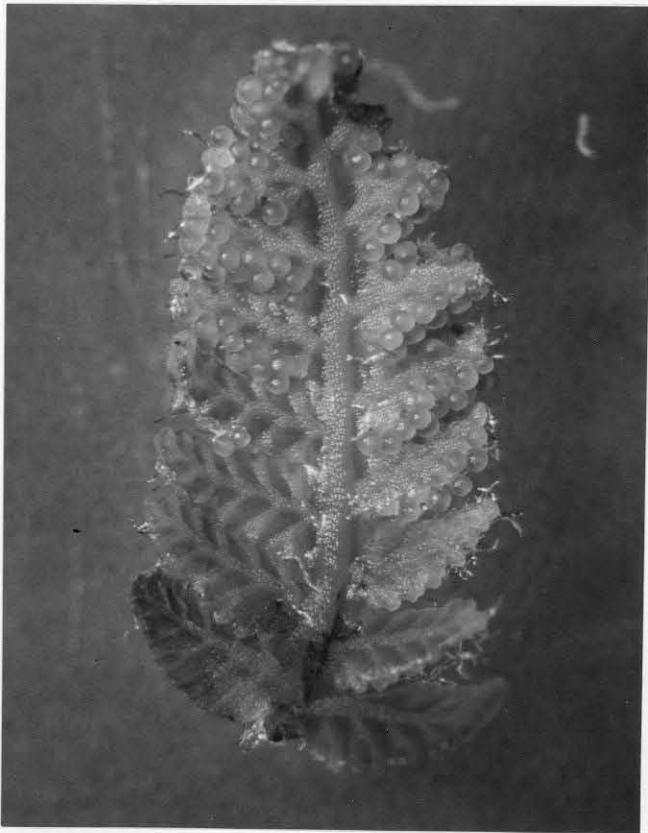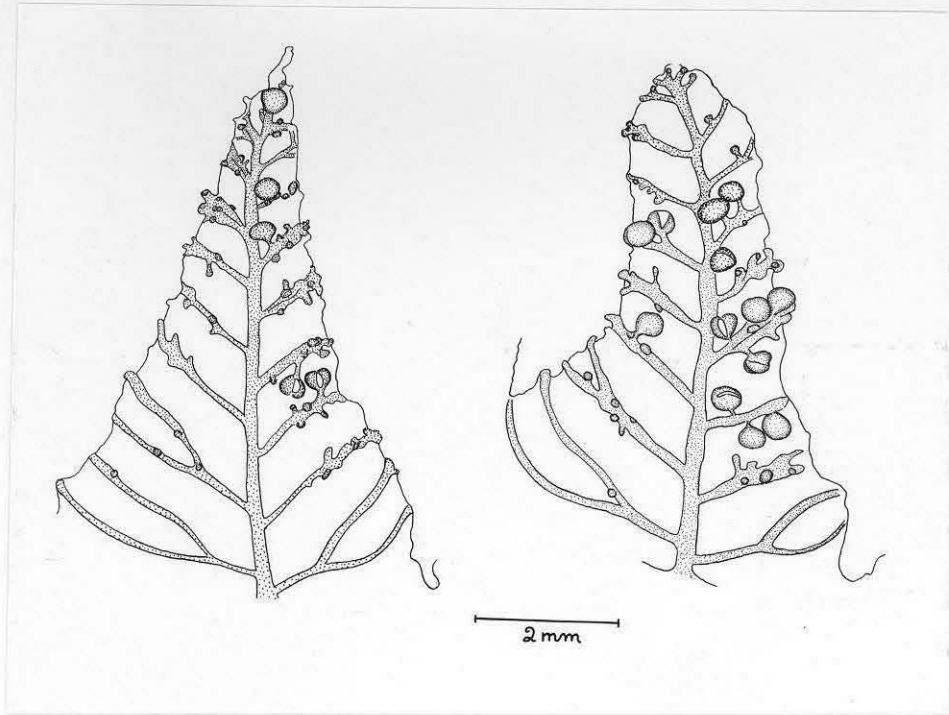
Figs. 40 and 41. Osmunda Claytoniana. Pinnae of
arrested sporophylls.
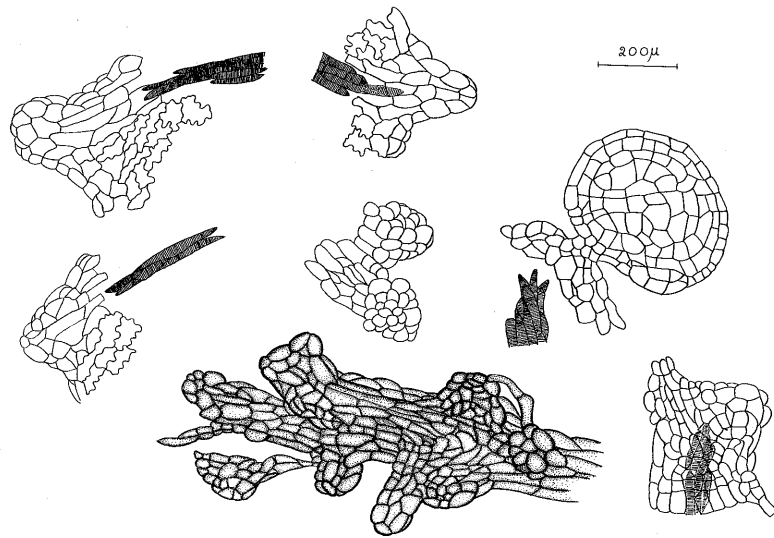
Fig. 42. Osmunda Claytoniana. Cell proliferations in pinnae of arrested sporophylls. At right, middle row, a sporangium, showing subjacent vascular supply.

Table I

Frequency of sporophylls with abnormal fertile pinnae in
natural populations of Osmunda Claytoniana in Ann Arbor,
Michigan

Groups of 30 fronds	# of sporophylls with abnormalities
1	17
2	17
3	14
4	19
5	15
6	14
7	17
8	20
9	20
10	18
11	16

development of the sporophyll in this species.

The abnormal pinnae are not equally common in the lower and in the upper limit of the region of the fertile pinnae of the sporophyll, as shown in Table II, in which the frequencies of the abnormal pinnae are discriminated by classes, according to the position (basal or apical) in which they occur in the sporophyll. So the abnormalities are more frequent in the apical limit of the region of the fertile pinnae of the sporophyll. This indicates that the fertile pattern of development is less stable in the apical limit of the region of the fertile pinnae. It is possible to think of the initiation of sporangia in the pinnae of the sporophyll, in this species, as being limited by different factors at the base and at the apex of the frond. In fact it is possible to induce the initiation of sporangia in all the apical pinnae of the frond, but not, so far, in the basal pinnae. This result will be discussed in the section dealing with the behavior of Osmunda Claytoniana.

Abnormalities in which the sorus or isolated sporangia are replaced by a callus-like proliferation, as those described for Onoclea and Osmunda, were reported for prothallial sporangia in Nephrodium dilatatum (114), for Ophioglossum vulgatum (Rabenhorst in 38), and, among other Pteridophytes, for Equisetum telmateia (178), for Lycopodium (38), and for Selaginella (38).

All these facts show that the sorus can be arrested and deviated in its initial development, producing a cell pro-

Table II

Frequency of abnormal fertile pinnae at the base, at the apex
and at both limits of the region of the fertile pinnae of
the sporophyll, in natural populations of Osmunda
Claytoniana in Ann Arbor, Michigan

Groups of 30 fronds	# of fronds with basal abnormal pinnae only (X_1)	# of fronds with apical abnormal pinnae only (X_2)	# of fronds with basal and apical abnormal pinnae (X_3)
1	2	11	4
2	4	9	4
3	2	6	6
4	10	5	4
5	5	9	1
6	1	9	4
7	1	9	7
8	4	14	2
9	4	11	5
10	2	13	3
11	4	9	3
$\sum x_i$	39	105	43
$\overline{x}$	3.5	9.0	3.9
$\sum \delta^2$	64.75	58.75	28.91
σ	2.4	2.3	1.6
$\overline{x}$ as % sample	3.7% to 19.7%	24.0% to 39.3%	7.6% to 18.3%

liferation at the leaf surface.

2. Sporangiasters and paraphyses.

Sporangiasters are sporangia that had their development arrested and deviated in a stage in which at least the pedicel and the capsule are clearly recognizable. In paraphyses development is arrested earlier and the appearance ranges from that of a unicellular hair to that of a sporangiaster (43). Figs. 43, 44 and 45 show several types of such organs for Onoclea sensibilis and for Osmunda Claytoniana. Facts of this nature have been reported for many species of ferns (20, 35, 36, 57, 69, 70, 71, 149).

There are three cases of arrest of development of sporangia reported in the literature that deserve a special mention because they disclose an interesting aspect of this phenomenon. One is the local arrest in development by albinism; the other is the differentiation of a glandular type of paraphysis observed in Polypodium vulgare and the third is the arrest by correlation between sporangia.

Local arrest in variegated plants.

Bateson has described a variegated plant of Adiantum cuneatum (28), which was subsequently studied in more detail by Andersson (9). The plant exhibits white areas, where chloroplasts rarely exceed 50% of the size of normal chloroplasts and have a pale green color. Cells either have small-pale chloroplasts or normal chloroplasts and there is no transition in size, nor in color, at the boundary between white and green regions of the leaf. When
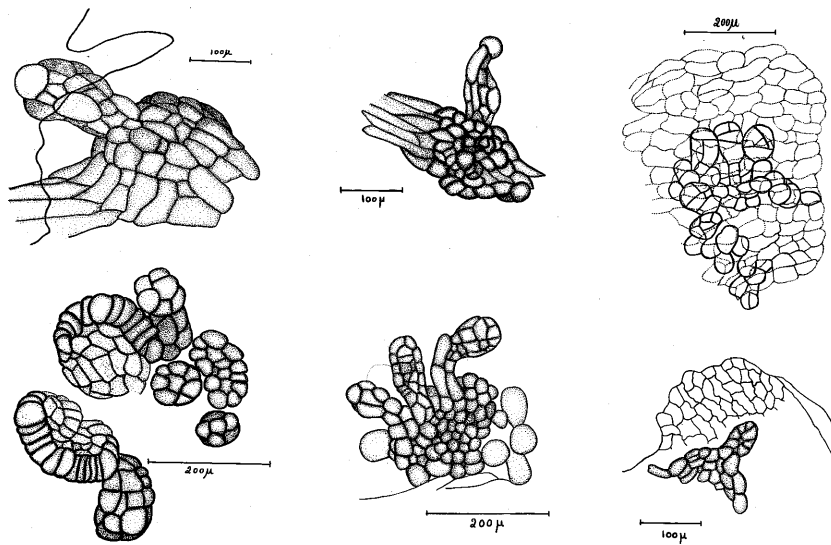
Fig. 43. Onoclea sensibilis. Sporangiasters in an arrested sporophyll.
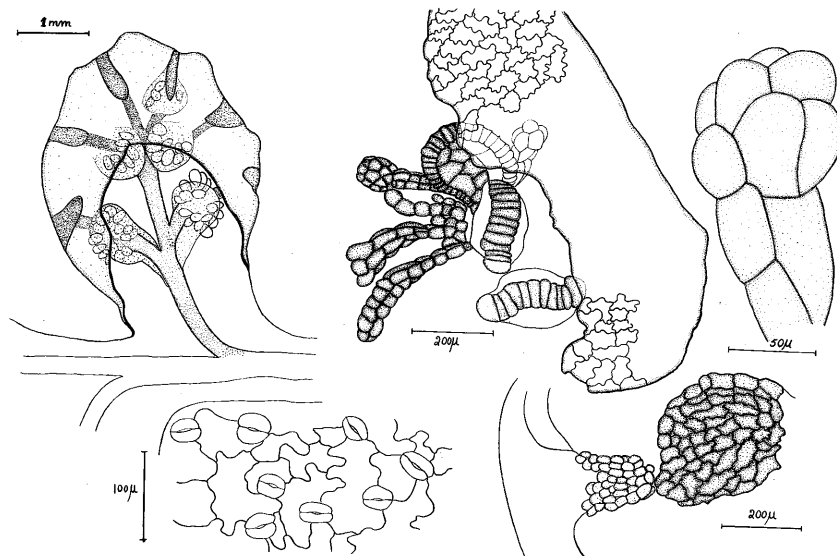
Fig. 44. Onoclea sensibilis. Sporangiasters. Arrested fertile pinna. (Left); sorus with sporangiasters and sporangia, individual sporangiaster (up, right), stomates (below and cell proliferation, with residual inclusium.
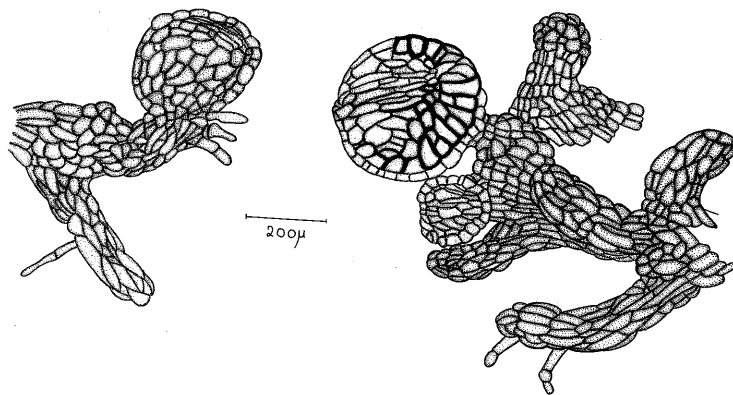
Fig. 45. Osmunda Claytoniana. Sporangiasters; at right: normal sporangium among sporangiasters.

the white sector reaches the leaf margin, where sori are developed in this species, sporangia are initiated both in the white and in the green sectors of the sorus, but only those initiated in the green areas complete their development. The sporangia initiated in the white area turn into sporangiasters. The study of the physiological differences between white and green patches in this plant may disclose some information on the factors involved in development of sporangia, inasmuch as other cases of variegation are known among ferns, in which the white areas initiate and develop normal sporangia and viable spores (12).

Partial arrest in development of the sorus in some strains of Polypodium vulgare.

Arrest in development of sporangia, forming glandular sporangiasters and paraphyses has been observed in Polypodium vulgare (20). Subsequently it was shown by Martens (130, 131, 132, 133, 134, 135) that these paraphyses occur in two different morphological types, which show definite geographical patterns. A cytological analysis by Manton has shown that both types bearing paraphyses belong to diploid populations (there are also tetraploid, hexaploid populations, as well as triploid and pentaploid populations) (126, 127). In this case the arrest in development of some sporangia of the sorus seems to be an inherited trait.

Arrest in development of sporangia by correlation with other sporangia.

The observation of development of sporangia in Lygodium (29) and in Anemia (110, 151, 172) shows that the apical

sporangia of the sorophore usually do not complete their development and remain arrested. Morphological changes following this arrest suggest that this effect could be caused by the older sporangia and not necessarily through trophic competition (172).

Suppression of development of microsporangia by developing macrosporangia has been clearly established by Pfeiffer (148), for Azolla. In this plant the sori contain an apical macrosporangium and basal potential microsporangia. Development of the macrosporangium starts first and when it reaches the stage in which the 32 spores are formed, two things may happen. First possibility: all of the 32 spores abort, the whole macrosporangium degenerates and this is quickly followed by development of microsporangia in basipetal succession. Second possibility: 31 out of the 32 spores of the macrosporangium degenerate; the remaining spore develops into the macrospore. In this case the young microsporangia, initiated at the base of the sorus, remain arrested in a very early stage and never complete their development.

3. Experimental arrest of the initiation and differentiation of sporangia.

a) Defoliation experiments.

Complete and partial suppression of the initiation of sporangia has been produced experimentally by defoliation in Onoclea sensibilis (21), in Matteucia struthiopteris (87),

in Anemia collina and A. flexuosa (112) In Anemia collina
defoliation ceases to be effective in this respect if a
single adult leaf is left in each plant (113).

b) Dark culture experiments.

Total suppression of sporangia can also be produced in
Anemia phyllitidis by culture of the plants in continuous
darkness (112).

c) Experiments with applied auxins.

Application of auxins (3-indole-acetic acid, 2,4-
dichlorophenoxyacetic acid and 2-naphthoxy-acetic acid) in
lanoline to the fertile pinnae of the young developing
fertile leaves, or as a solution given through the soil,
produces the replacement of sporangia by irregular cell
proliferations (111).

d) Experiments with continuous light at high tempera-
tures.

Replacement of sori by irregular cell proliferations
has been obtained by us experimentally in Salvinia rotundi-
folia by culture of plants at 30°C in continuous light in
the Earhart Plant Research Laboratory. This experiment
will be described in the section dealing with the behavior
of Salvinia rotundifolia.

e) Experiments with continuous sprays of water.

In Marsilea quadrifolia continuous spraying of plants
with tap water for 48 to 60 hours was found by Shattuck (164)

to arrest the development of macrosporangia, without affecting the microsporangia of the same sporocarp. More than 60 hours of spray was found to arrest the development of both types of sporangia. These results were interpreted as due to detrimental effects caused by a lowering of the temperature due to the water spray. As the experiments were not made with the appropriate controls with sprays of water at higher temperatures, the interpretation given is open to question and conclusions must await a reinvestigation.

f) Isolation of young fertile organs.

Williams (192) obtained reversion of the young fertile cones of Selaginella grandis to a vegetative development by the culture of isolated young fertile cones.

4. Sporangia replaced by vegetative buds.

Cases have been reported in which the sorus is totally or partially replaced by a vegetative bud in ferns (35), as well as in Isoetes (85) and in Lycopodium (88).

5. Sporangia replaced by gametophytes.

Production of aposporous gametophytes in adult leaves is by no means a rare event (see, for instance, 64). In some cases of apospory, the aposporous gametophytes are produced from young arrested sporangia (34, 35, 36).

6. Conclusions.

The above discussed facts, concerning alternative pathways of the differentiation of sporophylls, show that:

1) <u>The development of the sporangium is a catenary process, that may be arrested and deviated at any of its stages</u>.

2) <u>Initiation and development of sporangia can be experimentally suppressed in some plants by a variety of procedures, some of them involving climatic conditions, others using changes in internal physiological conditions</u>.

Both the lack of specificity of the topographic localization of the initiation of sporangia and the above mentioned conclusions suggest an attempt to describe the initiatfon of sporangia in terms of an interaction between internal and environmental factors. The first requirement for this purpose is to determine whether climatic factors, such as temperature, photoperiod and thermoperiod can affect the process in some plants.

PART IV

EFFECTS OF CLIMATIC FACTORS ON THE INITIATION OF
SPORANGIA IN SOME FERNS

1. Review of published data.

   a) Phenological information.

   The first fact that demands attention is the scarcity
of annuals among ferns. With the exception of some species
of Salvinia, Azolla, some Marsileaceae and some species of
Anogramma (79), all ferns are perennial plants. Among
tropical ferns a coincidence has been observed between a
relatively dry season and the time of production of sporo-
phylls (98). The best known cases of seasonal periodicity
in the production of sporophylls are those of Salvinia
natans and of the temperate species of Osmunda. Salvinia
natans was observed to produce sporophylls in the end of
August at Sendai, Japan (36°16' N latitude), when the length
of the day is approximately 10 hrs. 40 min. (142). Osmunda
cinnamomea differentiates sporangia before the fall (48)
and after the preceding spring (169). The same annual
periodicity was observed by us for Osmunda Claytoniana
growing in Ann Arbor, Michigan. The time of growth of the
already differentiated sporophylls of Osmunda cinnamomea
is progressively delayed in the year calendar, as we go
from the Antilles northward (168). This fact, as well as
the sporadic production of fertile leaves by plants of this

species in the fall (55), is not pertinent to the initiation
of sporangia, but to the growth of the fertile leaf, on
which the sporangia are already differentiated.

As is well known, the use of phenological information
as a hint for a preliminary choice of objects for studies
requires discretion, since experience has already shown
some of the pitfalls of an oversimplified attitude. Among
these are: 1) the existence, in some cases of several
limiting environmental factors, with non-coinciding annual
variations; 2) the existence, in some plants of different
processes leading to similar morphogenetic effects; 3) the
existence of additive requirements, which are satisfied in
succession in different times of the year; 4) the existence
of environmental requirements which are different for the
initiation of reproductive structures and for their develop-
ment. In natural conditions the interplay of these pro-
cesses, disclosed by physiological analysis, may be fairly
complicated so that the translation of phenological data
in physiological terms, and conversely, is seldom a straight-
forward matter.

b) <u>Experiments indicating a day-neutral behavior</u>.

Kaufhold (105) found that photoperiodism does not affect
the initiation of sporangia in the following species of ferns:
Blechnum spicant, Struthiopteris germanica, Dryopteris
spinulosa, Nephrolepis cordifolia, Polypodium aureum, and
Ceratopteris thalictroides.

Chouard (50), in long term experiments, has observed that Asplenium trichomanes, Asplenium viride, Asplenium septentrionale and Polystichum spinulosum are also independent of photoperiodism for the initiation of sporangia, although there is an annual periodicity in this process. As a consequence these species were classified by him as day-neutral or "photo-aperiodical," since they require light before and during the stage of reproductive differentiation, although this differentiation is neither accelerated, nor retarded, by photoperiodical treatments.

Recently Bloom and Voth (31) have tried, without success, to induce the formation of sporacarps in Regnellidium diphyllum by short-day and long-day treatments applied to plants grown in nutrient solutions specifically designed for the species. Although in this species photoperiodism is not the main environmental factor affecting sporocarp formation, this species is not day-neutral, as we shall see in the following.

c) Experiments establishing a short-day behavior.

As far as we are aware, the only information of this nature that can be found in the literature is in the work of Nakayama (142) with Salvinia natans. Plants of this species, cultivated in Knopp's nutrient solution, and submitted to continuous light, do not produce sori, whereas those submitted to short (7 hours) days initiate sori very quickly, as shown in Fig. 46.
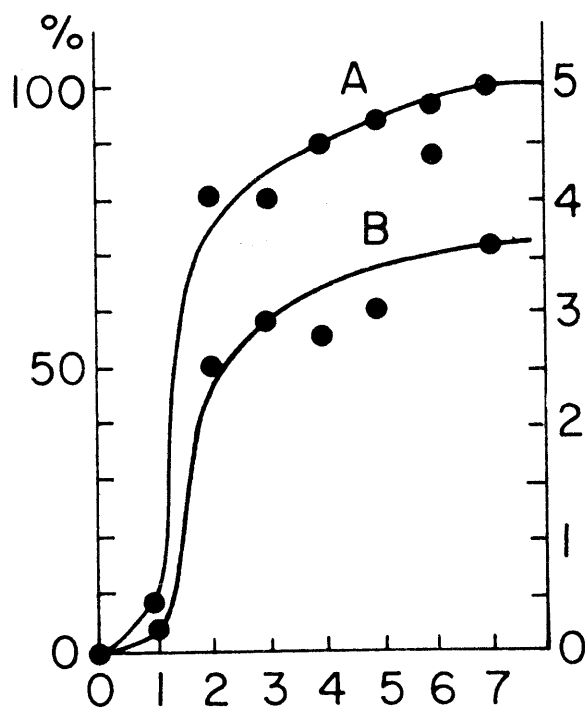
Fig. 46. Effect of long nights on the production of sori in Salvinia natans (from Nakayama (142)). Curve A: Ordinates: percentage of plants with sori; abcissae: number of long nights given. Curve B: ordinates: average number of sori per plant; abcissae: number of long nights given.

The experiments by this author indicate a critical night-length between 8 and 16 hours.

In this species the percentage of fertile plants (in samples of 50 plants each) was shown to be a crescent function of the number of floating leaves left in the plants, as shown in fig. 47. Also the average number of sori formed per plant is found to be proportional to the number of floating leaves per plant, as shown in Fig. 47.

Some experimental results indicate the possibility that a stimulus may be formed in the floating leaves and translocated to the young water leaves of the nodes kept without floating leaves by defoliation, where sori are initiated and developed. It was also demonstrated that no sori are initiated in photoperiodically induced plants grown in a nitrogen-deficient nutrient solution (142).

2. Behavior of Asplenium bulbiferum.

As was mentioned before this species offers some advantages as an object for studies on the initiation of sporangia, owing to the fact that it forms abundant buds in the leaves. These buds develop into adventitious plantlets, which are attached to the vascular system of the leaf. This peculiarity of organization makes it possible to obtain a uniform clone of plants for experiments and also provides the possibility of studying the transmission of induction for the initiation of sporangia from an adult plant to its adventitious plantlets.
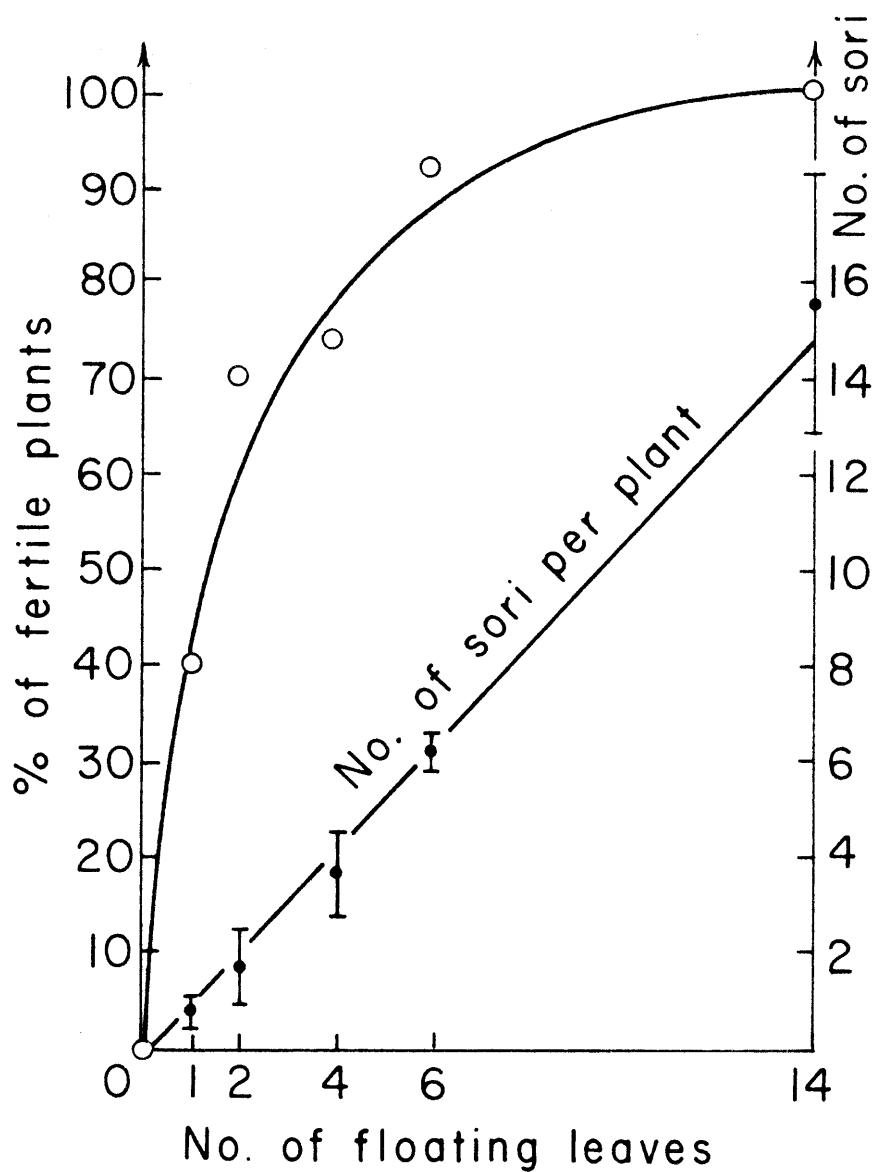
Fig. 47. Effect of the number of floating leaves on the production of sori in Salvinia natans in short-day conditions (from data of Nakayama (142)). Percentage of fertile plants and number of sori per plant as a function of the number of floating leaves per plant.

As was already noted, during development, the leaves of this plant change from a typical sterile shape, with broad pinnae (fig. 4) to a shape typical of the fertile blade, with narrow pinnae (fig. 5)

a) Effects of the photoperiod on the initiation of sporangia.

Experiment #1. (July 21, 1955-September 17, 1955.)

40 young plants growing at a nursery in Monrovia, all without fertile leaves, were introduced into the Earhart Laboratory and planted in a mixture of vermiculite and gravel. These plants were irrigated daily with the modified Hoagland's nutrient solution used in the Earhart Laboratory and cultivated at $20^{\circ}C$, with two different photoperiodic treatments. 20 plants received days of 8 hours of natural light, whereas the other 20 were given a total photoperiod of 16 hours (8 hours of sunlight + 8 hours of supplementary artificial, incandescent and fluorescent light, 400 ft.-c.). Results are shown in Table III and in fig. 48.

These results suggest a quantitative long-day behavior of this plant at $20^{\circ}C$.

Experiment #2. (September 17, 1955-November 5, 1955.)

The purpose of this experiment was to observe the effect of very short (4 hr.) days on the morphogenesis of the new developing leaves of adult fertile plants.

Nine adult fertile plants, previously cultivated at

Table III

Effect of photoperiod on the initiation of sporangia
in Asplenium bulbiferum at 20°C.

Dates	Days of treatment	8 hr. photoperiod		16 hr. photoperiod	
		# Sterile plants	# Fertile plants	# Sterile plants	# Fertile plants
July 21	0	20	0	20	0
July 26	5	20	0	19	1
Aug. 5	15	20	0	19	1
Aug. 12	22	20	0	18	2
Aug. 19	29	20	0	17	3
Aug. 29	39	18	2	6	14
Sept. 3	44	17	3	2	18
Sept. 17	58	12	8	2	18
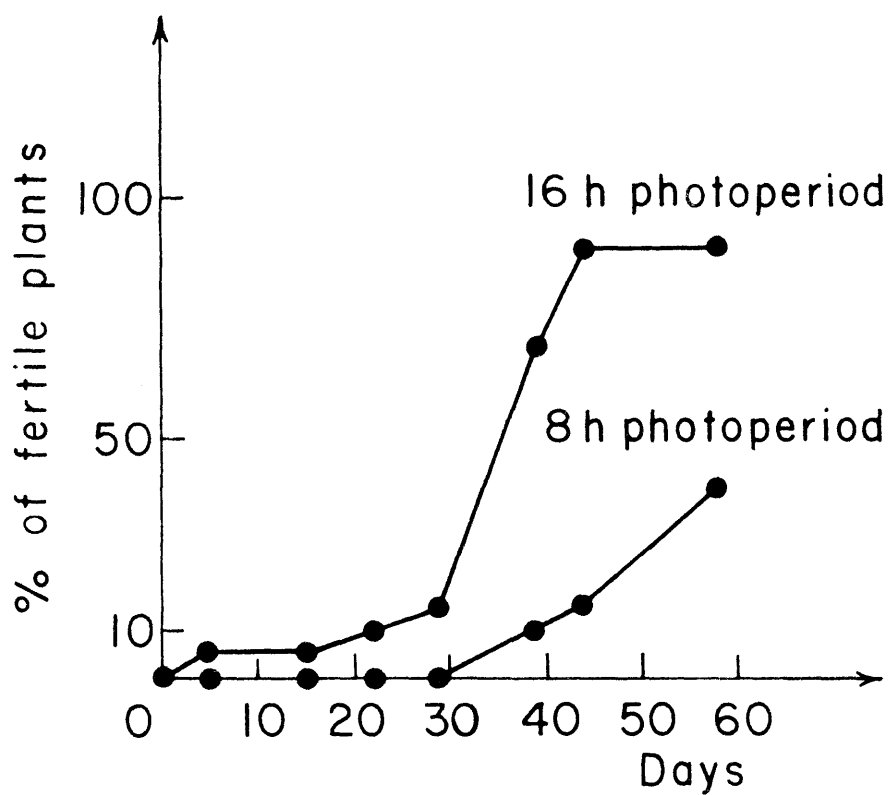
Fig. 48. Asplenium bulbiferum. % of fertile plants as a function of time in 8 hour and in 16 hour photoperiods at 20°C.

20°C, 16 hour photoperiods, as described in Experiment #1, were placed in days of 4 hours at the same temperature (20°C) and the new leaves, developed after the beginning of the treatment, were observed periodically. Results are presented in Table IV and in fig. 50.

Leaves developed during the treatment differ from leaves developed at 16 hour photoperiods by the absence of sporangia and also by a different shape, as shown in fig 49. Since this is the shape of the juvenile leaves and since it is known that such leaves can be skipped in the developmental sequence of some ferns by an artificial supply of sugars (188), the lack of initiation of sporangia in such extreme short days could be due not only to an excessive length of the night, but also to the excessive shortness of the day. It is known, for instance, that for Xanthium pennsylvanicum, for the same inductive dark treatment, the rate of flower development is minimum in the winter months (160). This effect was attributed to the low light intensity prevailing in these months and it was shown experimentally by Liverman and Bonner (123) that this effect can be overcome by administration of sugars and Krebs cycle acids to the plants during the inductive dark period. Therefore it was decided to observe the effect of the length of the night in cycles of 48 hours, since the use of this cycle allows treatments by long nights and long days simultaneously.

However, since the adventitious plantlets appear as

Table IV

Effect of 4 hour photoperiods on the initiation of
Sporangia in Asplenium bulbiferum at 20°C

Dates	Days of treatment	# of plants with the last leaf fertile
Sept. 17	0	9
Sept. 24	7	8
Oct. 1	14	8
Oct. 6	19	6
Oct. 16	29	4
Oct. 23	36	4
Oct. 30	43	1
Nov. 6	50	0

Fig. 49. Asplenium bulbiferum. Effect of 4 hour photoperiods at 20°C on leaf development. Left: fertile frond developed in 16 hour photoperiod. Right: sterile frond developed in 4 hour photoperiod.
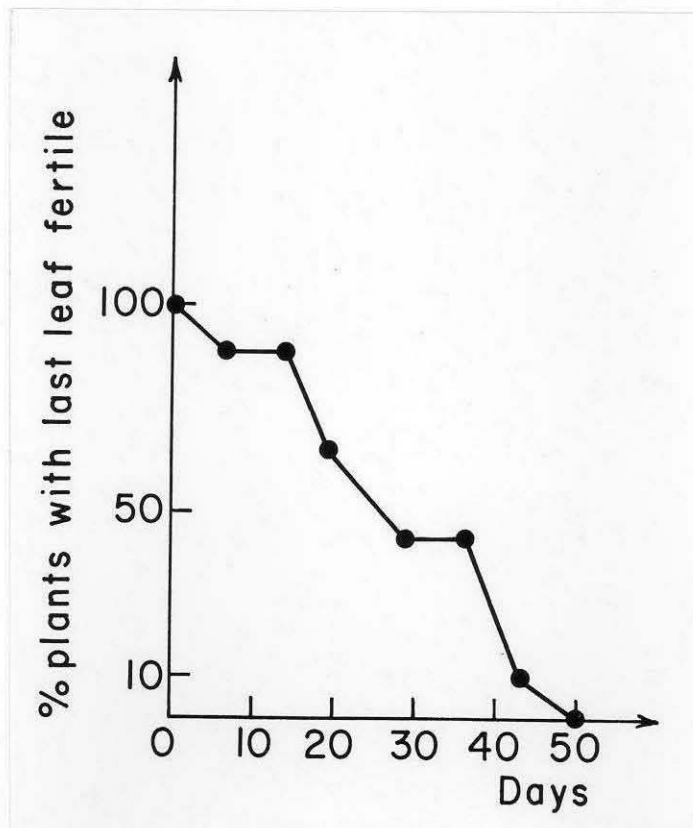
Fig. 50. Asplenium bulbiferum. % of plants with the last developed leaf with sporangia as a function of time, in 4 hour photoperiods at 20°C.

fertile and as sterile plants, before this experiment
could be done it was necessary to ascertain whether isolated
fertile plants continue the production of fertile fronds
after separation from the adult plant, in short and in long
days. If this were the case, fertile and sterile plantlets
would not be in comparable physiological conditions after
isolation from an adult plant and even sterile plantlets
could be already induced if attached to adult plants in long
days. Practically this would require the growing of adult
plants under nights of 24 hours, in 48 hour cycles, for
very long periods, in order to use their adventitious
plantlets as non-induced objects for the planned experiment.
Fortunately, a parallel series of experiments had been
started in order to investigate the behavior of adventitious
plants after isolation from the adult plant, and their
results made unnecessary the use of this time-consuming
precaution.

b) Behavior of adventitious plants after isolation
from the adult plant.

Experiment #3. (July 19, 1955-October 8, 1955)

Ninety-five adventitious plantlets with fertile leaves,
developed from an adult plant grown at 20°C from 8:00 a.m.
to 4:00 p.m. and at 14°C from 4:00 p.m. to 8:00 a.m.
natural photoperiod, were separated from the "mother-plant"
and planted in vermiculite moistened from below with nutri-
ent solution. Excessive evaporation was prevented by
shielding the plant containers with transparent plastic

covers. The isolated adventitious plants were kept in the
same conditions prevailing in the culture of their "mother-
plant."

Under these conditions roots and new leaves were
developed, but not a single fertile leaf, whereas, in the
same environmental conditions, the adult plant, as well as
their attached adventitious plantlets, continued producing
fertile leaves. The change in leaf morphology was already
apparent in the first leaf developed after separation from
the adult plant. Leaves produced after isolation of the
adventitious plants had much broader pinnae, typical of the
sterile leaves (see figs. 51, 52, 53).

Experiment #4. (September 9, 1955-January 31, 1956).

In the beginning of September, 1955 it was already
apparent in the plants of Experiment #1 that long day
conditions at 20°C accelerated the production of fertile
leaves in this species. On the other hand, it was also
apparent, in plants of Experiment #3, that separation of
adventitious plantlets from the adult plant leads to the
loss of the ability to initiate sporangia, under the condi-
tions of Experiment #3. Therefore, it was decided to start
another experiment, in which originally fertile and originally
sterile adventitious plantlets were separated from the leaves
of adult plants and given 16 hours photoperiods (8 hours
sunlight and 8 hours artificial light, as in Experiment #1),
at constant temperature (20°C).
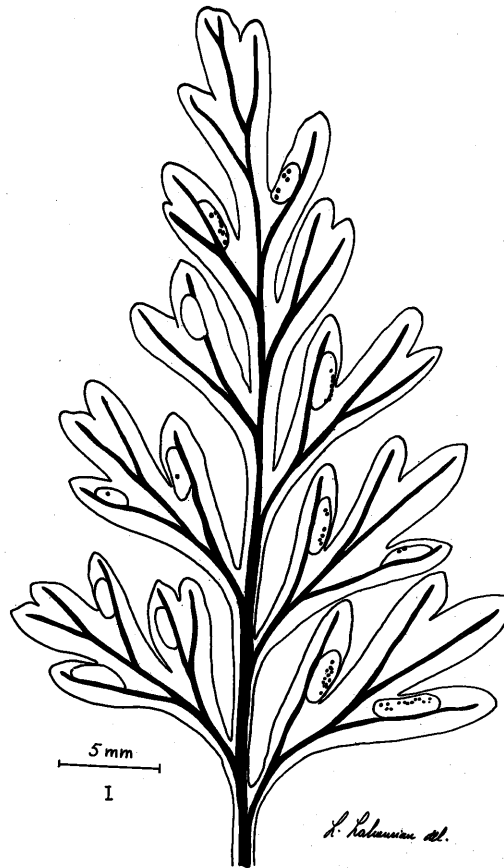
Fig. 51. Asplenium bulbiferum. Fertile leaf developed in adventitious bud, while still attached to adult fertile leaf. Dots in soral areas mark ripened sporangia.
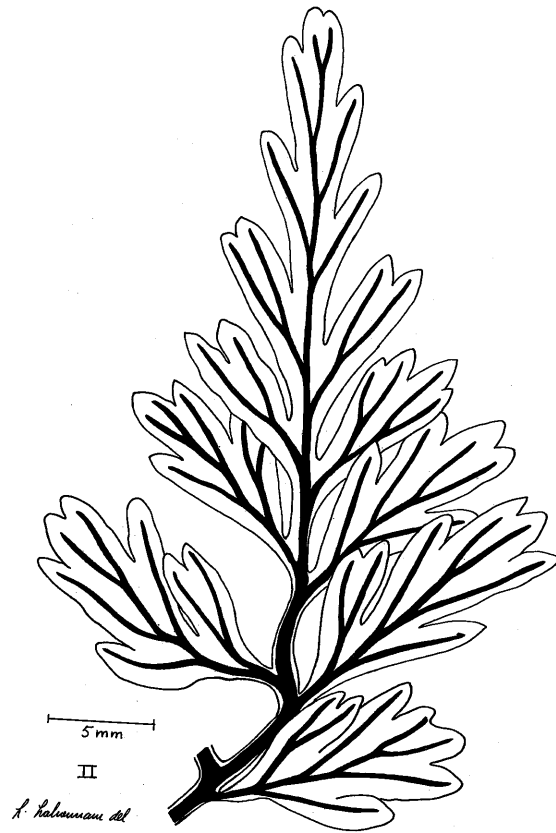
Fig. 52. Asplenium bulbiferum. First leaf of adventitious bud, developed after isolation of bud from adult plant.
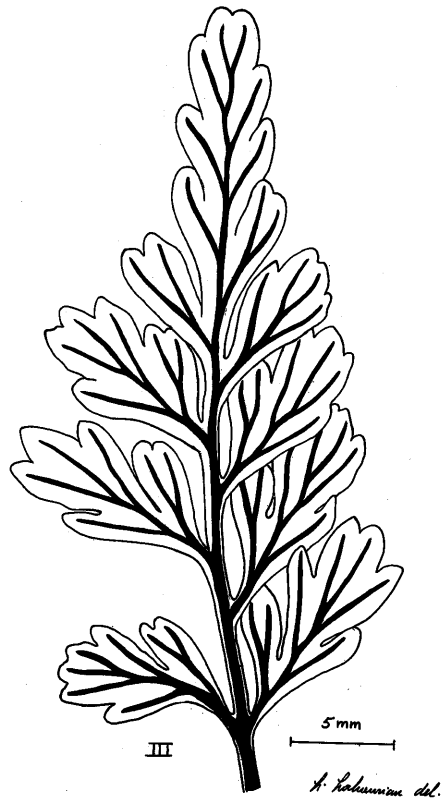
Fig. 53. Asplenium bulbiferum. Second leaf of
adventitious bud, developed after isolation of bud from
adult plant.

Ten fertile plantlets and 10 sterile plantlets, from the same source used in Experiment #3 were submitted to this treatment for a long period of time, in order to ascertain whether originally fertile plantlets would start initiation of sporangia earlier than originally sterile plantlets. The results are given in Table V. (See also fig. 54). The absence of fertile leaves in the period that follows immediately the isolation of the buds is not due to absence of new leaves in this period. The plants continued to produce new leaves, but these were sterile. These results indicate that the originally fertile plants have no advantage over the originally sterile plants in recovering the capacity of initiating sporangia under favorable photoperiodic conditions. It also verifies the observation that adventitious plants lose the ability to initiate sporangia upon separation from the adult plant. Since this capacity is not lost in fertile plantlets that remain attached to the adult plant, these results are taken as evidence that early initiation of sporangia in adventitious plants requires the vascular attachment to the leaf of the adult plant.

Since after isolation from the adult plant no residual effect was found of the previous induction of the initiation of sporangia, it is safe to use adventitious plantlets in order to test the effects of long nights in 48 hour cycles.

Experiment #5. (October 24, 1956-June 16, 1957).

Forty-eight adventitious plants were isolated from an

Table V

Effect of isolation of fertile and sterile adventitious
plants, grown in 16 hour photoperiods at 20°C, in
the production of sporangia.

Dates	Days of treatment	Originally fertile plantlets. # of plants with new fertile leaves	Originally sterile plantlets. # of plants with new fertile leaves
1955			
Sept. 9	0	-	-
Sept. 17	8	0	0
Sept. 24	15	0	0
Oct. 1	22	0	0
Oct. 8	29	0	0
Oct. 15	36	0	0
Oct. 22	43	0	4
Oct. 29	50	1	4
Nov. 5	57	3	5
Nov. 9	61	4	5
Nov. 12	64	4	6
Nov. 19	71	4	6
Nov. 26	78	4	6
Dec. 3	85	5	6
Dec. 16	98	6	6
Dec. 31	113	6	6
1956			
Jan. 31	144	10	10
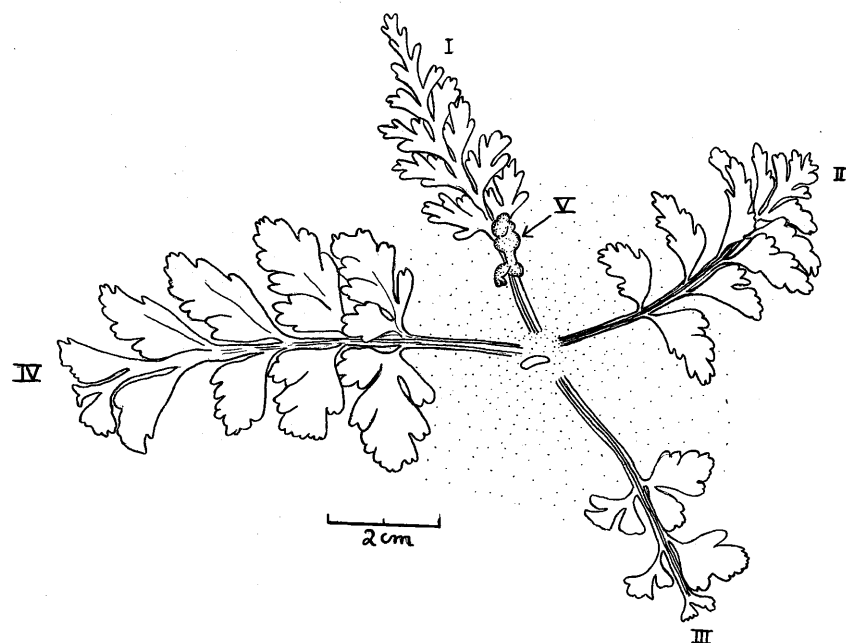
Fig. 54. Asplenium bulbiferum. An isolated
adventitious plantlet with 4 developed leaves: I. Fertile
leaf, developed when the bud was still attached to adult
leaf; II, III, IV sterile leaves developed after isolation
of the bud; V. Young leaf.

adult plant grown at 20°C, 16 hour photoperiod and rooted
in a moist chamber, in the same conditions (20°C, 16 hour
photoperiod). They were divided in 4 equal groups and
given the following treatments:

Group	Hours of sunlight	Hours of artificial light (500 ft. c.)	Hours of darkness
1	8	16	24
2	8	17	23
3	8	18	22
4	8	24	16

Results of periodic observations are shown in Table VI,
and in fig. 55.

These results show that, for the initiation of sporangia,
Asplenium bulbiferum behaves as a long-day plant with a
critical night-length of 23 hours in 48 hour cycles. The
initiation of sporangia in this plant, in natural, 24 hour
cycles at 20°C is not limited by photoperiodism in a
qualitative manner, since, in such cycles, nights can
scarcely be long enough to constitute a limiting factor
before the shortness of the day also becomes a limiting
factor.

3. Behavior of Osmunda Claytoniana.

   a) Annual cycle of development.

Osmunda Claytoniana is a species found in two separate
areas: East and Middle West of North America and Oriental

Table VI

Effect of the length of the night on the initiation of sporangia in young plants of Asplenium bulbiferum cultivated in 48 hour cycles at 20°C.

Dates	Days of treatment	24 hr. dark		23 hr. dark		22 hr. dark		16 hr. dark	
		# fertile plants	%	# fertile plants	%	# fertile plants	%	# fertile plants	%
1956									
Oct. 24	0	0	0	0	0	0	0	0	0
Nov. 13	20	0	0	0	0	0	0	0	0
Dec. 3	40	0	0	0	0	0	0	0	0
Dec. 23	60	0	0	0	0	0	0	0	0
1957									
Jan. 12	80	0	0	0	0	0	0	0	0
Feb. 1	100	0	0	0	0	0	0	0	0
Feb. 21	120	0	0	0	0	0	0	0	0
March 3	130	0	0	0	0	0	0	0	0
March 13	140	0	0	0	0	0	0	0	0
March 23	150	0	0	0	0	0	0	0	0
April 2	160	0	0	0	0	0	0	0	0
April 12	170	0	0	0	0	0	0	0	0
April 17	175	0	0	0	0	2	16	3	25
April 22	180	0	0	0	0	4	33	5	42
April 27	185	0	0	0	0	4	33	6	50
May 7	195	0	0	0	0	5	42	9	75
May 17	205	0	0	0	0	6	50	10	83
May 27	215	0	0	0	0	7	58	11	91
June 6	225	0	0	0	0	8	66	12	100
June 16	235	0	0	0	0	8	66	-	-
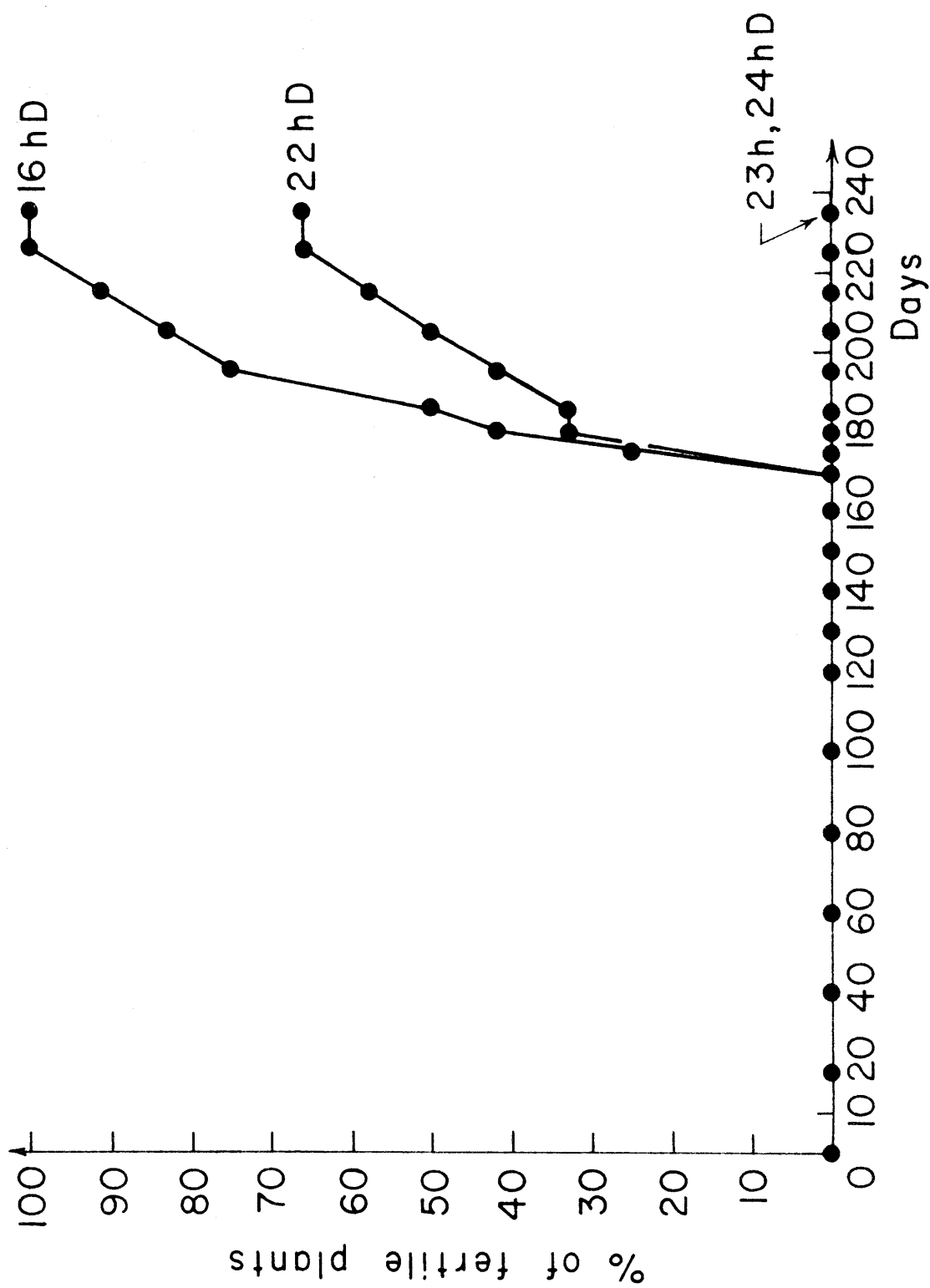
Fig. 55. Effect of the length of the dark period on the initiation of sporangia in Asplenium bulbiferum (48 hr. cycles).

Asia (79). It is a perennial plant with a well defined
annual cycle of development. In the spring the sporophylls
grow first and are followed, throughout the spring and the
summer by trophophylls. All adult leaves die in the fall
and growth is resumed in the following spring. A dissection
of a rhizome apex in the fall shows all types of leaves,
which are, in acropetal succession: cataphylls, sporophylls,
trophophylls and undifferentiated leaves.

Undifferentiated leaves.

These leaves show a flattened base, circinate vernation
and pinnae developing in acropetal succession. The pinnae
are club shaped, not flattened, white, with slightly wavy
margins. The length of the pinnae and the number of
marginal undulations decreases from the base to the apex
of the frond (see figs. 56, 57, 63).

Sporophylls.

The differentiation of these leaves, as far as the
fertile pinnae are concerned, is virtually completed in the
fall, when sporangia are already in a very advanced stage
of development. Fig. 58 shows a young fertile frond, with
a basal sterile pinnae and a fragment of a fertile pinna
drawn at the same scale.

The first fact that draws attention in this type of
leaf is its general organization, with a basal set of
sterile pinnae, an intermediate set of fertile pinnae and
an apical set of sterile pinnae (see figs. 32, 33).
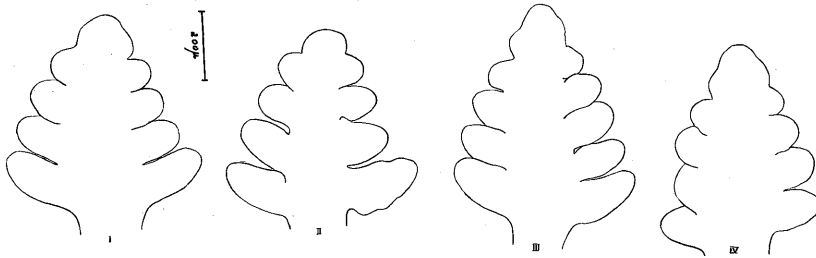
Fig. 56. Osmunda Claytoniana. Pinnae of an undifferentiated leaf.
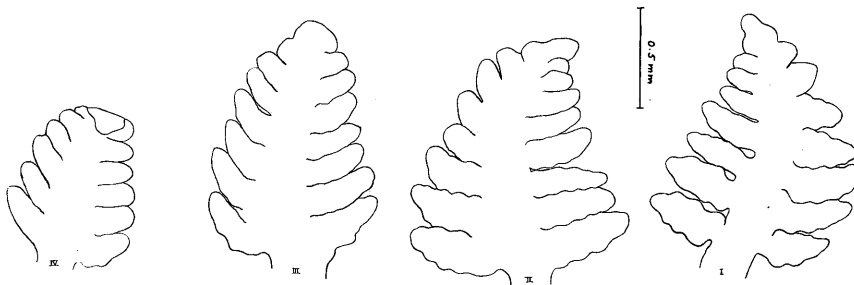
Fig. 57. Osmunda Claytoniana. Pinnae of an undifferentiated leaf.
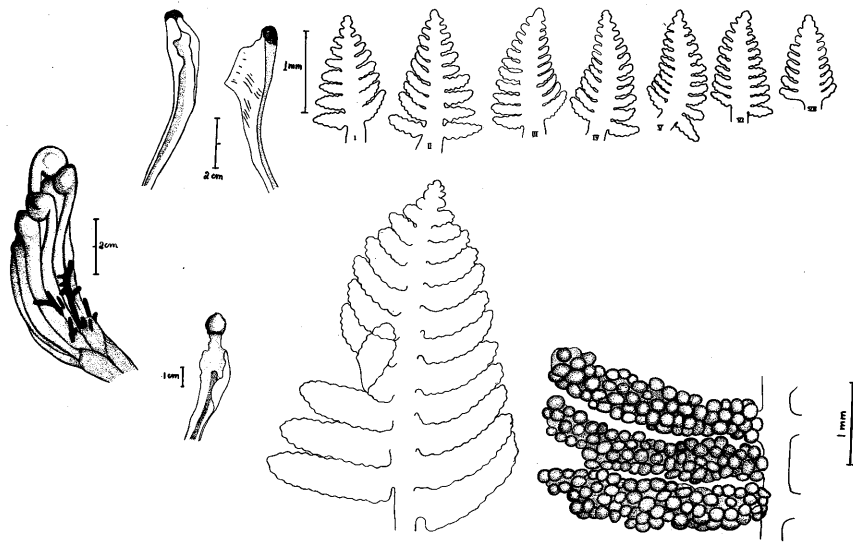
Fig. 58. Osmunda Claytoniana. Left: Apical bud
with young leaves and adventitious roots. Top row: Two
cataphylls and a series of pinnae of a cataphyll in
acropetal order. Bottom row: A young sporophyll, one of
its sterile pinnae and a fragment of a fertile pinna.

Dissection of young sporophylls reveals the existence of a double longitudinal gradient of development of their pinnae. Starting from a pinna which is usually the fifth development is progressively retarded both towards the base and towards the tip of the frond. This sequence of development is morphologically expressed by three features: the number of segments of the pinna, the shape of the margin and the color.

Fertile pinnae with already differentiated sporangia are conspicuous in the frond by a much deeper green color, as compared with the sterile pinnae of the same frond. This difference is already apparent in the young fertile pinna, when the sporangia are being initiated and the green color fades in successive pinnae further to the base and to the tip of the frond.

The shape of the margin of the pinnae shows a similar variation. The first morphological sign of the initiation of sporangia is the splitting of the marginal crenations by a plane parallel to the plane of the pinna blade, forming two lips at each crenation, which appear partially superimposed in face view (see pinna IV in figs. 59 and 60). Thus every crenation of the margin of the undifferentiated pinna is replaced, in the young fertile pinna, by a stacked pair of round marginal projections. Each of these marginal projections is a meristem that will produce a sporangium. During subsequent stages of development the sporangia gradually readjust their positions, sliding from the margin
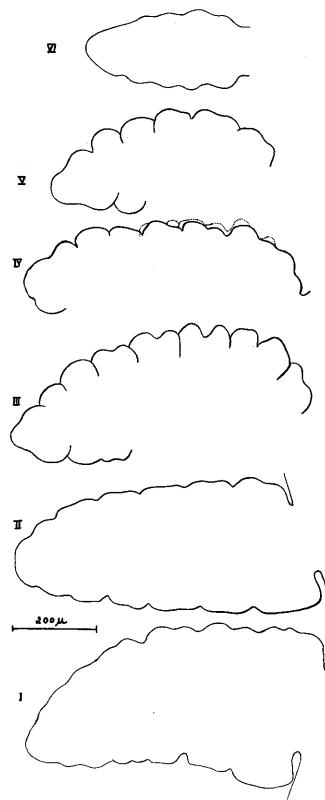
Fig. 59. Osmunda Claytoniana. Some of the basal
pinnae of a developing sporophyll, in acropetal order.
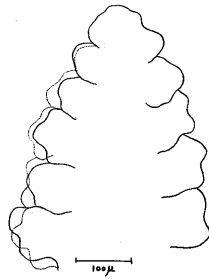
Fig. 60. Osmunda Claytoniana. Fertile pinna just before sporangia are initiated.

to the surface of the narrow blade of the fertile pinna. This produces the crowded appearance of the adult fertile pinna.

Observation of fertile pinnae in early stages of development is possible by the fact that sporophylls develop in acropetal sequence in the rhizome. The stages in development, as revealed by the above described changes in the shape of the margin of the pinna, show a gradual delay in different pinnae of the same frond, both basewards and tipwards.

Likewise, the number of segments of the pinna decreases towards the base and towards the tip of the frond.

The progressive delay of differentiation of fertile pinnae in both longitudinal directions makes understandable that the sporangial differentiation will become less stable as we consider pinnae more removed from the one that started differentiation first (usually it is the fifth). Thus, it is not surprising that the abnormal pinnae would be found so often in the limits of the fertile and sterile regions of the sporophyll.

Another conspicuous characteristic of the development of the sporophyll is that the pinnae of the apical part of the frond, following the fertile pinnae, are rather slow in their differentiation, remaining relatively undifferentiated when the basal pinnae are already clearly determined.

Trophophylls.

The differentiation of the pinnae of trophophylls is entirely similar to that of the sterile pinnae of the sporophylls. Fig. 61 shows the longitudinal sequence of pinnae of a young trophophyll. The color of the pinnae remains a pale yellowish green till the unfolding of the leaf, the blade is progressively flattened and cut in pinnate segments; the indentation of the margin is always smooth and undulated.

Cataphylls.

In this species cataphylls are generally similar to those described by Steeves and Wetmore for Osmunda cinnamomea (169). The base of such leaves is broader and more fleshy than in trophophylls and sporophylls and the lateral "wings" are much broader. The phyllopodium region is, as in the other types of leaves, densely covered with hairs (white in sporophylls, trophophylls and undifferentiated leaves, red-brown in cataphylls) and, upon dissection, the pinnae of such leaves are found to be brown and dead. Comparison of pinnae of cataphylls (fig. 62) with those of undifferentiated leaves (fig. 63), of young sporophylls and of young trophophylls, shows that, in different cataphylls, the pinnate part of the frond may have been killed in different stages, varying from those of very young undifferentiated leaves, to those of completely differentiated sporophylls, with fully developed sporangia.
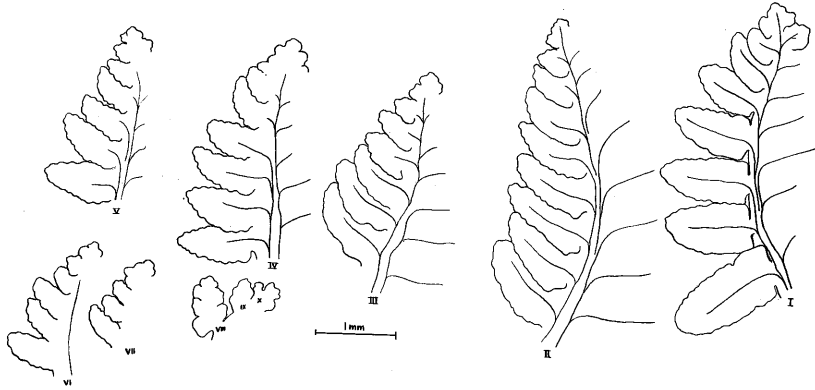
Fig. 61. Osmunda Claytoniana. Pinnae of a young trophophyll.
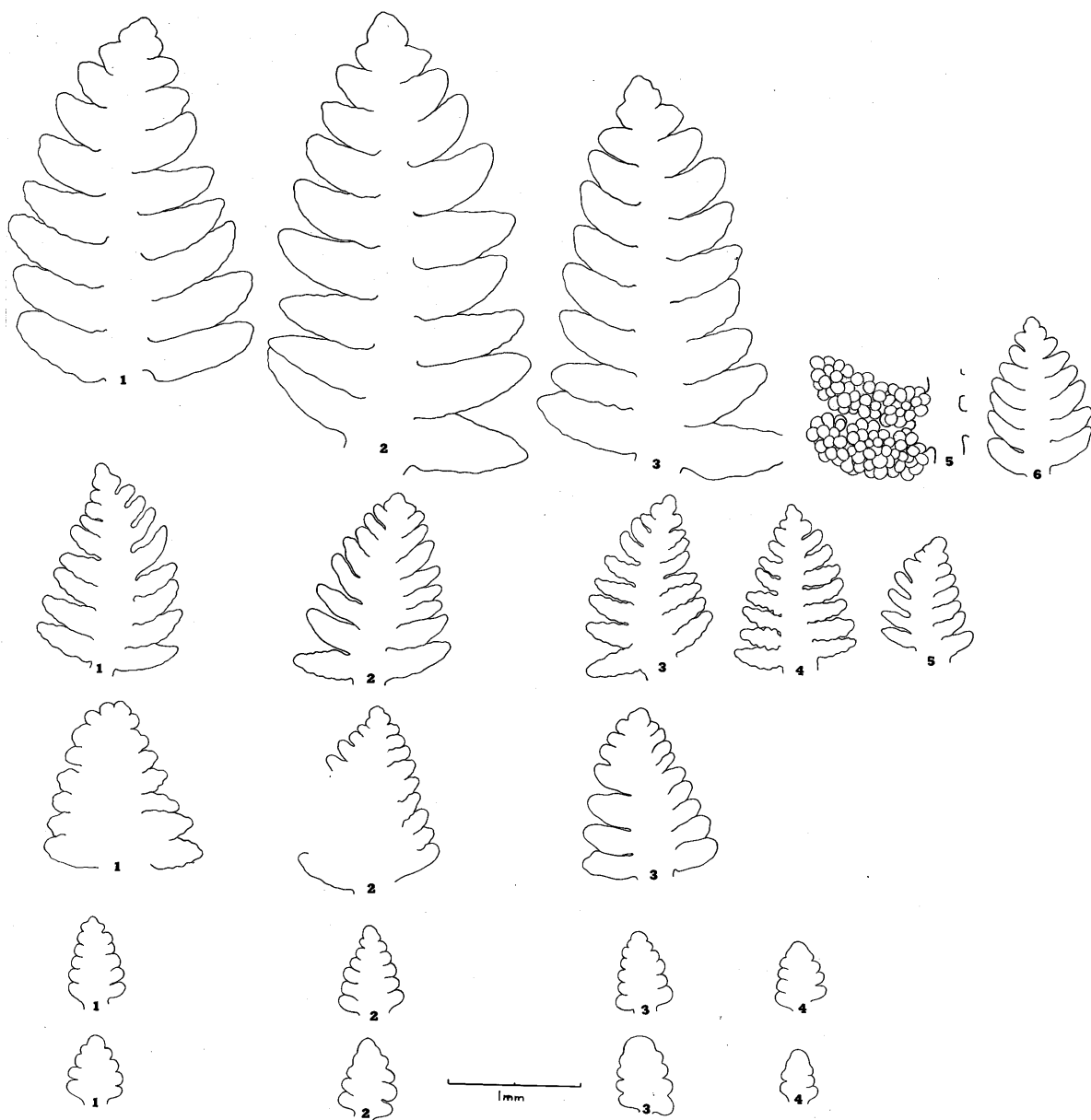
Fig. 62. Necrotic pinnae of different cataphylls of Osmunda Claytoniana, showing the fact that cataphylls may be made, in this species, from leaves in different stages of development.
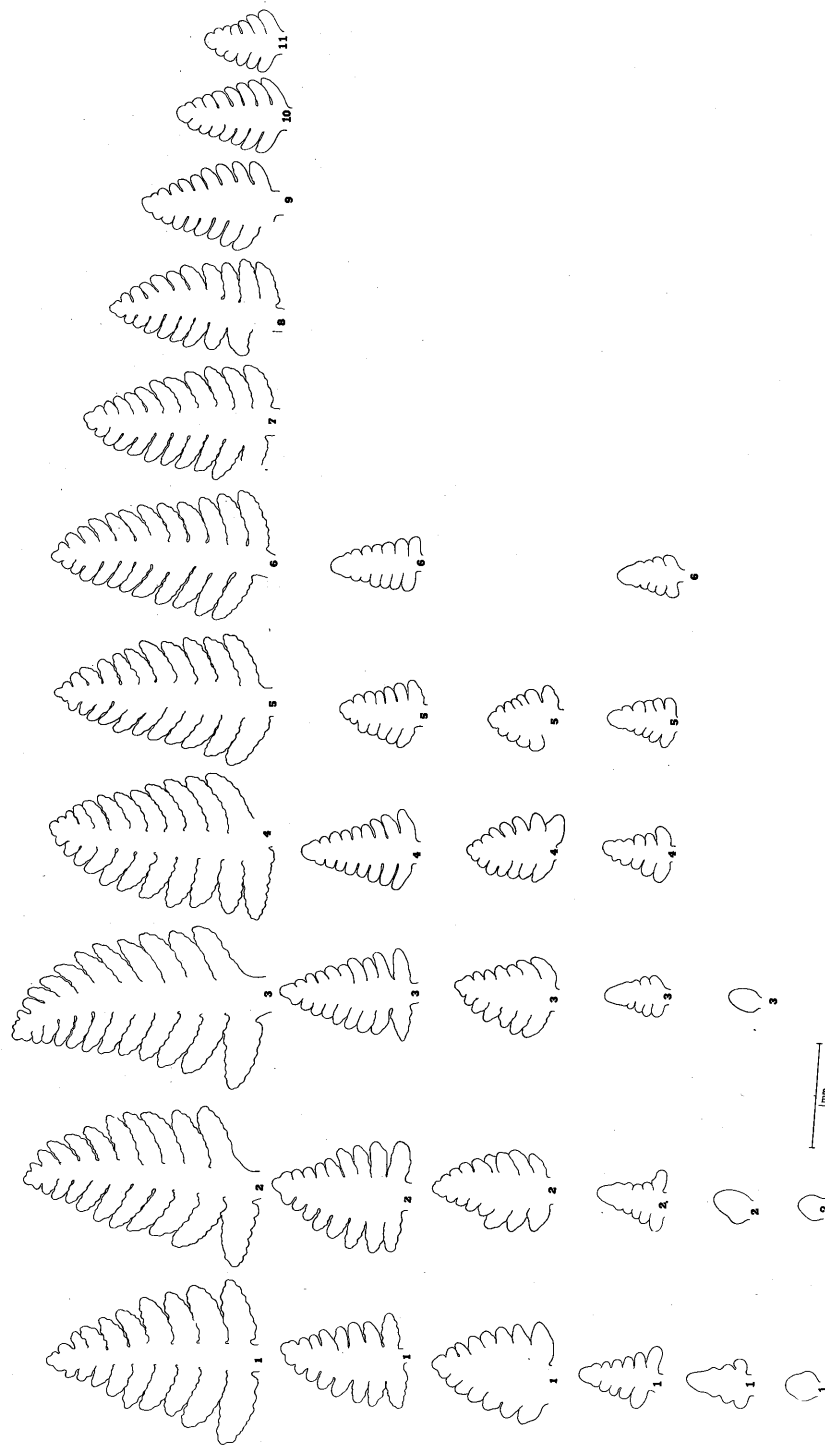
Fig. 63. Pinnae of undifferentiated young leaves of Osmunda Claytoniana.

All these types of cataphylls can be found in the rhizome
of plants developed under controlled conditions (17°C, days
of 8 hours). It is impossible to draw a line between the
different types of young leaves which bear necrotic pinnate
region. The cataphylls are then characterized by a much
broader leaf base and by the presence of necrosed pinnae.

Stage of differentiation of young leaves at the beginning of
the annual cycle.

In order to ascertain the stage of differentiation of
young leaves at the beginning of the annual cycle of
development 10 adult plants were dissected, from a shipment
harvested during the winter of 1956 originating from the
living collections of Isaac Longley Williams, Exeter,
New Hampshire. Results are presented in Table VII.

As may be seen the leaves differentiated in the pre-
vious year are followed by undifferentiated leaves only.

The same fact becomes apparent when plants harvested
before the end of the winter are cultivated in low
temperature. In these conditions differentiation of the
leaves is slowed down and the original winter-condition
is essentially retained. This may be seen by results of

Experiment #6. (March 20, 1957-June 14, 1957).

Nineteen plants from the source mentioned above were
harvested in the winter of 1957 and grown at the Earhart
Laboratory at 10°C, artificial light (400 ft. c.); 9 plants

Table VII

Types of leaf found in the rhizome apices of 10 plants
of Osmunda Claytoniana harvested during the winter

Plant #	Adult, fully differentiated leaves	Young leaves, with pinnae, but undifferentiated
1	17	6
2	9	5
3	10	6
4	8	5
5	11	8
6	15	4
7	14	6
8	14	5
9	13	5
10	8	4

were grown in days of 8 hours and 10 plants in days of 24 hours (continuous light). After 86 days the plants were harvested and individually dissected; the following results were obtained (see Table VIII). In the counting of the number of undifferentiated leaves we have included here all leaves beyond the stage in which the most advanced pinna is a single-lobe structure, as shown in the bottom of fig. 63.

The fact that at the end of the winter plants still have no differentiated young leaves, taken together with the fact that cataphylls, young sporophylls and young trophophylls are already differentiated in the fall shows that differentiation of leaves in this species is performed during the spring and summer months.

b) Relations between sporophylls and cataphylls.

1. In conditions that allow the development of these two types of leaves:

a) cataphylls precede sporophylls in the sequence of leaves in the terminal bud;

b) there may be undifferentiated leaves between the last cataphyll and the first sporophyll.

This is shown by the results of the following experiment:

Experiment #7. (October 24, 1956-March 23, 1957).

Twenty adult plants, from a shipment harvested at Exeter, New Hampshire, during the late winter of 1955-1956

Table VIII

Types of leaf developed in plants of Osmunda Claytoniana grown at 10°C for 86 days

C = cataphyll   T = trophophyll

Plant #	8 hr. photoperiod			24 hr. photoperiod		
	Adult leaves	Young, differentiated	Undiff.	Adult leaves	Young, differentiated	Undiff.
1	2	0	12	8	1(T)	8
2	3	0	9	9	1(T)	7
3	8	0	7	8	1(T)	8
4	8	0	5	6	1(C)	7
5	9	0	8	8	1(T)	6
6	4	0	9	7	0	6
7	5	1(C)	4	7	0	11
8	6	0	4	8	1(C)	13
9	5	0	5	7	2(T)	8
10	3	1(C)	4	7	0	7
	-	-	-	3	1(T)	6

were stored in moist vermiculite in a cold room at 4°C, days of 8 hours (artificial light), till the beginning of the experiment.*

On October 24, 1956, these plants were planted in individual crocks, in a mixture of vermiculite and gravel and irrigated daily with the laboratory nutrient solution. They were cultivated at 17°C, days of 8 hours (sunlight). The leaves differentiated between the spring and the fall of 1955 developed well, but they were not taken into account in judging the results of the treatment given, because they were determined before such treatment was started. After 86 days (January 18, 1957) 10 plants were dissected and the individual leaves examined in a dissecting microscope. The results presented in Table IX give, for each plant, the succession of leaf types in acropetal order in the rhizome tip.

Similar results are observed in:

Experiment #8. (October 24, 1956-January 18, 1957).

Twenty plants of the same source used in Experiment #7 were cultivated at 17°C, in continuous light (sunlight from 8:00 a.m. to 4:00 p.m. and supplementary artificial light 400 ft. c. for the rest of the time). After 86 days the plants were harvested and dissected. Results are given

---

*This delay was indispensable, since the experiment was designed as one of long duration and it would have to be interrupted if started before the summer of 1956, due to the temporary shut-down of the Earhart Laboratory for repairs.

Table IX

Types of leaf developed in plants of Osmunda Claytoniana
grown at 17°C, 8 hour photoperiods, for 86 days

C = cataphyll
S = sporophyll
U = undifferentiated leaf

Plant #	Adult leaves	Young leaves														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	15	C	C	U	S	S	S	S	S	S	S	U	U	U		
2	10	C	C	U	U	S	U	U	U	U	U	U	U			
3	17	U	U	U	U	U	U									
4	12	C	C	C	C	C	U	S	S	U	U	U				
	10	C	C	C	C	C	C	C	C	U	U	U				
5	11	C	C	U	S	U	U	U	U							
	8	C	U	U	U											
6	9	C	C	C	S	S	S	S	U	U	U					
	10	C	C	U	S	S	U	U	U							
7	10	C	C	C	U	U	U	U	U							
8	2	C	C	U	U	U										
	5	C	C	C	S	U	U	U	U	U						
9	18	C	C	C	C	S	S	S	S	S	S	S	S	U	U	U
10	12	C	C	C	C	C	C	C	U	S	S	S	U	U	U	

in Table X.

2. <u>In conditions that allow development of cataphylls and sporophylls, when the annual cycle of development ceases</u>:

    a) <u>undifferentiated leaves between the last cataphyll and the first sporophyll tend to disappear</u>;

    b) <u>occasionally the first sporophylls undergo a necrosis of the pinnae and thus become cataphylls</u>;

    c) <u>the sequence of leaves in the terminal bud is: cataphylls--sporophylls--trophophylls--undifferentiated leaves</u>.

This is shown by the results of the continuation of Experiment #7.

<u>Experiment #7 (continuation)</u>.

After 140 days of culture at 17°C, 8 hour photoperiod (March 12, 1957) all adult leaves developed since the beginning of the treatment were dead and no new leaves were growing. One hundred fifty days after the beginning of the experiment the 9 remaining plants were dissected (1 plant died). Results are shown in Table XI.

Comparison of this table with Table IX shows that there has been an increase in the number of cataphylls, at the expense of the "cataphyllization" of the undifferentiated leaves that, at the initial stages of the developmental period, stood between the last cataphylls and the first sporophylls. This shows that, in these conditions, the two

Table X

Types of leaf developed in plants of Osmunda Claytoniana grown at 17°C, continuous light for 86 days

C = cataphyll;   S = sporophyll;   U = undifferentiated leaf

Plant #	Adult leaves	Young leaves																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	10	C	C	C	C	U	S	S	S	U	U	U												
	8	C	C	U	U	U	U	U	U	U	U	U												
2	20	S	S	S	S	U	U	U	S	S	S	S	S	S	S	S	S	S	U	U				
3	17	C	C	C	S	S	S	S	S	S	U	U	S	U	S	U	U	S	U	U				
4	14	S	S	S	S	S	S	S	S	S	S	U	U	U	U	U								
	12	C	C	S	S	S	S	S	S	S	S	S	S	S										
5	19	S	S	S	S	S	S	S	S	U	S	U	U	U	U									
6	21	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	U	U	U					
7	14	C	S	S	S	S	S	S	S	S	S	S	S	S	U	U	U							
8	13	C	C	C	C	SC	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	U	U	U
9	11	C	C	S	S	S	U	U	U	U	U													
10	10	S	S	S	S	S	U	U	U															
	8	C	S	S	S	S	S																	

Table XI

Types of leaf developed in plants of Osmunda Claytoniana grown at 17°C,
8 hour photoperiod, for 150 days

C = cataphyll; S = sporophyll; T = trophophyll; U = undifferentiated leaf

Plant #	Adult leaves	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
																	Young leaves										
11	0	C	C	C	S	S	S	S	S	S	S	S	T	T	T	T	T	U	U	U							
12	0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	T	-	T	T	T	T	T	U	U	U
	0	C	C	C	C	C	S	U	S	S	S	S	S	S	S	T	U	U	U								
13	0	C	C	U	SC	S	S	S	S	U	U	U															
14	0	C	C	SC	SC	S	S	S	S	S	S	S	S	S	S	S	S	S	T	U	U	U					
15	0	C	C	C	C	C	S	S	S	S	T	T	T	T	T	T	U	U	U	U	U						
16	0	C	C	C	C	C	S	S	S	S	S	S	S	T	T	T	U	T	U	U	U						
17	0	C	SC	SC	S	S	S	S	S	S	T	T	T	T	U	U	U	U									
	0	C	C	C	S	S	T	T	T	T	T	U	U														
18	0	C	C	C	C	C	S	S	T	T	T	T	T	T	T	U	U	U									
19	0	C	SC	SC	C	C	C	C	C	C	C	C	U	C	S	S	S	S	S	T	U	U	U				
	0	C	SC	SC	SC	C	C	C	C	C	C	C	U	C	C	C	C										

types of leaf destiny (cataphyllization and differentiation as sporophylls) take place <u>simultaneously</u> in the apical bud of the stem, both types of leaf determination progressing towards the tip of the bud.

3. <u>It is possible to minimize the formation of cataphylls without stopping the differentiation of sporophylls.</u>

This is shown by the results of the following experiment.

<u>Experiment #9.</u> (October 24, 1956-January 18, 1957).

Twenty plants belonging to the same shipment described in the preceding experiment were cultivated at 26°C, in photoperiods of 8 hours (sunlight). The plants developed well and 10 of them were harvested, stored in a cold room (4°C, 8 hour photoperiod) and dissected 2 days later. Results are shown in Table XII.

A similar result was obtained in:

<u>Experiment #10.</u> (October 24, 1956-January 18, 1957).

Twenty plants (same source as in Experiments #7 and #8) were grown at 26°C, in continuous light (sunlight from 8:00 a.m. to 4:00 p.m. and artificial light the rest of the time 400 ft. c.). After 86 days 10 plants were harvested stored for 1 day in a cold room (4°C, 8 hours of light) and dissected. Results are given in Table XIII.

Considering the results of Experiments #7, 8, 9, and 10, together with the observations on the annual cycle of leaf development, we see that:

Table XII

Types of leaf developed in plants of Osmunda Claytoniana
grown at 26°C, 8 hour photoperiods, for 86 days

C = cataphyll
S = sporophyll
U = undifferentiated leaf

Plant #	Adult leaves	Young leaves														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	12	S	S	S	S	S	S	S	U	U	U					
2	12	U	S	S	U	U	U									
3	11	S	S	S	S	S	U	U	U							
4	14	C	S	S	S	S	U	U	U							
	15	S	S	S	U	U	U									
5	19	S	S	S	S	S	S	S	S	U	U	U				
6	21	S	S	S	S	S	S	S	S	S	S	U	U	U		
7	14	C	C	S	S	S	S	S	S	S	S	S	S	U	U	U
8	12	S	S	S	S	U	U	U								
9	13	U	U	U	U	U	U									
	10	S	U	U	U	U	U									
10	12	S	S	U	U	U										

Table XIII

Types of leaf developed in plants of Osmunda Claytoniana
grown at 26°C, continuous light, for 86 days

C = cataphyll
S = sporophyll
U = undifferentiated leaf

Plant #	Adult leaves	Young leaves															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	14	S	S	S	S	S	S	S	S	S	S	S	S	S	U	U	U
2	15	S	S	S	S	S	S	S	U	U	U						
	16	S	S	S	S	U	U	U									
3	14	S	S	S	S	U	U	U									
4	16	S	S	S	S	S	S	S	S	S	S	U	U	U			
5	18	S	S	S	S	S	S	S	S	S	S	S	U	U	U		
	18	U	U	U													
6	16	S	S	S	S	S	S	S	S	U	U	U					
7	13	C	U	U	S	S	S	S	S	U	U	U					
	20	C	C	C	U	S	S	S	S	S	S	S	U	U	U		
8	13	U	S	S	S	S	S	S	U	U	U						
9	18	C	S	S	S	S	U	U	U								
10	11	U	U	U	U	U	U										
	11	U	U	U	U	U	U										

a) cataphylls precede sporophylls in the annual set of leaves;

b) formation of cataphylls may be minimized by environmental conditions (26°C, both in short and in long-day conditions), without decreasing the formation of sporophylls;

c) occasionally sporophylls that are already differentiated can be transformed into cataphylls.

As the determination of a leaf as a sporophyll or as a cataphyll results from processes that may or may not take place simultaneously in the same bud and that, when they take place in the same bud, may or may not be superimposed in the same leaf, it is concluded that these are distinct processes.

c) Effects of photoperiod and temperature on the initiation of sporangia.

Data presented in Tables IX, X, XII, XIII are summarized in Table XIV.

These data indicate that reactions of this plant to photoperiod and temperature both for the determination of cataphylls and for the determination of sporophylls are of the quantitative type.

At 26°C cataphyll formation is scarce, irrespective of the photoperiod, whereas sporophyll formation is abundant, also irrespective of the photoperiod.

At 17°C cataphyll formation seems to be stimulated by short days, and sporophyll formation depressed.

The fact that, even in controlled conditions, the annual succession of leaves is maintained shows that it is

Table XIV

Effect of photoperiod and temperature on the differentiation
of leaf primordia in Osmunda Claytoniana

Temperature		26°C		17°C	
Photoperiod		24 hr.	8 hr.	24 hr.	8 hr.
Average # of differentiated leaves		8.1±1.4	5.9±1.1	12.9±1.8	7.2±1.8
Cataphylls	average # per plant	0.5±0.4	0.3±0.7	2.1±0.5	4.4±1.1
	% of the average # of diff. leaves	6%	5%	16%	61%
Sporophylls	average # per plant	7.6±1.2	4.9±1.1	10.8±1.7	2.8±0.9
	% of the average # of diff. leaves	94%	83%	84%	38%

(Errors given with the averages are standard errors of
of the mean)

not yet possible to correlate these experimental data with the observations made in plants developed in natural conditions. The results of these experiments show trends of variation of the two morphogenetic processes with temperature and photoperiod. An exact delimitation of the environmental requirements of these processes requires additional experimental data that could not be obtained within the time limits of this research.

There are two facts which suggest that the initiation of sporangia in this species is stimulated by high temperatures and long days.

The first is that this type of differentiation predominates in the young leaves of the terminal bud at 26°C, both in continuous light and in short (8 hour) days and at 17°C in continuous light.

The second is that in plants grown at 26°C, in continuous light, sporophylls are formed with all pinnae of the frond, except the basal set, differentiated as fertile pinnae (see figs. 64, 65, 66). This is not the normal condition in this species and shows an increment in the initiation of sporangia along the individual sporophyll. In these conditions some leaves display also, in the terminal pinnae, abnormalities that show a mixed pattern of development (fertile and sterile). (Fig. 67).

4. <u>Behavior of Salvinia rotundifolia</u>.

Living plants of this species were obtained from Florida and cultivated in the Earhart Laboratory.

Fig. 64. Osmunda Claytoniana. Plant with sporophylls fertile to the top (26°C, continuous light).

Fig. 65. Osmunda Claytoniana. Plant with sporophylls
fertile to the top (26°C, continuous light).

Fig. 66. Osmunda Claytoniana. Plant with sporophylls with all apical pinnae fertile.

Fig. 67. Osmunda Claytoniana. Plant with abnormal pinnae, partially differentiated as sporophylls.

According to Sadebeck's review of the genus Salvinia (159)
this species should be Salvinia minima Baker (leaves almost
round and almost sessile). Since the key for determination
of Salvinia species in the above mentioned review makes use
of rather imprecise characters, it was necessary to consult
a more thorough taxonomical work. Hertzog's review (95)
satisfies this requirement and is based on a broad world
collection. According to this review the material used in
the experiments is Salvinia rotundifolia Willd. (tall
papillae (1-3 mm), tapering in shape, with a terminal corymb
of 3-4 uniseriate hairs, with free end cells; papillae over
the surface of the leaf and not only in the margin, in rows
between the veins, furrowed adult leaves, lemon-shaped sori,
"sausage"-shaped placenta). In fact the two determinations
are not conflicting, since Salvinia minima Baker is now a
synonym of Salvinia rotundifolia Willd. Unfortunately the
numerous attempts made to obtain living specimens of
Salvinia natans for experiments failed repeatedly, but the
plants received dead made possible a morphological comparison
of the two Salvinias which confirm the fact that the plant
used in our experiments is not Salvinia natans.

Salvinia rotundifolia is found in Tropical America, from
Mexico to Southern Brazil (95).

Figs. 68 to 71 show the main features of the plant. The
water leaves, where sori are developed, are basically
constructed according to the vascular plan of the floating
leaves (fig. 71), in spite of the absence of anastomosing
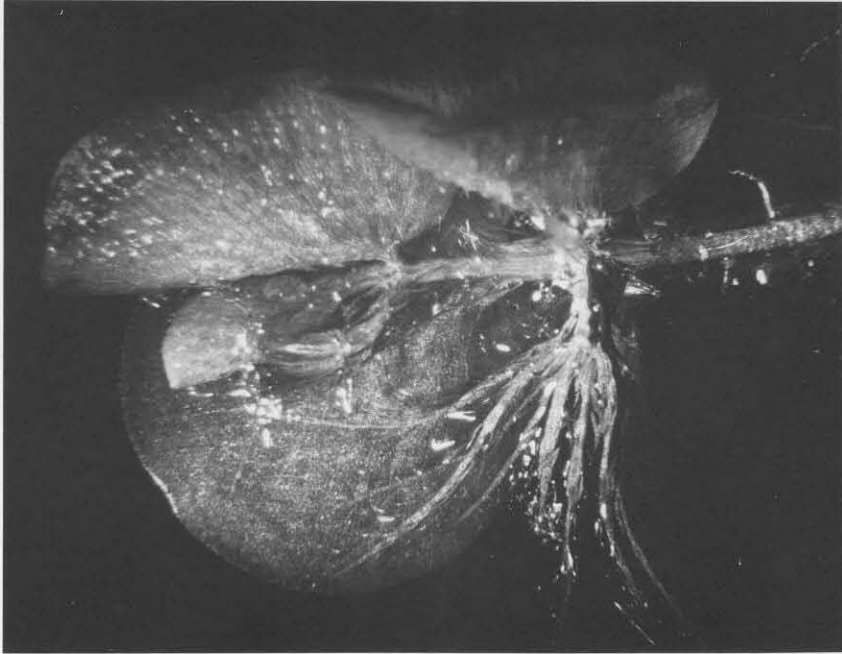
Fig. 68. Salvinia rotundifolia. Water leaf and floating leaves.
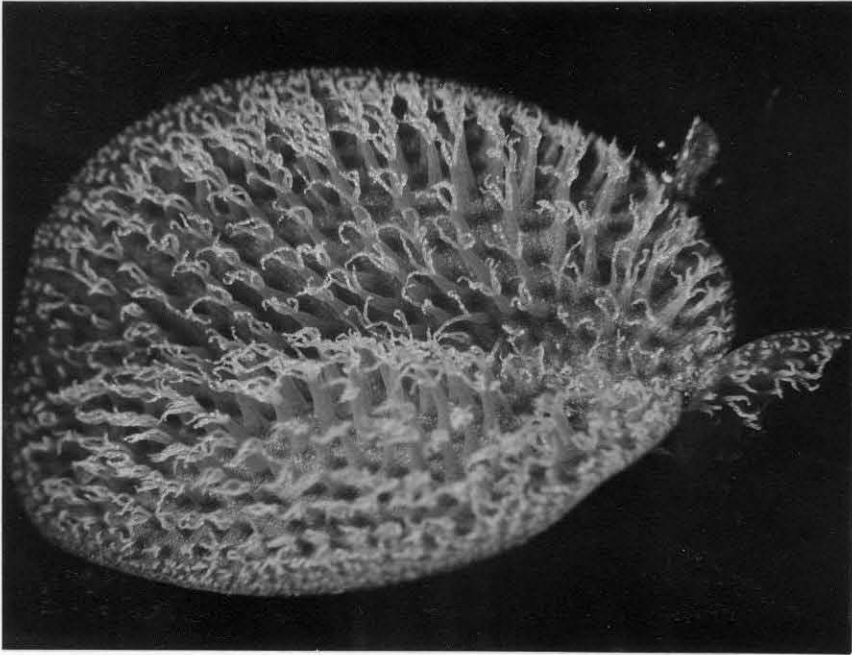
Fig. 69. Salvinia rotundifolia. Adaxial view of
floating leaf.
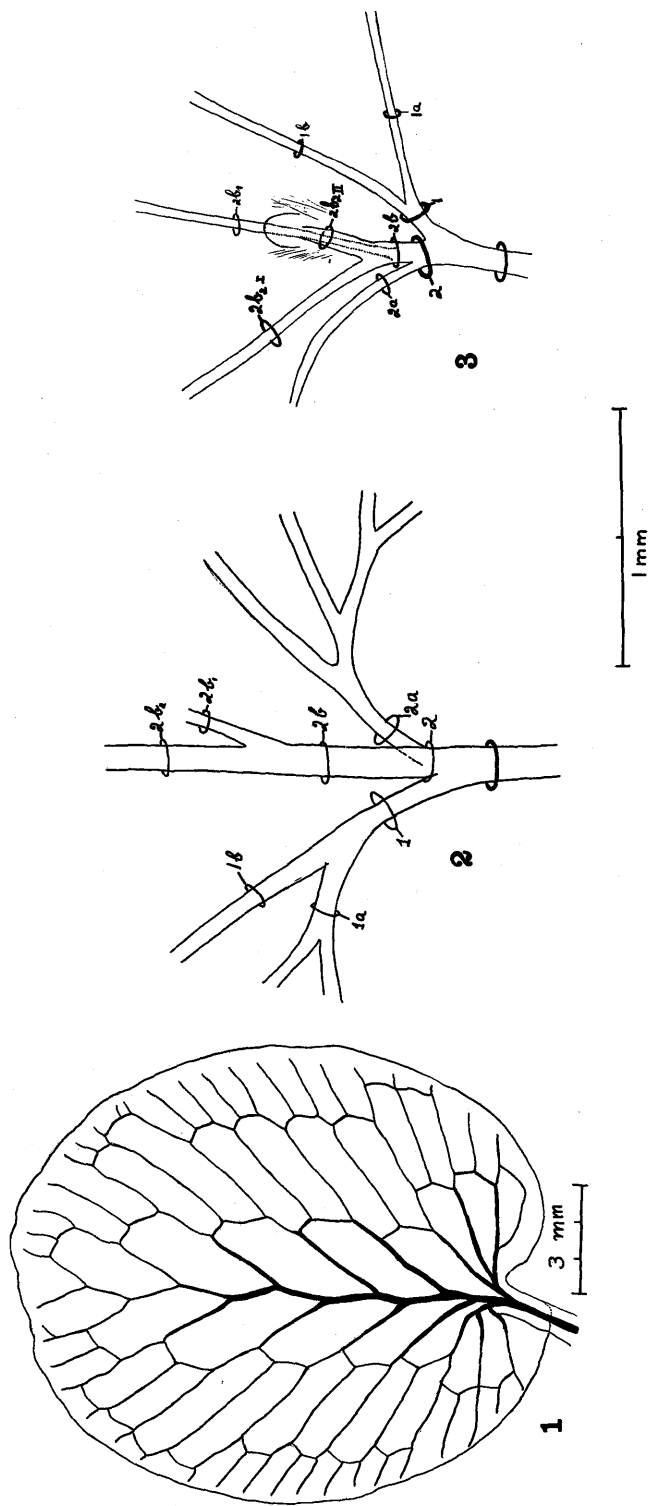
Fig. 70. Salvinia rotundifolia. Leaf Venation. 1: floating leaf (venation; 2: detail of the venation at the base of the floating leaf; 3: detail of the venation of the water leaf. The numbers in 2 and 3 refer to the order of vein branches.
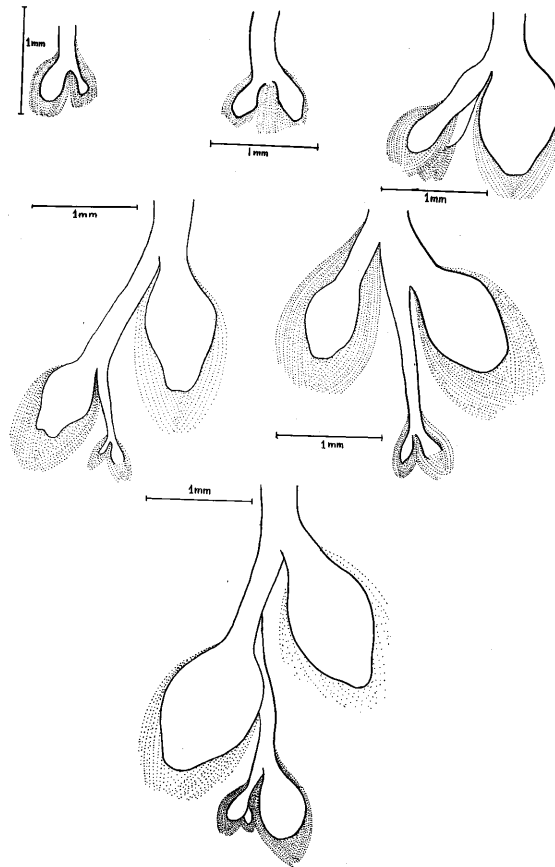
Fig. 71. Salvinia rotundifolia. Leaf segments with sori, in the axil of water leaf, showing several stages of soral development.

veins in the water leaves. The position of the sori thus
corresponds to that of the last dichotomies of the water
leaves.

a) <u>Effects of continuous light at different temperatures</u>.

As this species had never been studied before it was
necessary to investigate whether the behavior in continuous
light is uniform at different temperatures. The temperatures
referred to are air temperatures maintained by the air
conditioning system of the Earhart Plant Research Laboratory.

<u>Experiment #11</u>. (October 30, 1955-April 8, 1956).

<u>Effects of continuous light on the initiation of sporangia
at 30°C and at 26°C</u>.

All plants used in this and in the subsequent experi-
ment are originated from a single sterile plant, as
cuttings. The original plant was grown at 30°C, 24 hour
light. In the nodes this plant develops many axillary buds
that grow at a relatively high rate, developing into
branches, with new leaves. The breaking of the stem between
the nodes allows an easy and rapid process of vegetative
propagation. The plants were floating in crocks containing
the Earhart Laboratory nutrient solution. Periodical
observations were made by removal of a random sample of 20
plants. As the plants grown at 26°C, continuous light
(400 ft. c.) were used as a stock from which plants were
taken for the other treatments, it was necessary to prolong
the observation of this treatment over a long period of time,

in order to be sure that the plants of the stock were not induced to form sori. Results of observations are presented in Table XV.

Therefore, at these temperatures, there is no initiation of sporangia in continuous light.

Plants of 100 days of treatment showed some typical calluses replacing the sori, as can be seen in fig. 72.

Experiment #12.

Effects of continuous light at 30°C, 26°C, 20°C and 17°C.

The source of the plants was the same as the one used in Experiment #11; at the time the treatments of this experiment were started, Experiment #11 was not concluded and the assumption was made that the original plant was not induced to form sporangia. As this plant was kept at 30°C, continuous light, the subsequent results of Experiment #11 justify this assumption.

In this experiment plants were grown in the same nutrient solution and in the same light conditions as in the preceeding experiment, but at 5 different temperatures. Results are presented in Table XVI.

These results indicate that the effects of continuous light on the initiation of sporangia vary with the temperature. The effects of continuous light at 17°C were subsequently verified in:

Table XV

Effect of continuous light at 30°C and at 26°C on the initiation of sporangia
in Salvinia rotundifolia

Dates	Oct. 30	Nov. 9	Nov. 19	Nov. 29	Dec. 9	Dec. 19	Dec. 29	Jan. 8	Jan. 18	Jan. 28	Feb. 8	Feb. 28	March 19	April 8
Days	0	10	20	30	40	50	60	70	80	90	100	120	140	160
30°	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26°	0	0	0	0	0	0	0	0	0	0	0	-	-	-
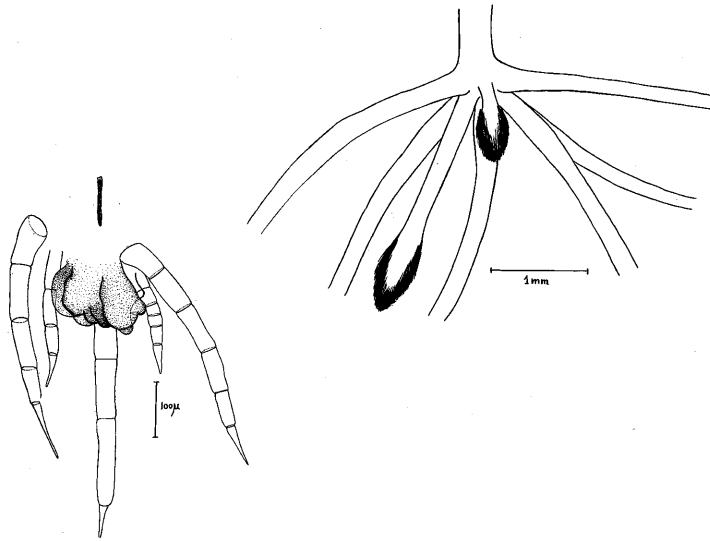
Fig. 72. Salvinia rotundifolia. 30°C, continuous light. Right: Sterile water leaf. Left: Cell proliferation replacing fertile leaf lobe.

Table XIV

Effects of continuous light at different temperatures on the initiation of sporangia in Salvinia rotundifolia

Temperatures	Days of treatment							
	0		10		20		30	
	Dates	# plants with sori	Dates	# plants with sori	Dates	# plants with sori	Dates	# plants with sori
30°	Oct.10 1955	0	Nov.9 1955	0	Nov.19 1955	0	Nov.29	0
26°	"	0	"	0	"	0	"	0
23°	Dec.10 1955	0	Dec.20 1955	0	Dec.30 1955	0	Jan.9 1956	0
20°	"	0	"	0	"	0	"	1(=5%)
17°	Jan.26 1956	0	Feb.5 1956	6(30%)	Feb.15 1956	6(30%)	Feb.25 1956	7(35%)

Experiment #13.   (March 5, 1957-June 10, 1957).

Eighteen plant cuttings, each with two floating leaves, were taken from a stock at 26°C, continuous light and cultivated in separate plastic containers perforated at the bottom and placed in a stainless steel tray containing Hoagland's nutrient solution. The nutrient solution was renewed weekly. The tray was immersed in a water bath maintained at such a temperature that the temperature of the nutrient solution was kept at 17°C. This was achieved by maintaining the water bath in a room in which the air temperature was 7°C and by heating the water bath by means of an electric heater controlled by a thermostat. The temperature of the water bath was kept uniform by continuous circulation maintained by a water pump immersed in the bath. A light fixture was suspended above the tray with two "warm white" GE fluorscent tubes and two incandescent bulbs, giving a total light intensity of 500 ft. c. at the level of the floating leaves. The air temperature just above the leaves was 18°C.

After 88 days the plants were harvested and individual leaves were observed under the dissecting microscope. The result follows:

> \# of plants with sori. 14 (78%)
>
> \# of plants without sori 4 (22%)

These results confirm the fact that this plant is able to initiate sporangia in continuous light at 17°C.

b) Effects of short days at different temperatures.

In these experiments days of 8 hours (sunlight) were used.

Experiment #14. (November 19, 1955-January 23, 1956).

All plants used in the experiment came from the stock at 26°C, continuous light, and were cultivated in the Earhart Laboratory nutrient solution. Results are shown in Table XVII.

These results, taken together with those of the experiments of growth of plants in continuous light at the same temperatures, indicate a short-day behavior at 26°C and at 23°C and a long day behavior at 17°C. At 20°C the plant initiates sporangia both in continuous light and in days of 8 hours, but initiation seems to be extremely slow in both conditions, suggesting a double photoperiodical limitation at these extreme photoperiodical conditions.

c) Effects of daily fluctuations of temperature.

Experiment #15. (November 19, 1955-January 23, 1956).

Plants of the stock at 26°C, continuous light, were used for this experiment. The photoperiod was given with artificial light (500 ft. c.). The treatments and results after 65 days of culture are given in Table XVIII.

Table XVII

Effects of 8 hour photoperiods at different temperatures

on the initiation of sporangia in

Salvinia rotundifolia

Temperatures	Photoperiod	Time of treatment	# of plants with sori in a sample of 20 plants	% fertile plants
26°C	8 hours	65 days	14	70%
23°C	8 hours	" "	12	60%
20°C	8 hours	" "	2	10%
17°C	8 hours	" "	0	0%

Table XVIII

Effects of daily fluctuations of temperature on the

initiation of sporangia in Salvinia rotundifolia

$17^{O}C$ day temperature and variable

night temperature

Day temperature	Photoperiod	Night temperature	# of plants with sori in a sample of 20 plants	% of fertile plants
$17^{O}C$	8 hours	$4^{O}C$	14	70%
$17^{O}C$	8 hours	$7^{O}C$	19	95%
$17^{O}C$	8 hours	$10^{O}C$	16	80%
$17^{O}C$	8 hours	$14^{O}C$	0	0
$17^{O}C$	8 hours	$17^{O}C$	0	0
$17^{O}C$	8 hours	$20^{O}C$	13	65%
$17^{O}C$	8 hours	$23^{O}C$	0	0
$17^{O}C$	8 hours	$26^{O}C$	0	0

Experiment #16. (November 19, 1955-January 23, 1956).

Plants of the same source as those of the preceding experiment were used. The same photoperiod was used, but phototemperature was varied with a constant night temperature (17°C).

Results after 65 days of treatments given are shown in Table XIX.

Results of these two experiments are summarized in fig. 73.

Experiment #17. (February 2, 1956-April 6, 1956).

The plants used came from the stock at 26°C, continuous light and, in all treatments, were given days of 8 hours (sunlight), followed by nights of 16 hours at different temperatures, as indicated in Table XX, where results of 65 days of culture in these conditions are given.

These results indicate that long nights cease to be effective for photoperiodic induction below 20°C. Comparison of these results with those of Experiment #15 suggests that the effects of long nights at low temperatures (7°C, 10°C), which are stimulatory for the initiation of sporangia when these cold nights alternate with short days at 17°C, are destroyed at 26°C.

The above results of experiments with Salvinia rotundifolia show that this plant is a favorable object for research on the induction of the initiation of reproductive structures, since the same plant displays a short-day process,

Table XIX

Effects of daily fluctuations of temperature on the

initiation of sporangia in Salvinia rotundifolia

17°C night temperature and variable

day temperature

Day temperature	Photoperiod	Night temperature	# of plants with sori in a sample of 20 plants	% of fertile plants
4°C	8 hours	17°C	0	0
7°C	8 hours	17°C	12	60%
10°C	8 hours	17°C	1	5%
14°C	8 hours	17°C	0	0
17°C	8 hours	17°C	0	0
20°C	8 hours	17°C	15	75%
23°C	8 hours	17°C	0	0
26°C	8 hours	17°C	0	0
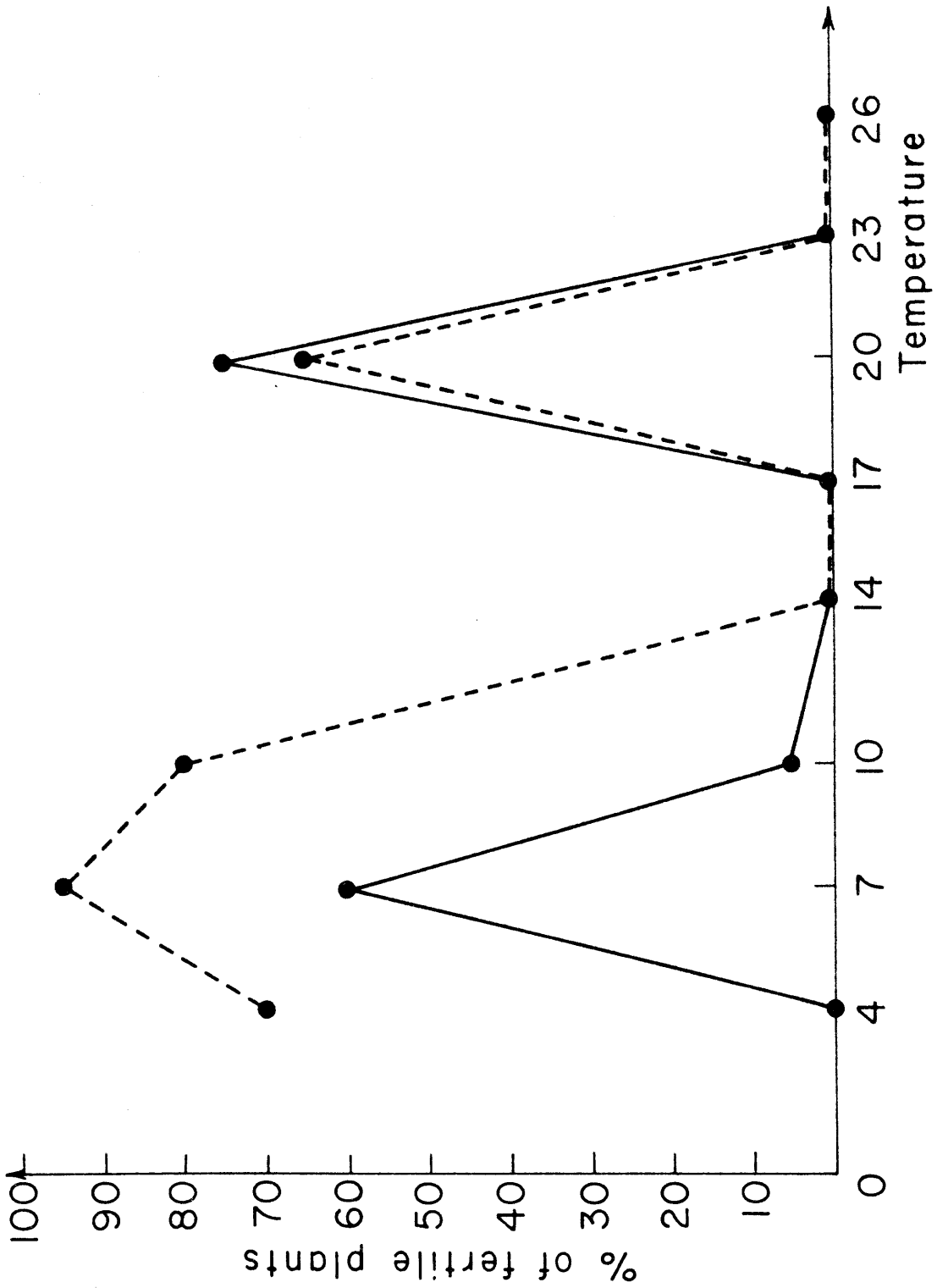
Fig. 73. Salvinia rotundifolia (Exp. #15 and 16). Solid line: nycti-
temperature 17°C, variable phototemperature. Interrupted line: variable
nyctitemperature, constant phototemperature (17°C).

Table XX

Effects of night temperature on the initiation of

sporangia in plants of Salvinia rotundifolia

grown in 8 hour photoperiods at 26°C

Day temperature	Night temperature	# of plants with sori in a sample of 20 plants	% of fertile plants
26°C	26°C	13	65%
26°C	23°C	5	25%
26°C	20°C	2	10%
26°C	17°C	0	0
26°C	14°C	0	0
26°C	10°C	0	0
26°C	7°C	0	0

a long-day process and thermoperiodical processes.

5. Behavior of Regnellidium diphyllum.

This plant is an endemic South American Marsiliacea
(66, 121). The time of ripening of the sporocarps under
natural conditions is reported as being from September to
November (162). There are no available records in the
literature as to the time of initiation of sporocarps, but,
judging from our results in the culture of this plant, it is
safe to estimate that 1-2 months are needed for development
of the sporocarp from initiation to ripening. This would
place the time of initiation of sporocarps in the months of
June and July. In the climate of the small area of natural
occurrence of this species this is the season with relatively
short days and with the lowest annual temperatures (140).

A recent investigation by Bloom and Voth (31) has shown
that culture of this plant for 21 days, both in short-day
conditions (days of 8 hours) and in long-day conditions
(days between 13 hours 54 minutes and 14 hours 40 minutes),
in a nutrient solution especially devised for the plant,
always failed to induce the initiation of sporocarps.
Comparison of this result with the production of sporocarps
in plants growing in shallow water (40 mm layer of water
above the soil), at the same time of the year, has led these
authors (31) to assume that the failure of experimental
plants to produce sporocarps was due to the fact that their
experimental plants were grown in relatively deep water

layers (140 mm). Lindmann had already observed (121) that sporocarps are only produced in leaves that are developed in shallow water, which have shorter petioles and smaller leaflets than those developed in deeper water. The same fact was reported for another Marsiliacea, namely Marsilia quadrifolia (164), but it was experimentally shown by Shattuck (164) that the maximum depth of water in which Marsilia quadrifolia initiates sporocarps depends on the conditions of exposure of the culture tanks to sunlight, being about 12 cm in diffuse light and 20 cm in direct sunlight under the conditions of this author's experiments. Therefore the effect of the depth of the water is not yet clearly established as an independent one and it is reasonable to assume that this may actually be a temperature requirement.

It is pertinent to this work to note that in Regnellidium each leaf has an axillary bud, which is prevented from immediate development by the apical dominance of the terminal bud (31). As soon as this effect ceases the lateral buds develop into branches, leading to a very ramified rhizome (121). This feature facilitates the vegetative propagation of the plant and is also of interest for the study of the initiation of sporocarps, since in our experiments we never observed the initiation of sporocarps in leaves of the main axis, but always in the leaves of the lateral branches (see fig. 74).
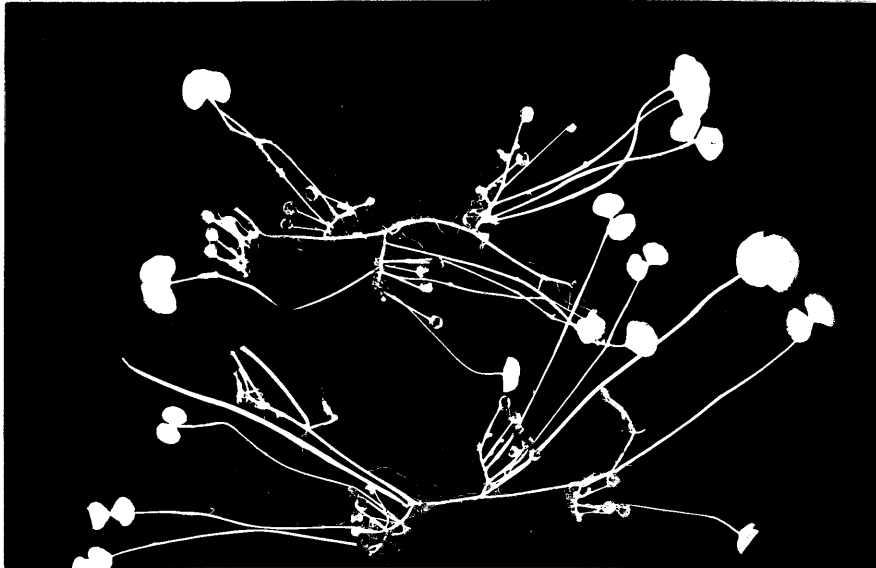
Fig. 74. Photograph of plants of Regnellidium
diphyllum. Cultivated at $20^{\circ}/14^{\circ}C$, 10 hour photoperiods.
The sporocarps are found <u>only</u> in axillary branches.

Experiment #18. (January 22, 1957-March 2, 1957).

Twenty cuttings with two adult leaves were taken from a sterile plant cultivated in mud, in a plastic container, and kept in a greenhouse at the Earhart Laboratory at 26°C from 8:00 a.m. to 4:00 p.m. and at 20°C from 4:00 p.m. to 8:00 a.m. Cuttings were also planted on mud covered with deionized water (depth of water layer 4 cm). They were divided in two groups. Group I (10 plants) was placed in a greenhouse in which air temperature is maintained at 17°C from 8:00 a.m. to 4:00 p.m. and at 11°C from 4:00 p.m. to 8:00 a.m. The total daily period of light was of 16 hours (8 hours of sunlight and 2 artificial light supplements after 4:00 p.m. and before 8:00 a.m.). Group II was placed in another greenhouse, where air temperature was maintained at 26°C from 8:00 a.m. to 4:00 p.m. and at 20°C from 4:00 p.m. to 8:00 a.m. The photoperiod was the same as in the case of group I and given similarly.

The plants at 26°/20° developed very well, whereas those at 17°/11° showed poor vegetative development. After 48 days (March 11, 1957) plants of group I were transferred to the same conditions to which plants of group II had been exposed all the time. Table XXI and fig. 75 present the results of periodic observations.

Therefore a preliminary period of 48 days in relatively low temperature, in long days, can induce plants to initiate and develop sporocarps at relatively high temperatures, at the same photoperiod.

Table XXI

Effect of seasonal thermoperiodicity in the production of sporocarps in Regnellidium diphyllum, in long-day conditions

Dates		Days	Group I				Group II			
			Temperatures		Photop. hr.	% fertile plants	Temperatures		Photop. hr.	% fertile plants
			8 a.m.-4 p.m.	4 p.m.-8 a.m.			8 a.m.-4 p.m.	4 p.m.-8 a.m.		
Feb.	1	10	17	11	16	0	26	20	16	0
Feb.	11	20	17	11	16	0	26	20	16	0
Feb.	21	30	17	11	16	0	26	20	16	0
Mar.	3	40	17	11	16	0	26	20	16	0
Mar.	11	48	26	20	16	0	26	20	16	0
Mar.	13	50	26	20	16	0	26	20	16	0
Apr.	2	70	26	20	16	30	26	20	16	0
Apr.	5	73	26	20	16	50	26	20	16	0
Apr.	8	76	26	20	16	80	26	20	16	0
Apr.	17	85	26	20	16	100	26	20	16	0
Apr.	22	90	--	--	--	--	26	20	16	0
Apr.	27	95	--	--	--	--	26	20	16	0
May	2	100	--	--	--	--	26	20	16	0

Fig. 75. Regnellidium diphyllum. Effects of seasonal thermoperiodicity on the production of sporocarps.

Since the low temperature treatment in this experiment was given as a daily thermoperiodicity with both temperatures at a relatively low level, it was necessary to investigate whether the same effect could be obtained by growing the plants for a period at constant low temperature and transferring them to the 26°/20° daily thermoperiodicity. This was attempted in the following experiment.

Experiment #19. (April 12, 1957- July 11, 1957).

Twenty cuttings each with two leaves were taken from sterile plants grown at 26°/20°, 16 hour photoperiods, as in the preceding experiment and cultivated in plastic containers, on mud and with deionized water added, at 10°C, 16 hour photoperiod, for 60 days. After this time (during which no sporocarps were observed) the plants were transferred to a greenhouse, where they received 26°C from 8:00 a.m. to 4:00 p.m. and 20°C from 4:00 p.m. to 8:00 a.m., and 16 hour photoperiod (8 hours of sunlight and 8 hours artificial light). In these conditions formation of sporocarps was observed as follows. (See Table XXII and fig. 76)

Experiment #20. (January 22, 1957-April 17, 1957).

Ten cuttings with two leaves were taken from sterile plants growing at 26°/20°, 16 hour photoperiod, as explained in the description of Experiment #19, and cultivated in a greenhouse in which the air temperature was maintained at 20°C from 8:00 a.m. to 4:00 p.m. and at 14°C from 4:00 p.m.

Table XXII

Effects of seasonal thermoperiodicity on the production

of sporocarps in Regnellidium diphyllum

Dates	Days at 26°/20°, 16 hours photoperiod, after 60 days at 10 C	% of plants with sporocarps
June 21	10	0
June 26	15	0
July 1	20	20%
July 6	25	50%
July 11	30	90%
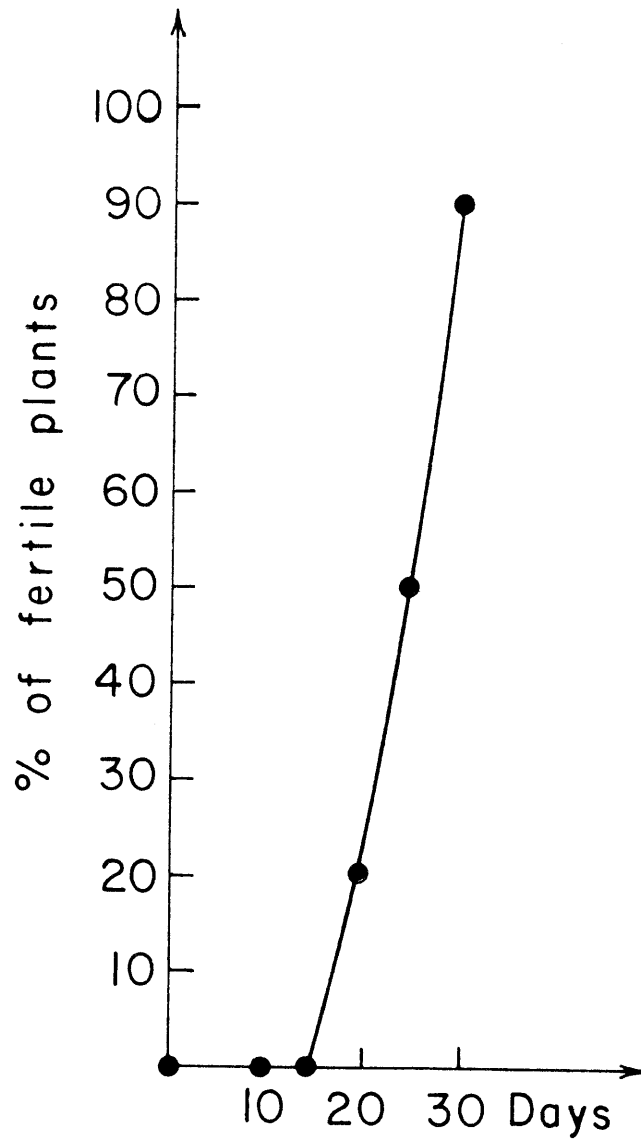
Fig. 76. Regnellidium diphyllum. Sporocarps as a response to low temperature treatment and subsequent growth in high temperature.

to 8:00 a.m. The photoperiod was the same as in the other greenhouse (16 hours) and given in the same manner. Results of periodic observations are given in Table XXIII and in fig. 77.

These results show that by a 3°C increase in the day and night temperatures, above those used in Experiment #18, it is possible to obtain simultaneously the induction of the initiation of sporocarps and their development. In this case, again, sporocarps were developed only on the lateral branches.

The effects of the daily periodicity at 20°/14°, as described in Experiment #20 are more accessible for study than those of Experiment #18, since in the first case both initiation and development can be obtained in a single treatment. In order to study the effects of photoperiod in this 20°/14° thermoperiodicity, the following experiment was set up:

Experiment #21. (April 12, 1957-June 6, 1957).

Ten cuttings were made each with two leaves, from plants growing at 26°/20°, 16 hour photoperiod, as explained for Experiment #18, and grown in the same thermoperiodicity used in Experiment #20. The photoperiod given, in this experiment, was of 8 hours and was made to coincide with the period at 20°C, by covering the plants at 4:00 p.m. and uncovering at 8:00 a.m. Therefore the whole period at 14°C was spent in darkness. Results of periodical observations are given in Table XXIV and in fig. 78.

Table XXIII

Effect of the daily thermoperiodicity $20^{\circ}/14^{\circ}C$ on

the production of sporocarps in Regnellidium

diphyllum, in long-days

Dates	Days of treatment	% of plants with sporocarps
Feb. 1	10	0
Feb. 11	20	0
Feb. 21	30	0
March 3	40	0
March 15	52	10%
April 1	69	40%
April 3	71	50%
April 6	74	60%
April 9	77	70%
April 10	78	80%
April 12	80	90%
April 17	85	100%

Fig. 77. Regnellidium diphyllum. Sporocarps as a response to the daily thermoperiodicity 20°/14°, in 16 hour photoperiod.

Table XXIV

Effects of the daily thermoperiodicity 20°/14°C on

the production of sporocarps in Regnellidium

diphyllum, in short days

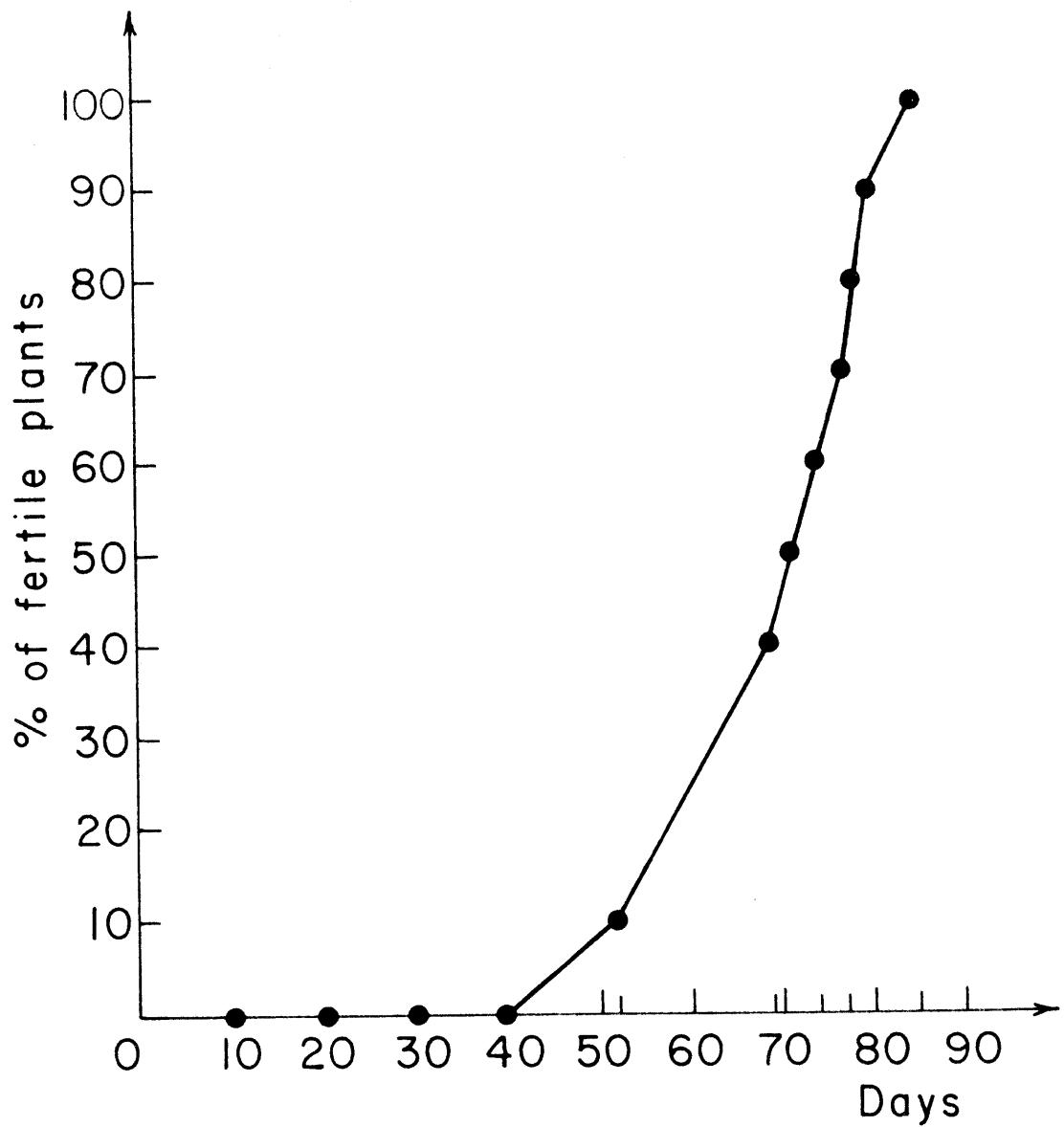| Dates	Days of treatment	% of plants with sporocarps
April 22	10	0
May 2	20	0
May 12	30	0
May 20	38	0
May 28	45	40%
June 2	50	70%
June 3	51	80%
June 5	53	90%
June 6	54	100%
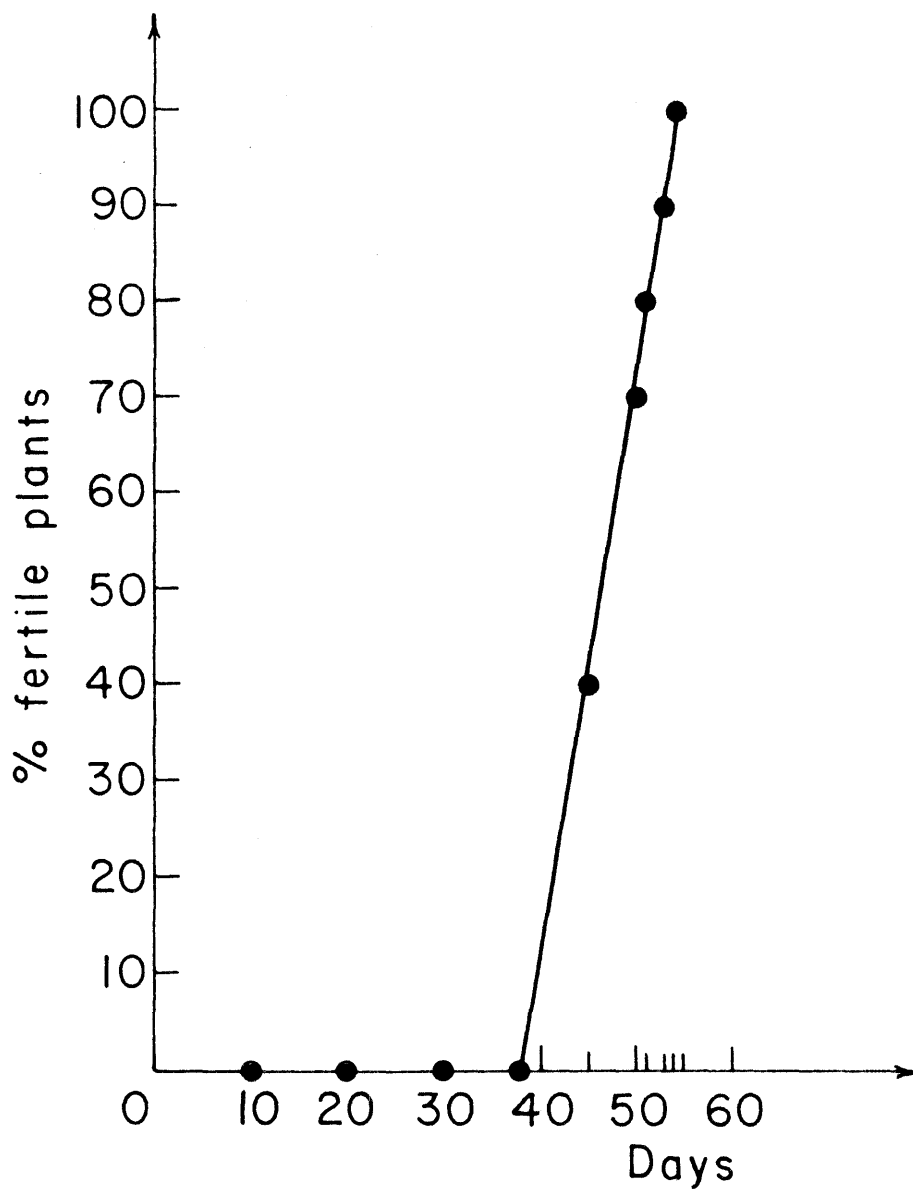
Fig. 78. Regnellidium diphyllum. Sporocarps as a
response to the daily thermoperiodicity 20°/14°, in 8 hour
photoperiods.

These results, compared with those of Experiment #20 show a considerable promotion of the process of formation of sporocarps by the lengthening of the dark period at 14°C. This fact suggested the possibility that formation of sporocarps might be dependent on a dark process, which would be more efficient at low temperatures. The fact that the time course of sporocarp formation is similar in Experiments #18 and #20, where the dark period at low temperature has the same duration, is consistent with this view. In order to make this study possible it is necessary to know if there is any induction of the initiation of sporocarps at 20°C, in short (8 hour) and in long (16 hour) days. This was tested by:

Experiment #22. (May 8, 1957-July 31, 1957).

Twenty cuttings were taken, with two leaves each, from plants growing at 26°/20°, 16 hour photoperiod, as explained in Experiment #18, and grown, in the already described manner (plastic containers, with mud and 4 cm water layer), at 20°C. One group of plants received 8 hour photoperiods (natural light) and the other, 16 hour photoperiods, by 8 hour supplementary artificial light (400 ft. c.). Results are given in Table XXV.

These results show that, in the experiments in which the formation of sporocarps is induced in the daily thermo-periodicity 20°/14° the inductive factor must be sought in the processes that take place at 14°C or in the daily temperature change.

Table XXV

Effect of photoperiodism at 20°C in the production

of sporocarps in Regnellidium diphyllum

Dates	Days of treatment	% of plants with sporocarps	
		8 hour photoperiod	16 hour photoperiod
May 18	10	0	0
May 28	20	0	0
June 7	30	0	0
June 16	40	0	0
June 27	50	0	0
July 7	60	0	0
July 17	70	0	0
July 31	84	0	0

The results of Experiments #18 and #19 show that sporocarps are not developed during the low temperature treatment. This indicates the existence of two partial processes, one which takes place in low temperatures (induction of axillary buds) and one which takes place at higher temperatures (20°C, 26°C). Further experimental studies are required in order to ascertain: a) whether both treatments are required for sporocarp initiation or if the second process is merely a requirement for speeding the development of the axillary buds; b) whether the induction at low temperatures is photoperiodically dependent (in a qualitative or in a quantitative manner) or not.

PART V

CONCLUSIONS

1.   Initiation and development of sporangia are potentially possible in many areas of the fern organism.

2.   Initiation and development of sporangia may be arrested in several stages, leading to a replacement of sporangia by a variety of alternative differentiations (cell proliferations, paraphyses, sporangiasters, vegetative buds and aposporous prothalli).

3.   The general types of behavior of Angiosperms, as regards the effects of climatic factors on flower initiation--short-day pattern, long-day pattern, thermo-periodical patterns--are extended to the initiation of sporangia in the species studied.

4.   Initiation of sporangia in the fern species studied was found to be interlocked with other morpho-genetic processes, such as heteroblastic development of leaves, vascular tissue differentiation, development of axillary buds and correlations between leaves.

PART VI

DISCUSSION

The fact that the usual site of initiation of sporangia in ferns is the leaf ties the problem of morphogenesis of sporangia to studies of leaf morphogenesis. In particular, since ordinarily only leaves of adult plants produce sporangia, the initiation of these organs in leaves may be considered as a special aspect of heteroblastic development of leaves. And, since it has been shown that there is a correlation between the initiation of sporangia and leaf veins, this aspect of the problem brings into discussion the interrelations between initiation of sporangia and the differentiation of vascular tissues.

Finally, since photoperiodic and thermoperiodic treatments were found to affect initiation of sporangia in some species of ferns, initiation of sporangia becomes connected with the general field of developmental effects of photoperiodism and thermoperiodicity.

This discussion is an attempt to connect the facts discovered with the general picture of these correlated fields of developmental physiology, with the purpose of deriving perspectives for further research.

Initiation of sporangia and the differentiation of vascular tissues.

The study of the differentiation of vascular tissues was started by cataloguing the variety of manners by which

such tissues can be built in vascular plants. As this variety was found to be very rich, particularly among ferns and Pteridophytes in general, the interest shifted to the origin of such structures. No matter how different they are in adult form and in position, vascular tissues have the common trait of being derived from undifferentiated apical meristems. Therefore it was natural that the causes of their differentiation should be sought in such meristems.

This approach was strengthened by the observation that the decapitation of the stem apical bud can cause correlative effects, such as the development of resting buds in the roots (Ophioglossum) or in the stems (Botrychium, Equisetum, Lycopodium, Osmunda) (194). Some facts pointed also to a correlative influence of the terminal bud of the stem, not by maintenance of apical dominance, but by directing development of organs of undetermined nature. This was found to be the case of the angle meristems of Selaginella, for instance. If the shoot apex is left, these meristems will eventually develop into rhizophores and the same happens as soon as the shoot apex is decapitated and replaced by a lanoline smear containing IAA. If there is only stem decapitation the angle meristem develops as a shoot, not as a rhizophore (193).

As early as 1915, the possibility was considered that vascular differentiation might be affected by the organizing effect of the basal, preformed, vascular tissue, as well as by the inducing effect of the apical meristem (194).

However, the emphasis was placed on the effects of the meristem when it was observed that the vascular tissue of growing lateral buds develops as an isolated group, that, later on, merges with the stem system. We have already pointed out that this is the case with the adventitious leaf-buds of Asplenium bulbiferum.

Experiments in which the apical meristem was isolated by incisions in planes parallel to the growth axis of the stem have shown that, in these conditions, the apical meristem differentiates an isolated group of vascular tissue (181, 182, 183). These results led Wardlaw to the view that differentiation of vascular tissues depends on the basipetal diffusion of substances produced by the apical meristem and that supply of water and of nutrients to the apical meristem takes place through the pith cells and "the thin layer of incipient vascular tissue" upon which the apical meristem resides.

Furthermore Gautheret (82) has obtained xylem and phloem differentiations in cambium tissue cultures and this type of differentiation has been observed in several successive subcultures. Camus (47) has shown that buds can induce incipient vascular differentiation in calluses upon which they are grafted. Ball (25, 26) and Wetmore (189), have obtained regeneration of complete plants from cultured inoculi consisting of pieces of the meristematic region of the shoot apex.

Although these experiments are very suggestive of a

vascular inducing capacity of the shoot apex, they do not
exclude the possibility of an effect of the previously
differentiated vascular tissues. The first result in which
differentiation of vascular cells was clearly shown to
take place in the complete absence of incipiently dif-
ferentiated cells in the inoculum was observed by Morel and
Wetmore (139) in tissue cultures of spores of Osmunda
cinnamomea. Another approach to the problem of vascular
tissue differentiation is represented by a series of
studies on the regeneration of cut vascular strands in sub-
apical internodes.

Sinnott and Bloch (166) observed that the vascular
regeneration in Coleus is basipetal, frequently in an
oblique direction, across the pith, and that the path of
the new bundle is first indicated by the planes of the walls
of the dividing pith cells and by the patterns of the lig-
nin bands in their walls. The observation of these pat-
terns, which start as granular cytoplasmic condensations
in the cell walls, indicates an alteration of cell polarity
as the first perceivable sign of vascular differentiation.

Jacobs (100) established, in Coleus sub-apical inter-
nodes, that defoliation and de-budding only affects the
predominantly basipetal xylem regeneration when the sup-
pressed parts are distal to the wounded area and that this
effect is maximal after stem decapitation. Wardlaw (180)
had also established that systematic destruction of leaf
primordia stops vascular differentiation in fern stem

apices. These results suggested to Jacobs the hypothesis that auxin is the limiting factor in xylem regeneration (100). Jacobs was able to show that physiological concentrations of IAA, applied to decapitated stems, quantitatively restore xylem regeneration (100). Furthermore it was possible to establish that the number of regenerated xylem strands is proportional to the amount of IAA transported in the tissue (acropetally and basipetally). The limiting factor in xylem differentiation appears to be the auxin transport capacity of the internode (101).

Subsequently Wetmore and Sorokin (190) were able to confirm Camus' results with bud grafts on callus tissue cultures and show, by control experiments with NAA, that auxin alone, in convenient concentrations, can induce tracheidal differentiation in such calluses. It was remarked however, that such induced tracheids do not show any organization resembling stem procambium (as was also the case in Camus' experiments).

Ball (27) was able to obtain differentiation of the tracheids in callus tissue cultures of Sequoia sempervirens and to show that different sugars supplied to the medium were not equally effective in tracheidal differentiation. Moreover, when sugars are given as C^{14}-labelled compounds the C^{14} is found to concentrate in the nodules where tracheids are differentiated. This result can be interpreted as meaning that differentiation of tracheids is hormonally controlled by other regions of the callus (27).

Jensen (102), considering the evidence that eugenol is a probable intermediate in lignin biosynthesis, that this biosynthesis requires a peroxidase system and that one such system has been shown by Galston to be enhanced by IAA, studied the correlation between IAA-induced peroxidase and lignin synthesis in roots of Vicia faba. Several regions (root cap, proepidermis, provascular tissues) were found to have peroxidase activity but the IAA-induced peroxidase activity was found only in the vascular tissue cells. If the root tips are incubated with IAA (induction of peroxidase activity) and sections of such tips incubated with eugenol and H_2O_2, formation of compounds, intermediates in lignin biosynthesis is observed.

The above-mentioned results, taken together, clearly establish that the basipetal differentiation of vascular tissue of the xylem is induced by the basipetal diffusion of IAA from the apical meristem.

Coming back to the correlation between the initiation of sporangia and the vascular tissues of the leaf, we must note first that the initiation of sporangia is simultaneous with the differentiation of the vein ends behind them. In highly compound leaves it is clear that before these vein-ends differentiate, the whole vascular system of the older leaf parts is differentiated. This is particularly easy to observe in Anemia (Euanemia), where the vascular systems of pinnae of 1st, 2nd and 3rd order is differentiated before differentiation of sporangia.

Differentiation of the latter start simultaneously with differentiation of the vascular tissue of the 4th order pinnae. Also in Asplenium bulbiferum the vascular connection between the adventitious bud and the adult leaf is formed <u>before</u> the bud produces sporangia in its leaves. Nevertheless, at the sub-marginal region of the developing fertile pinna of the sporophylls of the bud the ultimate vein ends and the sori differentiate simultaneously.

These facts suggest the following working hypothesis: acropetal stimuli from the adult leaves, carried by the veins and diffused through the undifferentiated cell path between the vein and the leaf margin start the initiation of sporangia; the sporangial meristems produce basipetal auxin gradients, which, in turn, stimulate vascular differentiation behind them.

The fact that, in many ferns, the sori occupy a definite position relative to some veins, but not to all veins (as can be seen clearly in Phyllitis scolopendrium) shows that the morphogenetic mechanism involved in these correlations is not a simple and direct one. This question can be attacked by developmental and experimental studies, using as objects the leaves of ferns that show special vascular differentiations peculiar to the sporophylls. Such is the case in Blechnum occidentale, where vein commissures parallel to the midrib are found only in the fertile leaves. Another favorable object is Pleopeltis, where tracheidal plexuses are found behind the sori. If such structures are

determined by the sporangia, they should be suppressed by early suppression of the sporangia.

On the other hand, Asplenium bulbiferum opens the possibility of graft experiments. If sterile adventitious buds can be grafted successfully on long-day leaves, they should develop sporangia in their leaves and the control grafts in leaves kept in 23 hour nights in 48 hour cycles at $20^{\circ}C$ (non-inducing conditions for the species) should not initiate sporangia in their leaves.

Besides the grafting experiments, adventitious buds can be cultivated in sterile nutrient agar. We know already that, after isolation from the leaf of the adult plant, such buds do not produce sporangia in their leaves for a long period, even under favorable photoperiodic conditions. Therefore, it is possible to try to replace this preliminary development by chemicals added to the basic mineral medium, both in favorable photoperiodic conditions and in inhibitory long nights.

Initiation of sporangia and heteroblastic development of leaves.

The fact that leaf shape, in many plants, changes throughout development (heteroblastic leaf development) was known for a long time, but understanding of the phenomenon started with Goebel's physiological interpretation (88), according to which: a) juvenile leaves are leaves arrested in their development; b) this arrest is caused by nutritional deficiencies during leaf development. Recent in-

investigations (4, 6, 7, 8, 189) have shown that Goebel's theory is essentially sound, although it needs to be completed with additional information, referring to other aspects and to mechanisms involved in heteroblastic leaf development.

As early as 1906 Diels (in 184) observed that conditions that promote flowering also promote progressive heteroblastic development of leaves in some species. This was also found by Harder and Witsch (in 184) to be the case in Kalanhöe Blossfeldiana. In this plant short days have a dual effect. They cause both flower initiation and changes in vegetative characters (such as increase in leaf thickness and shortening of stem internodes). All of these effects were shown to arise as a consequence of the photoperiodic treatment and it was found to be possible to limit such effects to restricted areas by limiting the area and the position of the photoperiodically treated parts (91). It is yet an open question whether these vegetative effects justify Harder's working hypothesis of a specific hormone-- metaplasin--but there seems to be little doubt that these heteroblastic photoperiodic effects are of a correlative nature. Photoperiodically induced changes in leaf shape were also observed by Sen Gupta and Santish (163).

Studies of Ashby (15,16) and of Ashby and Wangermann (17) led to the interpretation of heteroblastic development as being caused by aging of the apical meristem. This aging effect would involve correlation phenomena between the older leaves and the apex and between the

older leaves and the younger leaves.

Another view, emerging from the study of heteroblastic development of leaves of Acrostichum danaefolium, was presented by Crotty (58). It was observed in this plant that the progressive heteroblastic development coincides with a progressive delay in the time of the beginning of the differentiation of successive leaf primordia and coincides also with a progressive increase in size of these primordia before differentiation of pinnae. These facts are interpreted as due to the opportunity which the leaf primordium has to accumulate metabolites and growth factors before the beginning of differentiation. Correlations are also invoked, between older leaves and primordia in differentiation, leading to a conception of development as a sort of feed-back mechanism. Some of these interesting interpretations might be checked by chromatographic studies of successive leaf primordia, a method already in use for apical meristems (3, 5, 173, 174). Such studies should make use of chemical methods, as well as of bioassays, in order to test and identify the chromatographic fractions.

An interpretation of heteroblastic development, proposed by Alsopp (8), offers some similarities to Crotty's, but the size factor invoked by Alsopp refers to the apical meristem, not to individual leaf primordia. It is noted also that progressive heteroblastic leaf development coincides with progressive increase in the size of the stem apex; that reversion to juvenile stages coincides with a

decrease in size of the apex and that in the lateral buds
(which often develop juvenile leaves) the apices are smaller
than in the main axis.

Summarizing, we may say that heteroblastic leaf
development involves a nutritional aspect (that may refer
to the stem apex or to the individual leaf primordia) and
a correlative aspect. Effects of environmental conditions
may affect both sides of the question.

If now we apply this general information to the special
case of the heteroblastic development of leaves of Asplenium
bulbiferum, it is immediately apparent that the stimulatory
effects of long days on progressive changes in leaf shape
and on the rate of initiation of sporangia are the long-day
counterpart of the dual effects of short days observed in
Kalanchoe Blossfeldiana.

Asplenium bulbiferum is a very favorable object for
the experimental study of heteroblastic leaf development;
the rhizome apex is relatively large and the apices of
adventitious buds can be grown aseptically for pharmaco-
logical investigations on leaf development. The possibility
that so-called "ripening process" for the initiation of
reproductive structures and for the development of the
mature type of leaf would be the fulfillment of a nutri-
tional requirement of the apical meristem (morphologically
expressed by its size) can be tested in this plant.

Determination of cataphylls.

Another aspect of leaf development that usually

displays a periodic character is the formation of cata-

phylls. This type of leaf differentiation can be found in

many Angiosperms and Gymnosperms and in some ferns (33,

169).

The cataphylls of Osmunda cinnamomea have been studied

by Steeves and Wetmore. These authors have observed, in the

conditions of the climate of Massachusetts, the same

sequence of leaf types found by us in Osmunda Claytoniana

growing in natural conditions in Michigan and in controlled

conditions in the Earhart Laboratory. Cataphyll formation

was also found to progress acropetally in the rhizome, but

in Osmunda cinnamomea, it usually stops abruptly. It was

remarked that processes that lead to cataphylls seem to be

in opposition to those that lead to sporophylls (169, p.353);

incomplete sporangial differentiation in fertile leaves was

also observed. The experimental results of these authors

indicate that cataphyll formation may depend on the activity

of the adult leaves and that there may be an acropetal dis-

placement of the leaf types upon defoliation.

In Osmunda Claytoniana cataphylls can be characterized

by the following features:

    a) living leaf base and necrotic pinna region (crozier)
    b) laterally expanded leaf base
    c) necrotic pinnae have shapes and sizes that coincide
       with those of various stages of undifferentiated
       leaves and with those of several stages of young
       differentiated leaves, up to fertile pinnae with
       completely organized sporangia.

The observations of Steeves and Wetmore (169) on cataphylls of Osmunda cinnamomea show that in this species the process of "cataphyllization" of undifferentiated leaves goes much faster than in Osmunda Claytoniana, so that the undifferentiated leaves are caught by this process much before they develop their pinnae.

Since cataphylls were not observed by these authors in plants collected in Honduras, where much higher temperatures prevail, it is probable that in Osmunda cinnamomea also formation of cataphylls may be inhibited by high temperatures. It should be possible to force plants of Osmunda cinnamomea to form cataphylls more slowly by a moderate raise in temperature; under such conditions it will perhaps be possible to obtain the same overlapping of cataphyllization and sporophyll formation that we have observed in Osmunda Claytoniana at 17°C.

Steeves and Wetmore (169) report that "cataphylls are capable of developing in the absence of any expanding or mature fronds on the plant." No doubt the results of their experiments are very suggestive, but it seems to us that it would be desirable to reserve the term "cataphyll" to those leaves that have already a necrotic crozier. In these the crozier is dead and cannot be revived. What is established by Steeves' and Wetmore's experiments is the possibility of development of perspective cataphylls or of cataphylls that are incompletely determined as such. This indicates the existence of an active process that checks the development

of perspective cataphylls until the crozier becomes
completely necrotic. Since the preliminary results of
Steeves and Wetmore (169) indicate that this process
involves a correlation between adult leaves and leaf
primordia, it would be interesting to know whether photo-
periodism and temperature can affect this correlation.

On the whole our results with respect to cataphyll
determination in Osmunda Claytoniana agree with Goebel's
views that cataphylls are leaves arrested in the young
stage.

The situation seems to be different in the determin-
ation of cataphylls of Angiosperms, since Foster (71, 75,
76, 77) has established that their determination takes
place much earlier and in fact in the primordia. It would
not be too surprising if this difference in behavior in the
different groups of vascular plants is substantiated by
further research, since it was already shown by Wetmore
(189) that, for the regeneration from meristems, there are
differences between the two plant groups. What seems to be
common to these types of cataphylls is the fact that they
are determined, to a certain extent, by the environmental
conditions which prevail in the course of leaf development.
Whether the effects of these conditions become visible very
early (as in Angiosperm cataphylls), or later (as in Osmunda
cinnamomea), or very late, after pinnae differentiation
(as it happens in Osmunda Claytoniana) it seems to be a
matter of relative rates of different morphogenetic processes.

Further progress in the study of cataphyll determination in Osmunda can be obtained by a systematic study of Osmunda Claytoniana. In this respect it is essential to obtain information on the environmental conditions that determine cataphylls, on the correlative nature of the process and on its light dependence. The contrast of results of studies with whole plants in controlled conditions with the results of environmental treatments applied to excised leaf cultures by the technique of Sussex and Steeves (176), should also make clear whether the inhibition of leaf primordia, of undifferentiated leaves and of already differentiated young leaves involves only correlative effects with adult leaves or environmental effects on the developing leaf itself.

One of the yet unsolved problems of cataphyll determination lies in the fact that Osmunda Claytoniana is a perennial plant. If short days and low temperature were the only factors involved in the determination of cataphylls the plant would be a summer annual, since all leaf primordia would be "cataphyllized" in the fall. Other requirements must exist for cataphyll determination. It is possible that correlation effects with adult leaves and with the terminal meristem of the rhizome would respectively promote and inhibit the processes that lead to the cataphyllization of young leaves.

Initiation of sporangia and the physiology of development.

Our studies of effects of environmental factors on the
initiation of sporangia show that of the species of ferns
investigated, the most interesting is Salvinia rotundi-
folia. This fern displays a combination of features
which are unique. Both photoperiodic and thermoperiodic
processes, are involved in the initiation of sporangia.
In addition the photoperiodic processes show a qualitative
change with temperature.

There are a number of results reported in the litera-
ture in which photoperiodic processes have been shown to
be modified by temperature conditions. The simplest cases
of such a modifying effect of temperature are represented
by those in which the efficiency of the photoperiodic reac-
tions, particularly of the dark period, are found to have
a temperature dependence. Thus, it was shown by Garner
and Allard (81) that the time required for flowering by
some varieties of soybean, planted in Washington, all at
the same time of the year, in different years, varies
and these variations showed correlation with the tempera-
ture conditions in the different years. Further studies
by Parker and Borthwick (145) have shown that the important
temperature differences are those of the dark periods and
that, although the effects of a 16-hour dark period varies
with the temperature of the dark period (nyctitemperature),
8 hours dark periods are always ineffective for flower

induction, no matter what nyctitemperature is used. Simi-
lar results were obtained with the short-day plant Xanthium
pennsylvanicum (93, 124), and the most significant effects
were found to be those due to temperature of the leaf dur-
ing the dark period (146). Roberts and Struckmeyer (157)
grew several species in two separate greenhouses, in which
the nyctitemperatures were kept close to $14^{o}C$ and to $20^{o}C$,
respectively. A species of Salvia was found to require
short days to flower at the lower nyctitemperature and
failed to flower, both in short days and in long days at
the higher nyctitemperature. White clover showed long-
day requirement for flowering at the lower nyctitemperature
and no flowering (in short, nor in long days) at the higher
nyctitemperature. A species of Cleome, which behaved as
a short-day plant at the higher nyctitemperature failed to
flower both in short days and in long days at the lower
nyctitemperature. Evans (67) found that the quantitative
long-day plant Vicia faba, when grown in the Earhart
Laboratory in short (8 hour) photoperiods, with daily
thermoperiodicities at different levels, flowers faster
when the level of the thermoperiodicity is decreased. The
results of changing the nyctitemperature in the case of
Xanthium pennsylvanicum (146) show that the effect of the
long night induction can be decreased both by excessively
low and excessively high nyctitemperatures. On the other
hand, even at relatively constant nyctitemperature, there
is an optimal duration of the dark period for the induction

of flower initiation in this plant (122). This last fact
can be interpreted in several ways (122), but, in general,
it shows that not all processes that take place during
the dark period are favorable to flower initiation. There-
fore it is understandable that too low nyctitemperatures
can limit the rate of the promotive processes and too high
nyctitemperatures can increase the rate of the destructive
processes, whether they are simultaneous or not. It is
conceivable that the minimum and maximum nyctitemperatures
would not be the same for all plants, even in the short-day
class. Thus the results of Roberts and Struckmeyer with
Salvia might be due to too high nyctitemperatures and those
with Cleome to too low nyctitemperatures. On the other
hand it is known that one of the limiting factors for the
flowering of long-day plants is a destructive process that
takes place during the dark period (117). It is there-
fore conceivable that an increase in nyctitemperature, for
these plants, would increase the rate of this process, thus
producing detrimental effects on flower initiation. This
could explain the results of Roberts and Struckmeyer with
white clover and Evans' result with Vicia faba.

The behavior of Asplenium bulbiferum at $20^{\circ}C$, in our
experiments, with a critical long night of 23 hours in
48 hour cycles and its quantitative long-day behavior in
24 hour cycles could be due to the fact that $20^{\circ}C$ is suf-
ficiently low temperature to make the detrimental dark
process slow enough to require extremely long times to be

effective. If this interpretation is correct, the plant should possess a shorter critical night length at higher temperatures.

In all the preceding attempts of interpretation of the experimental results reported, the explanations suggested must be tested by quantitative studies in temperature controlled conditions. All that may be said at the moment is that the results do not contradict this interpretation, so that it can be kept, for the time being, as a useful working hypothesis.

Roberts and Struckmeyer (157) report that Maryland Mammoth Tobacco behaves as a short-day plant at the higher nyctitemperature (flowering only in days of approximately 9 hours), but flowers also in long-day conditions at the lower nyctitemperature (the plants received a supplement of 30-80 ft. candles of light "from before sunset until midnight"). On the other hand the Klondike Cosmos, var. "orange flare," that flowered only in short days with the lower nyctitemperature, flowered also in the above described long-day conditions at the higher nyctitemperature. As the authors did not conduct experiments with the same plants in continuous light at different temperatures (a fact that is understandable because air conditioning facilities for greenhouses were not available at the time these pioneer experiments were done), all we can conclude is that under the conditions of these experiments, there was a shortening of the critical long night for these plants, with increase

of the nyctitemperature. Reath and Wittwer (152) have observed that with 16°C nyctitemperatures "Alaska" and "Surprise" peas are day neutral, whereas with 10°C nycti-temperatures these forms behave as quantitative long-day plants.

Good examples of changes in photoperiodic behavior with temperature have been observed in the Earhart Laboratory in which day and night temperatures may be controlled. Thus Went (187) found that Marshall strawberries (Fragaria virginia-na X F. chiloensis) behave as short-day plants at temperatures above 10°C, but that the short-day behavior becomes much less pronounced at lower temperature. For example, 12 hour photoperiods are too long for flower initiation at 20°C and at 17°C, but are effective at and below 14°C, 16 hour photoperiods, that are ineffective at 14°C, are effective at 10°C and at 6°C. At the two latter temperatures the plants flower even in continuous light.

Another plant that quantitatively changes its photo-periodical behavior with temperature is the potato (Solanum tuberosum). Tuber formation in potato is usually considered to be a short-day process. This is true however only in high nyctitemperatures. At 10°C nyctitemperatures, 16 hour photoperiods are almost as effective as 8 hour photoperiods. At low temperatures tubers are formed even in continuous light (187).

Mathiola incana has been found to behave as a long-day

plant in the summer and as an indeterminate plant in the winter. This is due to the fact that it does not flower in short days at temperatures above 18°C. At temperatures below 18°C it flowers both in short and in long days (186).

The work of Paton with a late pea variety in the Earhart Laboratory (197) also establishes the fact that the photoperiodic response of this plant is quantitatively altered by changes in the temperature of the dark period: "In the absence of temperature variations during the dark period plants grown in 8 hour photoperiods (P_8) flower 25-60 days later than plants grown in 16 hour photoperiods (P_{16}). A temperature change of 10°-19°C during the dark period almost eliminates the photoperiodic response, P_8 plants initiating the first flower only 6-10 days later than the P_{16} plants."

The fact that Salvinia rotundifolia shows qualitative changes in photoperiodic behavior with temperature changes between 26°C to 17°C is of interest, since it represents an extreme of the series of cases referred to above. This property makes this plant a very favorable object for the study of the interrelations between partial processes in photoperiodism. For this purpose it will be desirable to determine the critical photoperiod at different temperatures and to clarify the situation at 20°C. At this temperature there is very slow initiation of sporangia both in continuous light and in short (8 hour) days and it is possible that this species, at this temperature, may be limited by two

overlapping reactions, requiring short days and long days, respectively.

The thermoperiodic behavior of Salvinia rotundifolia, shown by the results of Experiments #15 and #16, displays a pattern of reaction similar to that observed by Paton in the case of a late pea variety (147).

In general the environmental requirements for the initiation of sporangia on all species of ferns studied bring into consideration problems of interrelations between temperature and the photoperiodic processes, as well as problems of interrelations between the initiation of sporangia and other morphogenetic processes. The frequency with which these results appear in the study of similar processes in other plants, using the same techniques of culture of plants under controlled climatic conditions, suggests that this is not an isolated situation, although it might have been overlooked by the fact that equipment facilities rarely allow the study of interaction of factors and processes in a reasonably short time.

Concepts elaborated in different realms of morphogenesis are necessarily operational and, as such, they reflect the limitations of the techniques employed in these fields. Thus, the study of vascular tissue differentation and of heteroblastic leaf development place emphasis on the local circumstances prevailing in the meristems, whereas in the study of reproductive differentiation the accent is placed in correlations between the meristems

and the organs that perceive the environmental stimuli. Since these are actually two faces of the same phenomenon-- development--it is hoped that an approach that works with both aspects would bring to our knowledge new facts and new relationships that will unify scattered and even conflicting data in a simpler picture.

PART VII

BIBLIOGRAPHICAL REFERENCES

1. Albaum, H., Inhibitions due to growth hormone in fern prothallia and sporophytes, Amer. Jour. Bot. 25: 124-133, 43 figs., 1938.

2. _____, Normal growth, regeneration and adventitious outgrowth formation in fern prothallia, Amer. Jour. Bot. 25: 37-44, 12 figs., 1938.

3. Allsopp, A., Chromatographical study of meristematic plant tissues, Nature 161: 833, 83 figs., 1948.

4. _____, Experimental and analytical studies of Pteridophytes. XVII. The effect of various physiologically active substances on the development of Marsilea in sterile culture, Ann. Bot. NS 16: 165-183, 2 pl., 1952.

5. _____, The sugars and non-volatile organic acids of some archegoniates: a survey using paper chromatography, Jour. Exp. Bot. 2: 121-124.

6. _____, Experimental and analytical studies of Pteridophytes. XIX. Investigations on Marsilea. 2. Induced reversion to juvenile stages, Ann. Bot. NS 17: 37-55, 1 pl., 1953.

7. _____, Experimental and analytical studies of Pteridophytes. XXI. Investigations on Marsilea. 3. The Effects of various sugars on development and morphology, Ann. Bot. NS 17: 447-463, 2 pl., 1953.

8. _____, Juvenile stages of plants and the nutritional status of the shoot apex, Nature 173: 1032-1035, 1954.

9. Andersson, I., The genetics of variegation in a fern, Jour. Genetics, 13: 1-11, 2 pl., 1923.

10. _____, Note on some characters in ferns subject to mendelian inheritance, Hereditas, 9: 167-168, 4 figs., 1927.

11. Andersson-Kottö, L., A genetical investigation on Scolopendrium vulgare, Hereditas, 12: 109-178, 28 figs., 4 pl., 1929.

12. Andersson-Kottö, L., Variegation in three species of ferns (Polystichum angulare, Lastrea atrata, and Scolopendrium vulgare) Zeitschr. Ind. Abst. Ver. 56: 115-201, 5 figs., 1930.

13. _____, On the comparative development of alternating generations with special reference to ferns, Svensk. Bot. Tidskr. 30: 57-79, 1936.

14. Andersson-Kottö, L., and Gairdner, A. E., the Inheritance of apospory in Scolopendrium vulgare, Jour. Genetics, 32: 189-228, 7 pl., 2 tab., 1936.

15. Ashby, E., Studies on the morphogenesis of leaves. I. An essay on leaf shape, New Phytol. 47: 153-176, 13 figs., 1948.

16. _____, Studies on the morphogenesis of leaves. II. The area, cell size and cell number of leaves of Ipomoea in relation to their position in the shoot, New Phytol. 47: 177-195, 13 figs., 1948.

17. Ashby, E., and Wangermann, E., Studies on the morphogenesis of leaves. III. Preliminary observations on vegetative growth of Lemna minor, New Phytol. 48: 374-381, 4 figs., 1949.

18. _____, Studies on the morphogenesis of leaves. IV. Further observations on area, cell size and cell number of leaves of Ipomoea in relation to their position in the shoot, New Phytol. 49: 23-35, 7 figs., 1950.

19. Atkinson, G. F., A preliminary note on the relation between the sterile and the fertile leaves of Onoclea, Bot. Gaz. 19: 374-375, 1894.

20. _____, The study of the biology of ferns by the collodion method, XVII, 134 pp., ill., Macmillan and Co., New York and London, 1894.

21. _____, The relation between the sterile and fertile leaves of dimorphic fern, Fern Bull. 19: 81-84, 1896.

22. _____, The probable influence of disturbed nutrition on the evolution of the vegetative phase of the sporophyte, Amer. Nat. 30: 349-357, 1896.

23. _____, Some problems of sporophyll transformation among dimorphic ferns, Bot. Gaz. 22: 220-221, 1896.

24. _____, The relation between the sterile and fertile leaves of dimorphic ferns, Fern Bull. 19: 81-84, 1911.

25. Ball, E., Development in sterile culture of stem tips and subjacent regions of Tropaeolum majus L., and of Lupinus albus L., Amer. Jour. Bot. 33: 301-318, 16 figs., 1946.

26. _____, Segmentation of the shoot apex, Amer. Jour. Bot. 33: 817, 1946.

27. _____, Studies on the nutrition of the callus of Sequoia sempervirens, Ann. Biol. 31: 281-305, 20 figs., 1955.

28. Bateson, W., Genetic segregation, Proc. Roy. Soc. London, Ser. B 91: 358-368, 1920.

29. Binford, R., The development of the sporangium of Lygodium, Bot. Gaz., 44: 214-224, 34 figs., 1907.

30. Blake, S. F., A unilaterally fertile frond of Dryopteris thelypteris, Amer. Fern Jour. 23: 67-69, 1 fig., 1933.

31. Bloom, W. W., and Voth, P. D., Responses of Regnellidium diphyllum to the nutrient supply and photoperiod, Bot. Gaz. 117: 173-193, 16 figs., 1956.

32. Borthwick, H. A., M. W. Parker and P. H. Heinze, Influence of localized low temperature on biloxi soybean during photoperiodic induction, Bot. Gaz. 102: 792-800, 2 figs., 1941.

33. Bower, F. O., On the comparative morphology of the leaf in the vascular cryptogams and gymnosperms, Phil. Trans, Roy. Soc. London, 175: 565-615, 1884.

34. _____, On apospory in ferns (with special reference to Mr. Charles T. Druery's observations), Jour. Linn. Soc. Botany, 21: 360-368, 6 figs., 2 pl., 1886.

35. _____, Preliminary note on the formation of gemmae on Trichomanes alatum, Ann. Bot. 1: 183-184, 1887.

36. _____, On apospory and allied phenomena, Trans. Linn. Soc. London, 2 Ser. Bot. 2: 301-326, 1887.

37. _____, Studies on the morphology of spore producing members. IV. The Leptosporangiate Ferns, Phil. Trans. Roy. Soc. London, Ser. B 192: 29-138, 6 pl., 1899.

38. _____, Imperfect sporangia in certain Pteridophytes. Are they vestigial? Ann. Bot. 15: 225-267, 1901.

39. _____, The Ferns (Filicales), treated comparatively with a view to their natural classification. Vol. I Analytical examination of the criteria of comparison, X+359pp., 309 figs., University Press, Cambridge, 1923.

40. _____, The Ferns, idem, vol. 2 The Eusporangiatae and other relatively primitive ferns, VI+344 pp., 580 figs., University Press, Cambridge, 1926.

41. _____, The Ferns (Filicales), idem, Vol. 3 The Leptosporangiate Ferns, VIII+306 pp., 755 figs., University Press, Cambridge, 1928.

42. Brown, D. W., Apogamy in Phegopteris polypodoides, Bull. Torrey Bot. Club, 50: 17-34, 1918.

43. Buscaloni, L., Sui tricomi delle Felci con particolare riguardo alle parafisi, Malpighia, 28: 162-178, 271-284, 545-554, 2 pl., 1917.

44. Campbell, D. H., The Eusporangiatae, the comparative morphology of the Ophioglossaceae and Marattiaceae, VI+229 pp., 192 figs., Carnegie Institution, Washington, 1911.

45. _____, The evolution of land plants (Embryophyta), 731 pp., 351 figs., University Press, Stanford, 1939.

46. Camus, G., Mise en évidence de l'action differenti-atrice des bourgeons par la méthode des greffes, Compt. Rend. Acad. Sci., Paris, 221: 570-572, 2 figs., 1945.

47. _____, Recherches sur le rôle des bourgeons dans les phénomènes de morphogenèse, Rév. Cyt. et Biol. Végét. 11: 1-199, 1949.

48. Chamberlain, C. J., Winter characters of certain sporangia, Bot. Gaz. 25: 125-128, 1 pl., 1898.

49. Chavan, A. P., A morphological study of Cyathodium Barodae, Amer. Jour. Bot. 24: 484-491, 83 figs., 1937.

50. Chouard, P., Expériences de longue durée sur le photo-periodisme; leçons qui en découlent, Mém. Soc. Bot. France, 106-146, 1949.

51. Chowdhury, N. P., On the occurrence of superficial, abaxially placed sori in Osmunda Claytoniana, Jour. Indian Bot. Soc. 11: 137-145, 3 figs., 1 pl., 1932.

52. Chrysler, M. A., Abnormalities of Botrychium and certain other ferns, Bull. Torrey Bot. Club, 53: 279-289, 1926.

53. _____, An unusual Botrychium, Amer. Fern Jour.
    <u>32</u>: 87-88, 1 pl., 1942.

54. Chrysler, M. A., and Johnson, D. S., Spore production
    in Regnellidium diphyllum, Bull. Torrey Bot. Club,
    <u>66</u>: 263-279, 37 figs., 1939.

55. Clute, W. W., Fall fruiting of the cinnamon fern,
    Fern Bull. <u>15</u>: 39-40, 1907.

56. _____, Rare forms of ferns. IX. Four aberrants
    Osmundas, Fern Bull. <u>17</u>: 10-12, 6 figs., 1909.

57. Copeland, E. B., The comparative morphology of the
    San Ramon Polypodiaceae, The Philippine Jour. Sci.
    <u>2</u>: 1-76, 4 pl., 1907.

58. Crotty, W. J., Trends in the pattern of primordial
    development with age in the fern Acrostichum Danae-
    folium, Amer. Jour. Bot. <u>42</u>: 627-636, 4 figs., 1955.

59. Davenport, G. E., Onoclea sensibilis var. obtusi-
    lobata, Bull. Torrey Bot. Club, <u>8</u>: 109-111, 1881.

60. Dickason, F. G., A phylogenetic study of the ferns of
    Burma, The Ohio Jour. Sci. <u>46</u>: 73-108, 24 figs.,
    4 charts, 1946.

61. Diels, L., in: Engler, A. und Prantl, K., Die
    natürlichen Pflanzenfamilien, ITeil, Abt. 4, VI+
    808 pp., 481 figs., Wilhelm Engelmann, Leipzig, 1902.

62. Döpp, W., Karyologie, Chap. 8 in Verdoorn, F. (edit.),
    Manual of Pteridology, XX+640 pp., 121 figs.,
    Martinus Nijhoff, The Hague, 1938.

63. Drew, K. M., Rhodophyta, in: Smith, G. (edit.),
    Manual of Phycology, XI+375 pp., 48 figs., Chronica
    Botanica, Waltham, Mass. 1951.

64. Du Buy, H. G., and Nuernbergk, E. L., Growth, tropisms
    and other movements, Chap. 10 in Verdoorn, F. (edit.),
    Manual of Pteridology, XX+640 pp., 121 figs., Martinus
    Nijhoff, The Hague, 1938.

65. Duncan, R. E., Apogamy in Doodia caudata, Amer. Jour.
    Bot. <u>28</u>: 921-931, 1941.

66. Dutra, J., A flora Pteridophyta do Rio Grande do Sul,
    An. la. Reuniao Sul Amer. Botanica, Rio de Janeiro,
    <u>2</u>: 19-68, 1938.

67. Evans, L., The broad bean, Chap. 8 in: Experimental control of plant growth, F. W. Went (edit.), XVII+ 343 pp., 71 figs., 25pl., Chronica Botanica, Waltham, Mass.

68. Farmer, J. B., and Digby, L., Studies on apospory and apogamy in ferns, Ann. Bot. 21: 161-199, 1907.

69. Fée, A. L. A., Mémoires sur la famille des fougères, 2 vols. (Biblioteca Nacional, Rio de Janeiro), 1844.

70. _____, Genera Filicum, exposition des genres de la famille des Polypodiacées (Classe des Fougères), 5 me mém., 388 pp., 30 pl., V. C. Berger-Levrault et Fils, Strasbourg, 1850-52.

71. _____, Iconographie des espèces nouvelles décrites ou énumerées dans le Genera Filicum et revision des publications antérieures relatives à la famille des fougères, 6me, 7me et 8me mémoires, VI+138 pp., 27 pl., V. Berger-Levrault et Fils., Paris-Strasbourg.

72. Fernald, M. L., Critical plants of the upper great lakes region of Ontario and Michigan, Rhodora, 37: 197-222, 4 pl., 11 maps, 1935.

73. Ford, E. S., Additional fertile pinnules in the rattle-snake fern, Amer. Fern Jour. 25: 18-20, 1 pl., 1935.

74. Foster, A. S., Salient features of the problem of budscale morphology, Biol. Rev. Cambridge Phil Soc. 3: 123-164, 1928.

75. _____, Phylogenetic and ontogenetic interpretation of the cataphyll, Amer. Jour. Bot. 18: 243-249, 1931.

76. _____, Investigations on the morphology and comparative history of development of foliar organs. III. Cataphyll and foliage leaf ontogeny in the black hickory (Carya Buckleyi., var. arkansana), Amer. Jour. Bot. 19: 75-99, 1932.

77. _____, Leaf differentiation in Angiosperms, Bot. Rev. 11: 348-372, 10 figs., 1936.

78. Gairdner, A. E., Sporangia containing spermatozoides in Ferns, Nature, 131: 621-622, 1933.

79. Gams, H., Ökologie der extratropischen Pteridophyten, Chap. 12 in: Verdoorn, F. (edit.), Manual of Pteridology, XX+640 pp., 121 figs., Martinus Nijhoff, The Hague, 1938.

80. Garner, W. W., and Allard, H. A., Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants, Jour. Agric. Res. 18: 553-606, pl. 64-79, 1920.

81. _____, Photoperiodic response of soybeans in relation to temperature and other environmental factors, Jour. Agric. Res. 41: 719-735, 1930.

82. Gautheret, R. J., La culture des tissus, 202 pp., 43 figs., Gallimard, Paris, 1945.

83. Gilbert, B. E., Interrelation of relative day length and temperature, Bot. Gaz. 81: 1-24, 5 figs., 1926.

84. Glück, H., Die Sporophyllmetamorphose, Flora, 80: 303-387, 38 figs., 1 pl., 1895.

85. Goebel, K., Ueber Sprossbildung auf Isoetesblättern, Bot. Zeit. 37: 1-6, 4 figs., 1879.

86. _____, Beiträge zur vergleichende Entwicklungsgeschichte der Sporangien, Bot. Zeit. 38: 545-552, 561-571, 1 pl., 1880.

87. _____, Über künstliche Vergrünung der Sporophylle von Onoclea Struthiopteris, Protok. d. Fünften Gen Versamml. d. deut. bot. Gesels. LXIX-LXXIV, 1887.

88. _____, Organography of plants, especially of the Archegoniatae and Spermatophyta, Engl. trans. I. B. Balfour, vol. 2: Special Organography, 707 pp., 417 figs., Clarendon Press, London, 1905.

89. _____, Beiträge zur Kenntnis der Verwandtschaftsverhältnisse einiger javanischer Farne, Ann. Jard. Bot. Buitenzorg, 36: 105 (pl. 59-64), 1926.

90. _____, Pteridologische Notizen. 2. Neotenie und Sporophyllvariation bei Aneimia, Flora, 125: 457-471, 11 figs. 1931.

91. Harder, R., Vegetative and reproductive development of Kalanchöe Blossfeldiana as influenced by photoperiodism, Growth II (Sump. Soc. Exp. Biol.) VII 365 pp., ill., University Press, Cambridge, 1948.

92. Harris, S. K., Reports on the flora of Massachusetts. II. Rhodora, 35: 352-359, 1933.

93. Hastings, G. T., Rudimentary sporangia on the royal fern, Torreya 23: 10, 1923.

94. Heinricher, E., in 136, 1881.

95. Hertzog, R., Ein Beitrag zur Systematik der Gattung Salvinia, Hedwigia, 74: 257-284, 8 figs., 1935.

96. Holloway, J. E., Occurrence of tracheids in gametophytes of Tmesipteris, Abstr. Meet. Amer. Assn. Adv. Sci., 1938.

97. _____, The gametophyte, embryo and young rhizome of Psilotum triquetrum, Ann. Bot. NS 3: 313-336, 1939.

98. Holttum, R. E., The ecology of tropical Pteridophytes, Chap. 13 in: Verdoorn, F. (edit.), Manual of Pteridology, XX+640 pp., 121 figs., Martinus Nijhoff, The Hague, 1938.

99. House, H. D., Additions to the fern flora of New York State, Amer. Fern Jour. 23: 1-7, 1 fig., 1933.

100. Jacobs, W. P., The role of auxin in differentiation of xylem around a wound, Amer. Jour. Bot. 39: 301-309, 5 figs., 5 tabs., 1952.

101. _____, Acropetal auxin transport and xylem regeneration--a quantitative study, Amer. Nat. 88: 327-337, 2 figs., 1954.

102. Jensen, W. A., The histochemical localization of peroxidase in roots and its induction by indoleacetic acid, Pl. Phys. 30: 426-432, 4 figs., 1955.

103. Johnson, D. S., On the development of the leaf and sporocarp of Marsilea quadrifolia L., Amer. Jour. Bot. 12: 119-145, 3 pl., 1898.

104. Johnson, D. S., and Chrysler, M. A., The structure and development of Regnellidium diphyllum, Amer. Jour. Bot. 25: 141-156, 57 figs., 1938.

105. Kaufhold, A. W., Über der Einfluss der täglichen Beleuchtungsdauer und der Lichtintensität auf die Entwicklung einiger Archegoniaten, Beih. Bot. Centralbl. Abt. A 60: 641-678, 13 tabs., 1941.

106. Kupper, W., in 137, 1906.

107. Labouriau, L. G., Nota sobre algumas alomorfias dos esporófilos de espécies dos gêneros Lygodium, Osmunda, Botrychium, Tmesipteris e Blechnum, Rev. Bras. Biol. 7: 383-390, 7 figs., 1947.

108. _____, Nota sobre algumas alomorfias dos esporófilos de espécies dos gêneros Anemia, Blechnum e Polybotrya, Arqu. Jard. Bot. Rio de Janeiro, 8: 281-293, 10 figs., 1948.

109. _____, Contribuição ao estudo da morfogênese dos esporófilos em Anemia Sw. I. Teratologia dos esporófilos, Arqu. Jard. Bot. Rio de Janeiro, 8: 381-516, 98 figs., 1948.

110. _____, Contribution to the study of sporophyll morphogenesis in Anemia Sw. II. Morphological studies, Rev. Bras. Biol. 11: 367-391, 159 figs., 1951.

111. _____, Contribution to the study of sporophyll morphogenesis in Anemia SW. IV. Some effects of applied auxins, Rev. Bras. Biol. 12: 33-43, 60 figs., 1952.

112. _____, Contribution to the study of sporophyll morphogenesis in Anemia Sw. V. Correlation phenomena between fertile and sterile fronds, Rev. Bras. Biol. 12: 59-68, 17 figs., 1952.

113. _____, Contribution to the study of sporophyll morphogenesis in Anemia Sw. VI. Further studies on correlation phenomena between fertile and sterile fronds, PYTON, 2: 17-24, 54 figs., 1952.

114. Lang, W. H., On apogamy and the development of sporangia upon fern prothallia, Phil. Trans. Roy. Soc. London, Ser. B 190: 189-238, 5 pl., 1898.

115. _____, On the morphology of Cyathodium, Ann. Bot. 19: 411-426, 2 pl., 1905.

116. _____, On a variety of Scolopendrium vulgare that bears sporangia on the prothallus, Ann. Bot. 43: 355-374, 6 figs., 1 pl., 1929.

117. Lang, A., Physiology of flowering, Ann. Rev. Pl. Physiol. 3: 265-306, 1952.

118. League, E. A., and Greulach, V. A., Effects of day length and temperature on the reproduction of Vaucheria sessilis, Bot. Gaz. 117: 45-51, 3 figs., 1956.

119. Lawton, E., Regeneration and induced polyploidy in ferns, Amer. Jour. Bot. 19: 303-333, 22 figs., 5 tabs., 1932.

120. Lillibridge, A. E., Some abnormal forms of Botrychium simplex, Amer. Fern Jour. 16: 127, 1 pl., 1926.

121. Lindmann, C. A., Regnellidium, novum genus Marsiliacearum, Akiv f. Botnik, 3: 1-14, 10 figs., 1904.

122. Livermann, J., The physiology of flowering, Ann. Rev. Plant Physiol. 6: 177-210, 2 figs., 1955.

123. Livermann, J., and Bonner, J., Biochemistry of the photoperiodic response: the high intensity light reaction, Bot. Gaz. 115: 121-128, 5 tabs. 1953.

124. Long, E. M., Photoperiodic induction as influenced by environmental factors, Bot. Gaz. 101: 168-188, 2 figs., 1939.

125. Maheshwari, P., An introduction to the embryology of Angiosperms, 1st ed. X+453 pp., 216 figs., McGraw-Hill Book Co., New York, Toronto, London, 1950.

126. Manton, I., Polyploidy in Polypodium vulgare, Nature, 159: 136, 2 figs., 1947.

127. _____, Problems of cytology and evolution in the Pteridophyta, XI+316 pp., 279 figs., University Press Cambridge, 1950.

128. Marschal, E., and Marschal, Em., Aposporie et sexualité chez les mousses, Bull. Acad. Roy. Belgique I-3, in: R. van der Wijk, Morphologie und Anatomie der Musci, Chap. I in Verdoorn, Fr., 1932, Manual of Bryology, IX 486 pp., 129 figs., Martinus Nijhoff, The Hague, 1907.

129. Massart, J., Bouillenne, R., Ledoux, P., Brien P., Navez, P., Une mission biologique belge au Brésil, II, 67 p., 549 figs., Imprimerie médicale et scientifique, Bruxelles, 1929.

130. Martens, P., Les organes glanduleux du Polypodium virginianum (Polypodium vulgare var. virginianum). I. valeur systématique et repartition geographique, Bull. Jard. Bot. Bruxelles, 17: 1-14, 5 figs., 1943.

131. _____, Les organes glanduleux sporangiaux de Polypodium virginianum (P. vulgare var. virginianum), Bull. Soc. Roy. Bot. Belgique, 76: 37-38, 1944.

132. _____, Formations sporangiales et parasporangiales chez quelques fougères, Bull. Soc. Roy. Bot. Belgique, 79, 2me ser. 29 fasc. 1/2: 44, 4 figs., 1947.

133. _____, Les paraphyses de Polypodium vulgare et la

sous-espèce serratum, Bull. Soc. Roy. Bot. Belgique, 82: 225-262, 1949.

134.   , Les organes glanduleux de Polypodium virginianum. III. Nouvelles données systematiques, géographiques et histologiques, La Cellule, 53: 185-212, 3 figs., 1 pl., 1950.

135. Martens, P., et Pirard, N., Les organes glanduleux de Polypodium virginianum. II. Structure, origine et signification, La Cellule, 49: 383-406, 3 pl., 1943.

136. Maxon, W. R., A list of the ferns and fern allies of North America, north of Mexico, with principal synonyms and distribution, Proc. US Nat. Museum, 23: 619-651, 1901.

137. McVeigh, I., Vegetative reproduction of the fern sporophyte, Bot. Rev., 3: 457-497, 1937.

138. Mehra, P. N., A note on the occurrence of superficial sori in Osmunda Claytoniana, Jour. Indian Bot. Soc. 11: 146-147, 1932.

139. Morel, G., and Wetmore, R. H., Fern callus tissue culture, Amer. Jour. Bot. 38: 141-143, 5 figs., 1951.

140. Morize, H., O Clima do Brasil, (Contribuição ao estudo do), Min. Agric. Ind. Com., Observ. Nacional, 2 edição, 116 pp., Tip. Serv. Inf. Agr., Rio de Janeiro, 1927.

141. Murneek, A. E., and Whyte, R. O., Vernalization and Photoperiodism, a symposium, XV+196 pp., ill., Chronica Botanica, Waltham, Mass., 1948.

142. Nakayama, S., Experimental researches on photoperiodism. (1) Photoperiodic responses of Salvinia, Bot. Mag., (Tokyo) 65: 274-279, 2 figs., 4 tabs., 1952.

143. Ogden, E. B., The ferns of Maine, The Maine Bull. 51: 5-128, 10 pl., 1 map, 1948.

144. Pace, L., Some peculiar fern prothallia, Bot. Gaz., 50: 49-58, 11 figs., 1910.

145. Parker, M. W., and Borthwick, H. A., Effect of variation in temperature during photoperiodic induction upon initiation of flower primordia in biloxi soybean, Bot. Gaz. 101: 145-167, 1939.

146. Parker, M. W., and Borthwick, H. A., Influence of temperature on photoperiodic reactions in leaf blades of biloxi soybean, Bot. Gaz. 104: 612-619, 1 fig., 1943.

147. Paton, D., Thermoperiodic and photoperiodic control of floral initiation in a late pea variety, Abstract in Plant Physiol. 32: IX-X, 1957.

148. Pfeiffer, W. M., Differentiation of the sporocarps in Azolla, Bot. Gaz. 44: 445-454, 2 pl., 1907.

149. Pirard, N., Sporanges, paraphyses et organes connexes chez les fougères, La Cellule, 51: 155-184, 85 figs., 2 pl., 1947.

150. Porter, C. L., and Porter, M. W., The ferns and fern allies of Muskoka Lake region of Ontario, Amer. Fern Jour. 20: 18-21, 1950.

151. Prantl, K., Untersuchungen zur Morphologie der Gefässkryptogamen. II. Die Schizaeaceen, 161 pp., 8 pl., Wilhelm Engelmann, Leipzig, 1881.

152. Reath, A. N., and Wittwer, S. H., The effects of temperature and photoperiod on the development of pea varieties, Proc. Amer. Soc. Hort. Sci. 60: 301-310, 3 figs., 6 tabs., 1952.

153. Reed, C. F., The ferns and fern allies of Maryland and Delaware including District of Columbia, 286 pp., 72 pl., Reed Herbarium, Baltimore, Md., 1953.

154. Resende, F., Long-short day plants, Portugaliae Acta Biol. Serie A, 3: 318-321, 1952.

155. _____, Acerca do impulso floral em plantas de "dia longo-curto" e plantas de dia curto-longo, Rev. Fac. Ciencias Lisboa Ser. 2C, 3: 447-534, 1953.

156. Ridley, H. N., Teratological notes on plants. 3. Note on Equisetum maximum, var. serotinum (var. proliferum Milde), Jour. Linn. Soc. 20: 47-48, 1 fig., 1884.

157. Roberts, R. H., and Struckmeyer, B. E., The effects of temperature and other environmental factors upon the photoperiodic responses of some of the higher plants, Jour. Agric, Res. 56: 653-677, 46 figs., 1938.

158. Sachs, R., Floral initiation in Cestrum nocturnum. I. A long-short day plant, Plant Physiol., 31: 185-192, 2 figs., 5 tabs., 1956.

159. Sadebeck, R., Salviniaceae in Engler, A., und Prantl, K., Die natürlichen Pflanzenfamilien, I Teil, Abt. 4, VI+808 pp., 481 figs., Wilhelm Engelmann, Leipzig, 1902.

160. Salisbury, F., Kinetic studies on the physiology of flowering, Ph.D., Thesis, California Institute of Technology, 1955.

161. Salisbury, F. B., and Bonner, J., Interaction of light and auxin in flowering, Beitr. Biol. d Pflanzen, 31: 419-430, 1955.

162. Schultz, A., Contribuição ao conhecimento de Regnellidium diphyllum, Lindm., Lilloa, 17: 139-144, 2 figs., 1949.

163. Sen Gupta, J. C., and Santosh, K. P., Leaf heteromorphism and photoperiods in Sesamum orientale L., Nature, 160: 510, 1 fig., 1 tab., 1947.

164. Shattuck, C. H., On the origin of heterospory in Marsilia, Bot. Gaz. 49: 19-40, 4 pl., 1910.

165. Shurtleff, R., Botrychium dissectum, Amer. Fern Jour. 28: 25-28, 2 figs., 1938.

166. Sinnot, E. W., and Bloch, R., Visible expression of cytoplasmic pattern in the differentiation of xylem strands, Proc. Nat. Acad. Sci., Washington, 30: 388-392, 3 figs., 1944.

167. Small, J. K., Ferns of the vicinity of New York, 385 pp., The Science Press Printing Co., Lancaster, Pa., 1935.

168. Steele, W. C., Fall fruiting of Osmunda, Fern Bull. Torrey Bot. Club, 15: 39-40, 1902.

169. Steeves, T. A., and Wetmore, R. H., Morphogenetic studies on Osmunda cinnamomea L., some aspects of general morphology, Phytomorphology, 3: 339-354, 8 figs., 5 tabs., 1953.

170. Steil, W. N., Apogamy, Apospory and parthenogenesis in the Pteridophytes, Bot. Rev. 5: 433-453, 1939.

171. _____, Apogamy, Apospory and parthenogenesis in the Pteridophytes, II. Bot. Rev. 17: 90-104, 1951.

172. Stevens, W. C., On the development of sporangia and spores of Anemia phyllitidis, Ann. Bot. 25: 1059-1068, 2 pl., 1911.

173. Steward, F. C., R. H. Wetmore, J. F. Thompson, and
      J. P. Nitsch, A quantitative chromatographic study
      of nitrogenous compounds of shoot apices, Amer. Jour.
      Bot. 41: 123-134, 4 figs., 7 tabs., 1954.

174. Steward, F. C., Wetmore, R. H., and Pollard, J. K.,
      The nitrogenous compounds of the shoot apex of
      Adiantum pedatum, Amer. Jour. Bot. 42: 946-948, 1955.

175. Straszewski, H. R., in 41.

176. Sussex, I. M., and Steeves, T. A., Growth of excised
      fern leaves, Nature, 172: 624-625, 1953.

177. Tryon, R. M., D. W. Dunlop, N. C. Fasset, and M. E.
      Diemer, The ferns and fern allies of Wisconsin, 158
      pp., 215 figs., The University of Wisconsin Press,
      Madison, 1953.

178. Tschudy, R. H., The significance of certain abnormali-
      ties in Equisetum, Amer. Jour. Bot. 26: 744-749,
      18 figs., 1939.

179. Velenowsky, J., Vergleichende Morphologie der Pflanzen,
      I Teil, 277 pp., 200 figs., Rivnac, Praga, 1905.

180. Wardlaw, C. W., Experimental and analytical studies
      of Pteridophytes. 9. The effect of removing leaf
      primordia on the development of Angiopteris evecta
      Hoff. Ann. Bot. NS 10: 221-235, 14 figs., 1 pl., 1946.

181. _____, Experimental investigations of the shoot apex
      of Dryopteris aristata Druce, Phil. Trans. Roy. Soc.
      London, Ser. B, 232: 343-384, 76 figs., 5 pl., 1947.

182. _____, Further experimental observations on the
      shoot apex of Dryopteris aristata Druce, Phil. Trans.
      Roy. Soc. Ser. B, 233: 415-451, 44 figs., 7 pl., 1949.

183. _____, The comparative investigation of apices of
      vascular plants by experimental methods, Phil. Trans.
      Roy. Soc., Ser. B, 234: 583-602, 27 figs., 5 pl. 1950.

184. _____, Phylogeny and morphogenesis (contemporary
      aspects of botanical science), VIII 536 pp., 173
      figs., Mc Millan & Co., London, 1952.

185. Weatherby, C. A., A list of varieties and forms of
      the ferns of eastern North America, Amer. Fern Jour.
      25: 45-51, 95-100; 26: 11-16, 60-69, 74-99, 130-136;
      27: 20-24, 51-76, 1935.

186. Went, F. W., The effect of temperature on plant growth, Ann. Rev. Plant Physiol. 4: 347-362, 1953.

187. _____, The experimental control of plant growth, XVII+343 pp., 71 figs., 25 pl., Chronica Botanica, Waltham, Mass., 1957.

188. Wetmore, R. H., Carbohydrate supply and leaf development in sporeling ferns, Science, 118: 578, 1953.

189. _____, The use of in vitro cultures in the investigation of growth and differentiation in vascular plants, Abn. and path. plant growth, Brookhaven Symp. Biology, 6: 22-40, 33 figs., 1954.

190. Wetmore, R. H., and Sorokin, S., On the differentiation of xylem, Jour. Arnold Arboretum, 36: 305-317, 2 figs., 6 pl., 1955.

191. Wettstein, Fr. von, et al., Tratado de Botanica Sistematica, trad. esp. P. Font Quer, 4th ed., XIX, 1039 pp., 709 figs., Editorial Labor, Buenos Aires, 1944.

192. Williams, S., An analysis of the vegetative organs of Selaginella grandis Moore, together with some observations on abnormalities and experimental results, Proc. Roy. Soc. Edinb. 57: 1-24, 9 figs., 2 pl., 1931.

193. _____, Correlation phenomena and hormones in Selaginella, Nature, 139: 966, 1 fig., 1937.

194. _____, Experimental morphology, Chap. 3 in: Verdoorn, F. (edit.), Manual of Pteridology, XX+ 640 pp., 121 ill., Martinus Nijhoff, The Hague, 1938.

195. Yamanouchi, S., Apogamy in Nephrodium, Bot. Gaz. 45: 289-319, 1908.

196. Zanoni, G., Sulla fisiologia dello sporofillo di alcune Filicinee, Boll. d. Musei di Zool. e Anat. Comp. 17: 5-33, 6 tabs., 9 figs., 1937.

197. _____, Il sistema ossidoriduttivo acido ascorbico-dehidroascorbico e la funzione sporangifera, Bull. Musei e d. Iss. d. Univers. Genova, 21: 3-24, 1941/2.