

0°C. Rb* was absorbed by the roots in a form easily removed by exchange for inert isotopes in the bathing solution. P*O₄ was absorbed non-metabolically in a form with difficulty removable by exchange for isotopes in the outside solution.

The non-metabolic uptake of trace amounts of Rb and PO₄ is to a major degree confined to the first few millimeters from the root tip. In this re-

gion the uptake represents an accumulation in that the concentration per unit volume of tissue is greater than in the bathing solution.

The application of the procedure used to the general problem of ion uptake is discussed.

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LITERATURE CITED

- BROOKS, S. C. 1937. Selective accumulation with reference to ion exchange by the protoplasm. *Trans. Faraday Soc.* 33: 1002-1006.
- BROYER, T. C., AND R. OVERSTREET. 1940. Cation exchange in plant roots in relation to metabolic factors. *Amer. Jour. Bot.* 27: 425-430.
- HAMILTON, J. G. 1942. The use of radioactive tracers in biology and medicine. *Radiology* 39: 541-572.
- HELMHOLTZ, A. C., CHARLES PECHER, AND PERRY R. STOUT. 1941. Radioactive Rb from deuteron bombardment of Sr. *Phys. Rev.* 59: 902.
- HOAGLAND, D. R. 1940. Salt accumulation by plant cells with special reference to metabolism and experiments on barley roots. *Cold Spring Harbor Symposia* 8: 182-194.
- , AND T. C. BROYER. 1936. General nature of the process of salt accumulation by roots with description of experimental methods. *Plant Physiol.* 11: 471-507.
- , AND ———. 1940. Hydrogen ion effects and the accumulation of salt by barley roots as influenced by metabolism. *Amer. Jour. Bot.* 27: 173-185.
- , AND ———. 1942. Accumulation of salt and permeability in plant cells. *Jour. Gen. Physiol.* 25: 865-880.
- JACKSON, V. G. 1922. Anatomical structure of the roots of barley. *Ann. Bot.* 36: 21-39.
- JENNY, H., R. OVERSTREET, AND A. D. AYERS. 1939. Contact depletion of barley roots as revealed by radioactive indicators. *Soil Sci.* 48: 9-24.
- MACHLIS, LEONARD. 1944. The respiratory gradient in barley roots. *Amer. Jour. Bot.* 31: 281-282.
- OVERSTREET, R., T. C. BROYER, T. L. ISAACS, AND C. C. DELWICHE. 1942. Additional studies regarding the cation absorption mechanism of plants in soil. *Amer. Jour. Bot.* 29: 227-231.
- PREVOT, P., AND F. C. STEWARD. 1936. Salient features of the root system relative to the problem of salt absorption. *Plant Physiol.* 11: 509-534.
- STEWART, F. C., P. PREVOT, AND J. A. HARRISON. 1942. Absorption and accumulation of rubidium bromide by barley roots. Localization in the root of cation accumulation and of transfer to the shoot. *Plant Physiol.* 17: 411-421.

OBSERVATIONS ON CHYTRIDIACEOUS PARASITES OF PHANEROGAMS.

I. PHYSODERMA MENYANTHIS DE BARY¹

F. K. Sparrow

CHYTRIDIACEOUS FUNGI are predominantly aquatic organisms which reach their greatest development in fresh waters of the world. Here, as parasites of algae and microscopic animals and their eggs, and as saprophytes of algae and decaying organic debris, they contribute to the great cycle of disintegration so necessary to a balanced environment.

Certain of these fungi have, somehow, become adapted to existence within the living cell contents of certain terrestrial or semi-terrestrial phanerogams and have seemingly become obligate parasites on these hosts. The pathological effects induced by these chytrids are well known and familiar. Most commonly they manifest themselves in the formation of pronounced, often brightly colored warts, galls, spots, streaks or powdery pustules, composed for the most part of great numbers of the thick-walled, often dark-colored resting spores.

As early as 1833 Wallroth (1833) had described as *Physoderma (Urophlyctis) pulposa*, a chytrid found on members of the Chenopodiaceae. Schröter

(1883) later studied the same species and recorded very briefly an interesting observation, namely, that in addition to the well-known endobiotic resting stage, visible in aggregate as pustules, warts, etc., there was also formed a superficial epibiotic zoosporangial stage, invisible save by microscopic observation. The vegetative system of this newly discovered thin-walled sporangial structure, in marked contrast to the extensive, endobiotic one on which the dark resting spores were formed, was short, bushy and distinctly monophagous. Similar, thin-walled sporangia were subsequently found by Büsgen (1887) in *Physoderma Butomi*, parasitic on *Butomus umbellatus* and in *Physoderma maculare* on *Alisma Plantago-aquatica* by Clinton (1902). Büsgen noted that upon the germination of the thick-walled resting spores zoospores were produced which gave rise, apparently without copulation, either to the strongly polycentric endobiotic phase which bore the resting spores, or to so-called "ephemeral sporangia." These sporangia were essentially like those described earlier by Schröter in *P. pulposa*. They liberated at maturity zoospores which were like those produced by the thick-walled rest-

¹ Received for publication November 3, 1945.

Contribution from the Botany Department, University of Michigan, No. 830.

ing spores save that the oil globule was somewhat smaller. After discharge the sporangia exhibited an unusual capacity, at least among chytrids, for internal proliferation, and a second and sometimes a third sporangium were successively formed. The zoospores, so far as Büsgen could determine, produced only new ephemeral sporangia and he concluded that these thin-walled reproductive structures were simply a means of dispersal of the fungus.

Clinton's subsequent observations on the parasite of *Alisma* confirmed beyond question the regular occurrence in *Physoderma* of these two contrasting epibiotic, monocentric, and endobiotic, polycentric phases. He was inclined to believe that the epibiotic stage was formed primarily on the aquatic, and the endobiotic on the aerial leaves of *Alisma* although, like Büsgen, he found instances in which the two occurred side by side. Clinton suggested that the endobiotic phase may have developed from zoospores which after settling down on the host leaf had become exposed to air, whereas the epibiotic or "ephemeral" sporangia developed from zoospores which had remained submerged at all times during their development. One feature is apparent from Clinton's observations to which no reference is made, namely, that in the sequence of development of the fungus, the epibiotic phase appears to precede the endobiotic.

The object of the present paper is to give an account of a series of observations initiated primarily to determine whether or not this little-known monocentric stage is present in *Physoderma Menyanthis* in which it has not been reported and, if possible, to determine the significance of this stage in the life history.

Through the kindness of Prof. F. T. Brooks of the Botany School, Cambridge University, it was possible to obtain living material of the fungus. The writer wishes to express his thanks to Professor Brooks for this material.

Materials and methods.—The material of *Physoderma Menyanthis* was obtained from a stand of *Menyanthes trifoliata* growing in a damp, boggy swale bordering a prehistoric dike in the vicinity of the River Lark near Mildenhall, Suffolk, England. Abundant infection had been noted at this site during the summer of 1931 by Dr. W. J. Dowson of the Botany School, and a similar infection was found the following year by Professor Brooks and the writer. At the time of collection in October the host plants were already dead and in some cases partly disintegrated. The resting spores of the fungus were unquestionably fully mature. The fungus has subsequently been found in fair abundance in several places in Michigan.

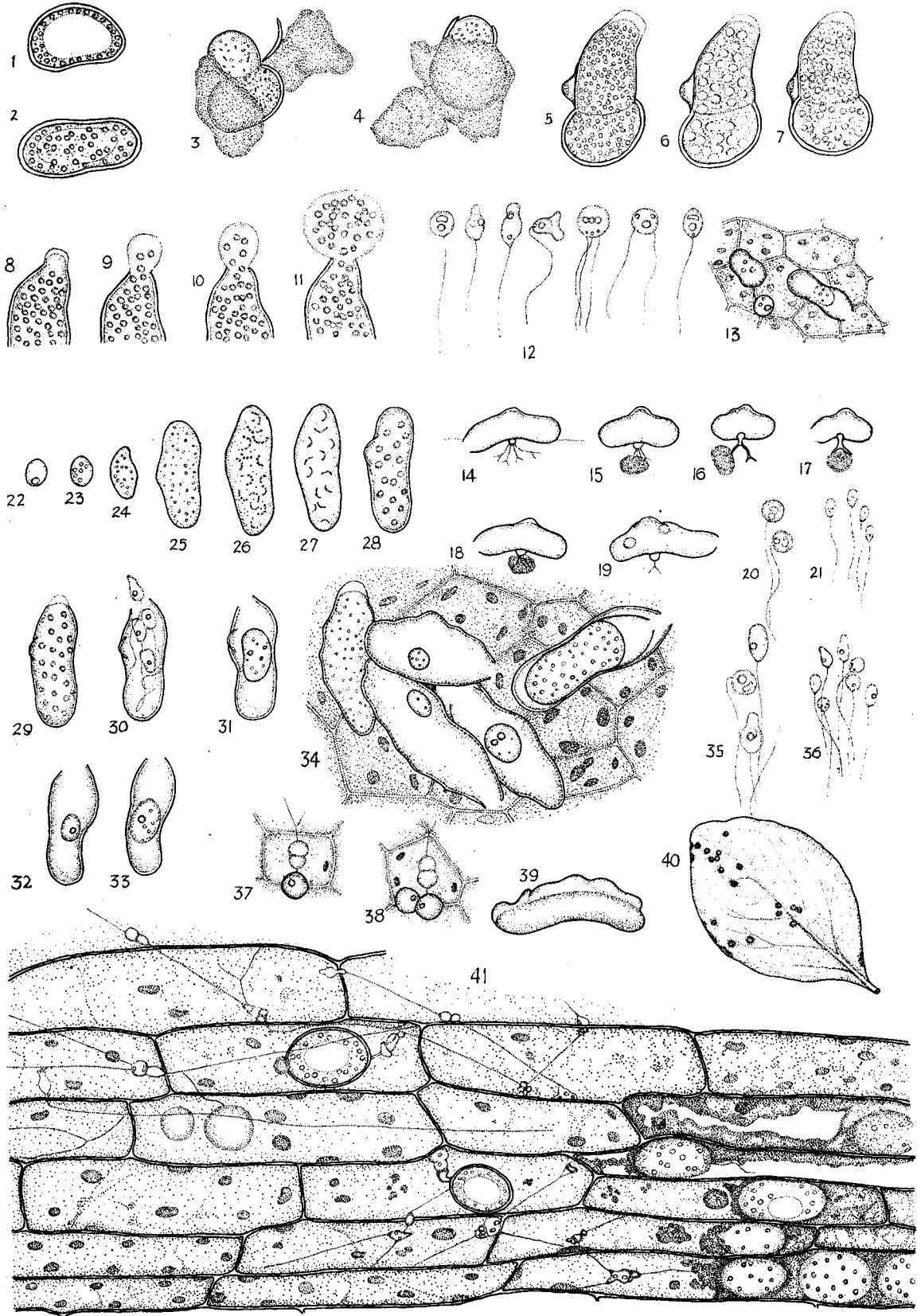
Since the germination of the resting spores was the obvious point at which to initiate the proposed study, and since it was found that they would not germinate immediately after collection, it was necessary to place the spores under conditions that would

favor this process. From their natural habitat it was presumed that if the resting spores were kept moist and subjected to prevailing winter temperature conditions, they should germinate after their natural "after-ripening" period. Accordingly, dead infected leaves were placed in cheese-cloth bags and stored in perforated tin boxes between layers of wet sphagnum and dead *Menyanthes* leaves obtained from the place of collection. These boxes were left outside on the roof of the Botany School throughout the mild English winter, water being added from time to time to keep the contents moist. Periodic germination tests indicated that by mid-December the resting spores were capable of germination and from then on until the supply was exhausted in March, excellent material for germination was available.

In preparing the resting spores for germination the following methods were employed. Small clumps of debris consisting of dead host tissue and resting spores were teased apart in thin films of water on cover glasses. When considerable separation had been achieved and larger bits of host debris removed, the cover glasses were ready for use in hanging drop preparations. Open watch glasses containing dissected clumps of spores in a relatively deep layer of distilled water were also used but gave inferior results.

Resting spores of *Physoderma Menyanthis* were exceedingly slow in germinating when taken directly from the host tissue, placed in hanging drop cultures and left at laboratory temperature (17°C.), approximately seven days being necessary. However, it was found that those stored for two weeks at near freezing temperature in an ice box could then be induced to germinate within two days after removal. Since seeds of *Menyanthes trifoliata* were not available, in order to obtain host material it was necessary to force plants from underground rootstalks.² Several methods were used for inducing infection. In the first, drops of water containing zoospores from germinated resting spores were placed by means of a brush on undisturbed young leaves of the host and the whole plant covered with a large bell jar in order to prevent rapid evaporation. Those portions of the leaf beneath the drop were subsequently removed and examined for sporangia. Parts of undisturbed leaves were also allowed to dip into small dishes of water containing germinating resting spores. Another, and the most useful method for observing the actual infection and development of the ephemeral sporangia was the following. Small chips of living leaf or stem tissue were mounted on a cover glass in a thin film of water containing germinating resting spores, care being taken that the epidermal surface of the host material was next to the surface of the glass. The preparations were then inverted and set up in van Tieghem cells in the usual

² It is a pleasure to acknowledge my indebtedness to Mr. Humphrey Gilbert Carter, Keeper of the University Botanic Garden, Cambridge, for supplying rootstalks of *Menyanthes*.



manner in diffuse daylight at room temperature. Under these conditions, the bulk of the host tissue remained alive for approximately a week, a time sufficient for the observations. Using this set-up, the activities of the zoospores and their subsequent fate could be followed directly and with relative ease.

PHYSODERMA MENYANTHES.—Descriptions of the resting stage of this species and of the lesions caused by it on the host plant have been published by a number of investigators, including deBary (1864), Lüdi (1901), Büsgen (1887), and Clinton (1902).

Infected leaflets of *Menyanthes* bearing the resting spores show, irregularly distributed, slightly raised circular areas which vary from pin point size to 1.5 mm. in diameter (fig. 40). Although distinct and rounded in outline on the lamina of the leaflet, when occurring on the margin and petiole, they may become confluent and somewhat elongate. There is no splitting or shredding of the adjacent host tissue, which in most cases appears to undergo nearly normal growth. When young the spots are somewhat pinkish but at maturity assume a dark chocolate color.

The resting spores are aggregated within the pustulate area where they occur in dense groups, linear series or occasionally separate. Usually they are fused with dark brown, disintegrated host material, so much so that it is difficult to obtain single resting spores. No trace remains of the vegetative system on which they were formed. A striking feature of the spores especially when compared, for example, with those of *P. Zeae-maydis*, was their irregularity in shape. In general they were somewhat elongate ovoid and slightly flattened on one face (fig. 1, 2). Their precise shape was usually obscured by their tendency to form together with host tissue dense aggregations. The wall is pale to chestnut brown, smooth and relatively thin. The contents bear a large central oil globule surrounded by finely granular protoplasm within which are numerous minute hyaline globules. If pits are present at all, as stated by Jones and Drechsler (1920), they must be exceedingly minute

for none was demonstrated after treatment with the usual reagents.

GERMINATION OF THE RESTING SPORE.—The first indication of germination is the beginning of fragmentation of the large oil globule. As this successively separates into smaller and smaller globules, the protoplasm becomes more distinctly granular and homogeneous and bears the minute, refractive, somewhat angular remains of the globule. The wall of the resting spore then splits, probably circumcissally and there is formed a slight hyaline protrusion (fig. 3, 4). Due to the presence of large amounts of residual host material around the resting spore, it is not usually possible to detect with certainty the precise method of dehiscence. In occasional instances it appears that a cap is thrown back, but often no such well-defined structure can be observed, a fact also noted by Clinton (1902) in this species.

During the ten to thirty hours following the opening of the resting spore, there is very slowly formed a stubby, finger-like, often somewhat curved, thin-walled, hyaline sac usually termed a sporangium. As this structure slowly elongates, the more or less evenly distributed refractive granules making up its contents coalesce to form the highly refractive globules of the individual zoospores (fig. 5-7). Meanwhile, at the somewhat blunt apex of the protrusion there appears, almost coincident with the completely homogeneous granular stage of the contents, an arc-like zone of clear protoplasm. This increases in thickness and forms the conspicuous discharge papilla. At maturity this is differentiated into a distal region of very high refractivity from which may occasionally be observed emerging a downwardly-directed central peg, and a less refractive region bearing transient vacuoles which is bounded proximally by the granular cytoplasm. During maturation the walls of the sporangium become sharper in outline, apparently thickening, and the conspicuous globules assume a regular arrangement in the contents. There is not, however, any visible evidence of the cleavage of the cytoplasm into zoospores. At the

Fig 1-41.—*Physoderma Menyanthis*. Fig. 1-21, $\times 800$; fig. 22-39, $\times 1100$; fig. 40, natural size; fig. 41, $\times 800$.—Fig. 1. Resting spore in optical section.—Fig. 2. Another, in surface view.—Fig. 3-4. Early stages in germination of the resting spore.—Fig. 5-7. Stages in the aggregation of the fatty materials to form the globules of the zoospores. An operculum is visible.—Fig. 8-11. Initial stages in the discharge of the zoospores.—Fig. 12. The four to the left, various shapes assumed by the normal zoospores. The next two, abnormal incompletely cleaved zoospores. Right hand one, ellipsoidal normal zoospore showing globule, nuclear cap and basal structure near posterior flagellum.—Fig. 13. Various stages of development of the ephemeral sporangia on the surface of a bit of *Menyanthes* tissue.—Fig. 14-19. Discharged ephemeral sporangia showing various shapes.—Fig. 20. Zoospores from germinated resting spores.—Fig. 21. Zoospores from ephemeral sporangia (same magnification).—Fig. 22-28. Successive stages in the development of an ephemeral sporangium.—Fig. 29. Mature ephemeral sporangium.—Fig. 30. The same discharging its zoospores. The lowermost structure will give rise to a new sporangium and is not a zoospore.—Fig. 31. Same sporangium showing at a later time the developing secondary sporangium.—Fig. 32-33. Similar to the preceding.—Fig. 34. Group of ephemeral sporangia on surface of host tissue. The left one is mature, the three central ones have discharged their first generation of zoospores and the secondary sporangia are developing. The right hand one is producing a tertiary sporangium, two others having previously emptied their contents.—Fig. 35. Zoospores from resting spores.—Fig. 36. Zoospores from ephemeral sporangia for comparison (same magnification).—Fig. 37. Initial stage in formation of the endobiotic, polycentric system; the first turbinate organ has been formed.—Fig. 38. Two encysted zoospores, one of which has formed the first turbinate organ.—Fig. 39. Unusually gibbose ephemeral sporangium.—Fig. 40. Pustules formed by the endobiotic phase on leaflet of *Menyanthes*.—Fig. 41. Portion of the leaf tissue of *Menyanthes* artificially inoculated with the fungus showing the polycentric endobiotic system, and resting spores. The turbinate cells are irregular in shape and so filled with strongly refractive material as to obscure their true shape.

moment of discharge, the double contour of the papilla is lost, the refractive material suddenly expands and the zoospores emerge (fig. 8 to 11). These are swept out passively and rapidly and form a globular motionless mass imbedded in the material of the discharge papilla. When a third of them or, in some instances, nearly all have emerged, the material causing them to aggregate appears to become dissipated in the medium. They then fall apart into a loose group for an instant before darting away and assuming individual motility. Under less favorable environmental circumstances, nearly all of the zoospores escape individually from within the germ sporangium by flagellar action.

The zoospore thus produced is typically chytridiaceous (fig. 12), somewhat ovoid, 8–9 μ long by 5 μ in diameter, with a conspicuous, eccentric, protruding colorless refractive globule and a long posterior flagellum. In both living and stained material the characteristic nuclear cap is unusually conspicuous. Under poor environmental conditions abnormal bi-, tri- and multi-flagellate zoospores are commonly produced (fig. 12). Movement of the normal spores may be characterized as an even swimming interspersed with occasional hops and sudden stops during which amoeboid motion may be assumed.

After a variable period of swarming of not over 24 hours duration, the length doubtless dependent upon environmental conditions prevailing in the medium, the spores come to rest in the water, lose their flagella and encyst. If they are not in contact with living tissue of the host, usually no further development occurs. If, however, they settle down on the outer surface of the epidermis of the host, the formation of the so-called "ephemeral sporangia" ensues.

DEVELOPMENT AND STRUCTURE OF THE EPHEMERAL SPORANGIA.—A series of van Tieghem cells was set up as previously described and maintained at approximately 15.5°C. Bits of the outer tissue of the winter bud of *Menyanthes* were placed in the water film swarming with recently liberated zoospores from germinated resting spores. A control series was also prepared, the bits of host tissue being suspended in sterile water alone.

Typically, after 24 hours the zoospores from germinated resting spores had come to rest (fig. 22). Little change was noticed during the ensuing 24 hours in those resting on the outer host surface. The succeeding 24 hours, however, witnessed the initiation of development of the young monocentric thalli. The first visible change in the contents of the encysted zoospore is the fragmentation of the oil globule (fig. 23). Almost coincidentally, there is initiated a gradual enlargement and elongation—primarily in a plane parallel with host surface—of the spore body on the ventral or "belly" face (fig. 24). A dorsal portion of the spore body, however, apparently remains rigid and persists as a more or less conspicuous hump or papilla (fig. 14 to 19, 30), a type of development recalling that found in sporangia of

Chytridium Schenkii and related species, and noted previously by Büsgen in *P. Butomi*. Owing to the opacity of the host, early stages in the development of the rhizoidal system could not be followed. It is reasonable to suppose, however, that the usual penetration tube is produced which bores through the wall and contacts the living host contents, and from this the small apophysis and short bushy, rhizoidal system, structures easily observed on developing sporangia (fig. 14 to 17), are formed.

The sequence of protoplasmic changes undergone by developing ephemeral sporangia is essentially like that observed in the sporangium formed at the germination of the resting spore, the most noticeable feature being the organization of the globules of the zoospores (fig. 22 to 28).

At maturity the colorless, ephemeral sporangium bears a prominent discharge papilla and in dorsal view is somewhat irregularly slipper-shaped in outline. Laterally, it is pronouncedly gibbose (fig. 14 to 19, 39), the persistent unexpanded portion of the original zoospore case being a conspicuous feature. It measures 24–52 μ in length by 8 μ in greatest width, the majority being 20–40 $\mu \times$ 8–10 μ .

The zoospores, of which there are usually 20 to 30, escape upon the deliquescence of the papilla. As in the discharge of zoospores from germinated resting spores, the first liberated swimmers are imbedded in the hyaline material of the papilla and move passively from the sporangium. Later ones, however, usually escape in amoeboid fashion by their own efforts (fig. 30). They are somewhat ellipsoidal and are provided at the time of emergence with a single posterior flagellum 15–18 μ in length. They differ from the zoospores produced by the germinated resting spores primarily in size. Whereas those formed at the germination of the resting spores are 8–9 μ long by 5 μ in diameter, those produced by the ephemeral sporangia are 5–7 $\mu \times$ 3–5 μ . The flagella are proportionally shorter in the smaller spores. Highly characteristic features of the larger zoospore are the conspicuous, eccentric oil globule and dull gleaming nuclear cap. Zoospores from ephemeral sporangia, however, bear a minute refractive globule, and the nuclear cap although probably present is not evident. In their movement they resemble the larger zoospores, alternately darting evenly and swiftly through the water and suddenly crawling in amoeboid fashion over the substratum.

After discharge there is often observed within the sporangium at the point of its attachment to the rhizoidal system a structure resembling a quiescent zoospore (fig. 13, 32). After approximately 24 hours this body has doubled in size (fig. 31, 33 to 34) and in another 24 hours has become a mature sporangium. Zoospores similar in size and shape are eventually discharged and a third reproductive structure may be formed (fig. 34). Such sporangial proliferation has also been noted by Büsgen (l.c.) in *P. Butomi* and by Clinton (l.c.) in *P. maculare*, where as many as 5 to 6 "nested" sporangia may be formed.

Clinton declared that these secondary sporangia arose by successive swelling of the apex of the septum separating the rhizoidal system from the sporangium. The present observations seem, rather, to point to the necessarily nucleated rudiment being laid down at the time of formation of the zoospores, although further observations will be necessary before this point can be settled with certainty.

FATE OF THE ZOOSPORES FROM EPHEMERAL SPORANGIA.—Both Büsgen and Clinton ascertained that the zoospores formed by ephemeral sporangia produce new sporangia of the same type. To obtain information on this point in *Physoderma Menyanthis*, a series of van Tieghem cells was prepared as before with zoospores from germinated resting spores and bits of living host tissue. Suitable controls were also set up. After three days, all the swarmers had come to rest and ephemeral undischarged sporangia were present in abundance. The bits of host tissue were then removed from the van Tieghem cells and washed in several changes of sterile water to remove any swarmers which might possibly have persisted. They were then placed in new van Tieghem cells in sterile water together with fresh bits of host tissue especially notched for future identification. After three to four days the notched bits of tissue bore abundant ephemeral sporangia which must necessarily have been formed from zoospores discharged from the ephemeral sporangia on the original bits of *Menyanthes* tissue. It can be seen, therefore, that this phase of the fungus can be multiplied not only by the zoospores of the initially formed sporangium but by zoospores from secondarily produced sporangia formed by internal proliferation and by zoospores from new ephemeral sporangia. Furthermore, according to these observations, two types of swarmers from two different structures form similar sporangia, namely, the zoospores from germinated resting spores and those from ephemeral sporangia (fig. 20, 21, 35, 36).

Because of the opaque nature of the host tissue, little could be observed of the effect of the ephemeral thalli on the host cells. Although some disintegration of the chloroplasts was occasionally seen, it was evident that, unlike the endobiotic stage, no hypertrophy, discoloration, etc., was produced. Such effects as were found seemed confined to the particular host cell involved. Under more favorable conditions of observation, such, for example, as those afforded by young seedlings, it is hoped that further, more concise information can be obtained on this point.

One of the primary objectives of this study was to determine whether zoospores from ephemeral sporangia were not, in fact, gametes, which fused to form a zygote which in turn established the strongly polycentric endobiotic system. It seemed more likely that these swarmers, rather than those from the resting spores, would be the ones to function in this manner. Occasional cases of zoospores with two flagella, such as noted by Ojerholm (1934) in *P.*

Zea-maydis, were seen in both types of swarmers studied (fig. 12), but they were never observed in the act of fusing and must be regarded as abnormal, "giant" spores occasionally formed in the sporangia of most aquatic Phycomycetes. If such a fusion does occur—and further investigations may yet substantiate it—unfavorable environmental conditions or possible incompatibility of the swarmers may have nearly or completely prevented it under the conditions of observation.

THE ENDOBIOTIC PHASE.—At approximately the time the first formed ephemeral sporangia had discharged their zoospores and secondary sporangia were being formed, the initial stages in the formation of the endobiotic, polycentric phase were observed. Several instances were noted in which a zoospore cyst, approximately 4–5 μ in diameter, somewhat larger than that formed by the quiescent zoospore from the ephemeral sporangium, was resting on the outer surface of the host. Directly beneath it there was faintly visible a two-celled turbinate organ or "Sammelzellen" connected to it by a delicate rhizoid (fig. 37, 38). Distally, this turbinate structure, so characteristic a feature of the mature polycentric rhizoidal system, gave rise to a branched rhizoid, as yet of limited extent. Since the host tissue rarely remained viable for more than a week, further studies on the extension of this thallus throughout the host cells was impossible. Enough was observed, however, to determine that, in contradistinction to the ephemeral thallus, this one was definitely polycentric (fig. 37, 38).

Since the fate of individual swimming spores, of which there were many in each van Tieghem cell, could hardly be followed, it was impossible to ascertain the origin of the epibiotic cysts from which the polycentric, endobiotic rhizoidal system had been derived. These cysts are definitely larger than those of individual zoospores from ephemeral sporangia (4–5 μ in diameter as compared with 2.5–3 μ) and in this respect are similar to the cysts of zoospores from germinated resting spores (5 μ). However, the large epibiotic cysts were always found four to five days after setting up the van Tieghem cells, at which time the vast majority of those zoospores from germinating resting spores had either disintegrated, or if resting on host tissue, had produced ephemeral sporangia which had already discharged their first "generation" of zoospores. If these cysts were indeed from the zoospores produced at germination of the resting spores, then the rate of development of the endobiotic phase must be exceedingly slow.

Since further stages in the life history of the parasite could not be studied by the van Tieghem cell method, it seemed nonetheless of interest to carry the cycle to completion, even if methods less susceptible to direct observation would have to be employed.

Menyanthes plants were forced in the greenhouse, the pots standing in water under large bell jars to insure a high humidity. Since zoospores from eph-

meral sporangia could not be obtained in sufficient quantity to be utilized as an inoculum, resting spores were germinated and the active zoospores painted on parts of the leaves and stems with a camel's hair brush. Other methods of application were tried but this one seemed the most successful. Both upper and lower surfaces were treated and the areas where the inoculum was applied were circumscribed with a line in India ink. It was found very difficult to keep the liquid bearing the zoospores in place because of the strongly cutinized nature of the surface. In four of twelve areas thus treated, there appeared after four weeks, pale, pink, then reddish-brown pustules primarily on the upper surface of the host leaf. Young areas when removed and teased apart showed the typical polycentric rhizoidal system bearing numerous highly refractive turbinate cells and the typical resting spores in various stages of maturity (fig. 41).

Further work on this stage of the fungus was not feasible at the time and is reserved for a future study.

SUMMARY

The present paper describes certain phases in the life history of a chytrid parasite of a bog-inhabiting phanerogam, *Menyanthes trifoliata*. The fungus,

Physoderma Menyanthis, winters over in the resting spore stage. At germination of the resting spore, it cracks open and a thin-walled, finger-like structure is produced within which are formed zoospores. These swimmers may eventually settle down on the surface of living host tissue, penetrate it, and form a monophagous, bushy rhizoidal system within. The epibiotic body of the spore steadily enlarges and forms at maturity a thin-walled, so-called "ephemeral" sporangium. This forms zoospores which are somewhat smaller than those formed at the germination of the resting spores. These zoospores apparently form only new ephemeral sporangia. New ephemeral sporangia may also be formed by internal proliferation of discharged ones.

About five days after the swarming of the zoospores from germinated resting spores, the first evidences of the thallus on which will be borne the dark resting spores is observed. Contrasted with the epibiotic thallus this one is polycentric, bearing numerous turbinate organs and produces visible hypertrophy of the host.

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LITERATURE CITED

- BARY, A. DE. 1864. Beiträge zur Morphologie und Physiologie der Pilze. Erste Reihe. *Protomyces* und *Physoderma*. Abhandl. Senckenbergisch Naturf. Ges. 5: 137-232, pls. 26-31.
- BÜSGEN, M. 1887. Beitrag zur Kenntniss der Cladochytrien. In Cohn, Beitr. Biol. Pflanzen, 4: 269-283, pl. 15.
- CLINTON, G. P. 1902. *Cladochytrium Alismatis*. Bot. Gaz. 33: 49-61, pls. 2-4.
- JONES, F. R., AND CHARLES DRECHSLER. 1920. Crownwart of alfalfa caused by *Urophlyctis Alfalfae*. Jour. Agric. Res. 20: 295-324, pls. 47-56.
- LÜDI, R. 1901. Beiträge zur Kenntniss der Chytridiaceen. Hedwigia 40: 1-44, pls. 1-2.
- OJERHOLM, ELIZABETH. 1934. Multiciliate zoospores in *Physoderma Zeae-maydis*. Bull. Torrey Bot. Club 61: 13-18.
- SCHRÖTER, J. 1883. Untersuchungen der Pilzgattung *Physoderma*. Jahres-bericht Schles. Gesell. Vaterland. Cultur. 60: 198-200.
- WALLROTH, F. G. 1833. Flora Cryptogamica Germaniae 2: 1-923. Nürnberg.

ISOLATION OF 3-INDOLEACETIC ACID FROM IMMATURE CORN KERNELS¹

A. J. Haagen-Smit, W. B. Dandliker, S. H. Wittwer, and A. E. Murneek

THE PERIODIC presence of natural auxin-like substances within the reproductive parts of many higher plants might conceivably provide a key to the experimental elucidation of problems concerned with seed and fruit formation and development, the physiology of which is little understood (Murneek, 1939). A powerful stimulus to plant growth following pollination and subsequent fertilization in the tomato has been demonstrated by Murneek (1926). That the occurrence of hormones in reproductive tissues of corn is associated with the critical developmental processes of gametophyte and seed formation has been suggested by Wittwer (1943). These periods of hormone production, which seem to follow the union of chromosomes in the synaptic reaction and of the fusion of nuclei in the syngamic process, are accompanied by parallel or simultane-

ous growth phenomena in the corn plant which give rise to two maxima in the growth curve as outlined by Briggs, Kidd and West (1920). Consequently, our interest has been focused on the nature of active hormone substances so abundantly present in immature seeds, perhaps causal for the overall acceleration in plant growth.

3-indoleacetic acid has been isolated from the auxin liberated by the alkaline hydrolysis of corn meal and dormant corn kernels by Haagen-Smit *et al.* (1941) and Berger *et al.* (1944), respectively. The auxin liberated in this way constitutes the so-called "bound" or "precursor" auxin. The fraction which occurs in a free and biologically active form after mere extraction with water or organic solvents has not been previously isolated from either dormant or immature corn.

¹ Received for publication November 9, 1945.