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Observations on Chytridiaceous Parasites of Phanerogams

XVII. Notes on a *Physoderma* Parasitic on *Asclepias incarnata* *

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Summary. A. *Physoderma* found parasitic on *Asclepias incarnata* L. is noteworthy for the behavior of its zoospores from epibiotic sporangia. These, which bore either a colorless or ochraceous salmon colored globule, apparently lacked the capacity to produce new epibiotic sporangia and exhibited a marked tendency to fuse in pairs. The fate of the fused product could not be followed directly. Seedlings exposed to these, however, eventually had formed within them the polycentric endobiotic system bearing resting spores. The latter germinated after dehiscence of a lid. There is some evidence for believing the fungus is confined to *Asclepias*.

In a previous publication (Sparrow, 1961) the principal features of a *Physoderma* parasitic on *Asclepias incarnata* L. in the vicinity of the University of Michigan Biological Station at Douglas Lake were outlined, without illustrations. The present paper adds not only figures of the morphological features of both its endobiotic and epibiotic systems but notes on certain of its biological features as well.

Our material has come from a single shallow-water site and although we have searched assiduously for it elsewhere over the years in Cheboygan and Emmet counties we have never encountered it.

Infected plants are uniformly those which have their bases resting in water. During years of relatively dry springs no infection is found. There are two very distinct manifestations of infection. On the lower stem (the upper, above-water parts were never attacked) infected areas appeared as dark red streaks or dark brownish-black elongate, separate or confluent depressions or "dimples" up to 1 cm long by 0.5 cm wide. These areas were always covered by host epidermis and never open and powdery. On the lowermost part of the stem at the crown, a second type of host reaction is found. There are most often groups of relatively large somewhat transversely ovate galls up to 2 cm broad by 0.5 cm high which at maturity of the fungus split open and expose dark-

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brown powdery masses of resting spores (Fig. 1). Such structures are undoubtedly heavily infected adventitious buds from which normally new growth would occur. Some information on these basal galls was obtained from infected seedlings carried for some months in the greenhouse. Seedlings (24) exposed to epibiotic swarmers were planted in pots and grown under a 16-hour light regime. Fourteen of these survived and within 10 days infection was evident. During this period the first two internodes above the cotyledons elongated and the cotyledons themselves shriveled and dropped off. Infection was limited to the internode below and the one above the cotyledons and most markedly at the cotyledon node itself. Symptoms included marked reddish discoloration, hypertrophy and obvious asymmetry of the stem.

Fig. 1. Lower nodes of stem of *Asclepias incarnata* showing galls caused by infection of buds by *Physoderma* sp.

The basal nodes and especially the cotyledonary node of *Asclepias incarnata* are unusually active bud formers. In fact, buds form on buds. Observations on healthy plants indicate that development of these buds to form tillers is the means by which the plant produces its characteristic clumps. Infection of this area by the *Physoderma* results in slow hypertrophic development of the nodal tissue to form a large irregular lobate mass with buds scattered over its surface. Inside the tissues the endobiotic thallus continues development, its growth keeping pace with the hyperplastic growth that it stimulates. Gall development was followed for 5 months after which, when they were about 2 cm broad, they were sacrificed in a futile attempt to get them into tissue culture. Where the basal bud is not attacked, renewal of growth occurs, and clusters of plants are found sometimes exhibiting two seasons of infected stems. In pustules on dead culms of the previous year's growth, better than 90% of the *Physoderma* resting spores sometimes had germinated. In others (possibly not inundated) up to 50% were ungerminated but viable, for most of these germinated when placed in water. If all adventitious buds were attacked growth is completely suppressed. Since there

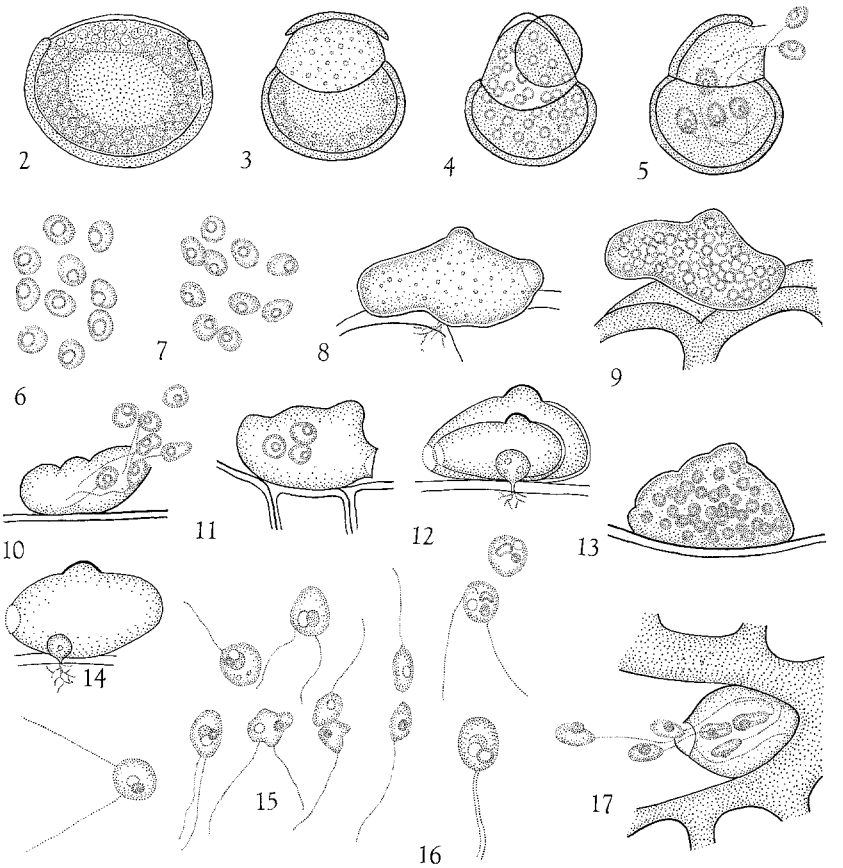
is great variation in degree of host plant development even in uninfected plants, no generalizations can be made of the effect of the parasite when only the stem is invaded. Our impression is that it is relatively slight.

A brief study of prepared slides of infected areas of the stem indicates that the cortex, phloem, cambium and undifferentiated xylem elements are invaded. Cork cambial activity is stimulated in the immediate area of infection. When the fungus extends beyond the somewhat elongated cortical cells and reaches the cambium, the latter is disrupted in its activity, and its elements increase in size and come to resemble cortical parenchyma. Subsequently, undifferentiated (but not mature) secondary xylem cells are invaded, as is the wood ray parenchyma for a limited distance. Meanwhile, the vascular cambium beyond the periphery of the infection has continued to function providing more susceptible cells than were present at the time and point of the original infection. For the most part, however, increase in size rather than numbers of cells is responsible for the hypertrophy.

Endobiotic System

This consists of a series of rhizoids which ramifies from cell to cell in the host tissue and bears on it turbinate cells and, eventually, dark, thick-walled, relatively large resting spores (Fig. 18, 19). No stages in establishment of the infection were observed and hence the nature of the primary turbinate cell and how it gives rise to the first elements of the vegetative system are not known. Secondary turbinate cells were broadly ovate, $9.4-12.6 \times 6.3\,\mu$ and one-celled, or divided by delicate (usually transverse) septa into 2—3 cells (Fig. 18). A few bore longitudinal as well as transverse septa (Fig. 19). The rhizoidal system was little if at all branched and emerged from 1—4 places on the turbinate cell, primarily on the distal surface. Peelings of streaked or dimpled infected areas of the host stem showed no noticeable distortion of the host cells and no lysis of tissue.

The rudiments of the most conspicuous element of the mature endobiotic system, the resting spores, originate singly as spheres terminating short tubular, usually lateral, rarely apparently terminal, outgrowths from turbinate cells (Fig. 20 a and below). When they are relatively small there may be observed on some, but not all, 1—3 stubby, dichotomously branched haustorial outgrowths or "antler-like" processes (Fig. 18). As the spore rudiments increase in size these outgrowths tend to disappear. Coincident with increase in size, the contents of the resting spore rudiments undergo a sequence of changes. This involves a gradual increase in the size and number of refractive bodies (Fig. 18). Before reaching approximately mature size, the rudiment, apparently by centripetal thickening of its limiting membrane, forms a rigid, pale



Figs. 2—17. *Physoderma* sp. on *Asclepias incarnata*. Figs. 2—5. Stages in germination of resting spore. Fig. 6. Group of quiescent resting spore zoospores. Fig. 7. Same, of zoospores from epibiotic sporangia. Note smaller size of globules when compared with those of Fig. 6. Fig. 8. Immature epibiotic sporangium with colorless globules. Fig. 9. Mature one. Fig. 10. Discharge of epibiotic sporangium. Fig. 11. Empty one with several unescaped zoospores. Fig. 12. Three times proliferous sporangium showing rhizoidal system. Fig. 13. Mature sporangium with ochraceous salmon colored globules. Fig. 14. Once proliferous sporangium with rhizoidal system. Fig. 15. Epibiotic sporangial zoospores showing from right to left stages in quick fusion of zoospores of different globule color. Fig. 16. Product of fusion about to swim off; the parallel flagella will quickly appear as one structure. Fig. 17. Discharge of zoospores with colored globules. All figures $\times 825$

brown wall around itself. This becomes 1.5—2.0 μ thick and is pale amber-colored at full maturity (Fig. 19). One to several supraequatorial pits can be seen in some but not all cleared spores. At maturity the

resting spore content bears a large central vacuole which is surrounded by numerous nearly likesized refractive globules (Fig. 19 above). One, less often, several resting spores are formed in large host cells, usually those of the cortex. Rarely, they are formed in the epidermis. They are circular in outline in face view, and in edge view ellipsoidal, with one face somewhat flattened, and are 15.6—25.0 × 21.8—28.0 μ (av. 21.7 × 25.5 μ).

Germination of the Resting Spores

Inasmuch as details of the germination process have been published by Sparrow, Griffin and Johns (1961) in a *Physoderma* on *Agropyron repens* and since those of the *Asclepias* fungus did not differ significantly they will not be repeated here. The sequence of events culminating in zoospore discharge is shown in Figs. 2—5. It will be noted that as in all true species of the genus observed by us, save apparently *Physoderma menyanthis*, a broad operculum is dehisced as the endosporangium protrudes from the body of the spore.

The resting spore zoospores are posteriorly uniflagellate, approximately 5.0 × 3.0 μ and slightly elongate when in motion and bear a conspicuous, colorless globule. After several hours of motility they come to rest on the surface of the host plant and develop in a manner shortly to be described.

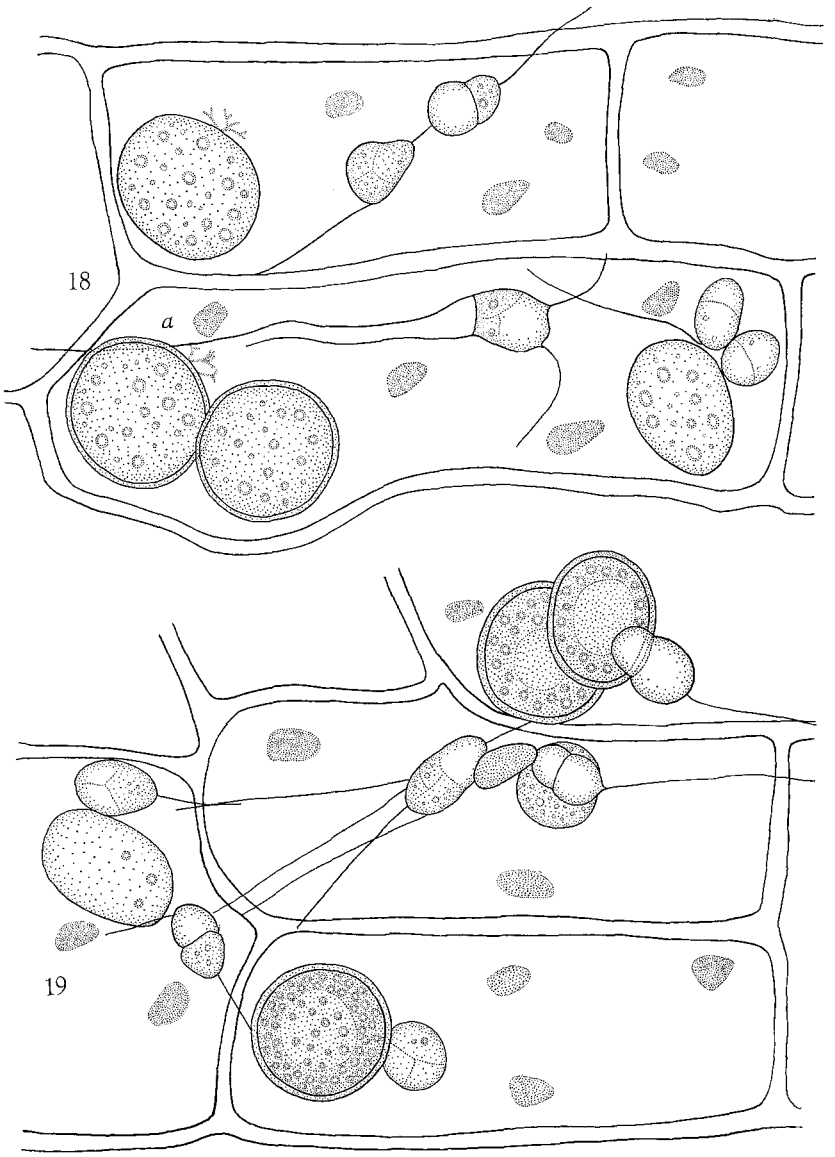
One feature of interest was noted with respect to the resting spores, namely, that they often failed to germinate in water without the presence of the host plant.

Epibiotic Stage

If seedlings or bits of host tissue are introduced into water in which resting spore zoospores are swimming (our material was in diffuse daylight at 20° C) the *epibiotic*, monocentric "ephemeral" sporangial stage is induced. No precise developmental time schedule was followed but it was found that 80 hours after sowing resting spores in water, they had germinated and great numbers of immature epibiotic sporangia had already formed on the host tissue. The latter structures bore either colorless or ochraceous salmon (Ridgeway) contents, the two types occurring in approximately equal numbers[1].

Epibiotic sporangia (Fig. 8—14,17) 27.0—29.0 μ long by 9.0—14.0 μ high by 14.0 μ at greatest width were sessile, stout, elongate, and gibbose by reason of the persistence of an unexpanded apical portion of the cyst of the resting spore zoospore, and were transversely placed on the outer surface of the host. Within the latter and at the tip of a narrow penetra-

1 Of 210 mature or nearly mature epibiotic sporangia observed on 6 seedlings of *A. incarnata* from 2 dishes, 112 (53%) were colorless and 98 (47%) were colored.



Figs. 18—19. *Physoderma* sp. on *Asclepias incarnata*. Endobiotic system within host tissue. Fig. 18. Resting spore at *a* and immature one above it bear haustorial processes. Fig. 19. Several-celled turbinate cells and resting spores in various stages of development. All figures $\times 825$

tion tube there was a very delicate, bushy complex of relatively short, branched rhizoids (Fig. 12, 14). After a series of protoplasmic changes, essentially like that described by Sparrow, Griffin and Johns (loc. cit.), somewhat fusiform swarmers $5.0-7.0 \times 3.0\ \mu$ were discharged from a single pore $7.0\ \mu$ in diameter formed at the somewhat narrower apex of the sporangium (Figs. 10, 17).

The zoospores were distinctly fusiform when in motion and possessed a single, ochraceous salmon or colorless eccentric refractive globule and posterior flagellum. All zoospores from a single sporangium bore the same color globule. Pigmentation of the colored globule soon faded during the motile period and the distinction between the two types was almost lost. Furthermore, they were scarcely if all at distinguishable in size from resting spore zoospores (Fig. 6, cf. 7). A distinct difference in the size of the globule of the two zoospores was, however, noted, those from epibiotic sporangia being smaller (Fig. 7). Many instances of internal proliferation of epibiotic sporangia were observed (Fig. 12, 14).

Behavior of Epibiotic Zoospores

Unlike zoospores of epibiotic stages of all other members of *Physoderma* studied by us, those formed by the *Asclepias* parasite consistently failed to form new epibiotic structures. That is, unlike epibiotic zoospores of *Physoderma menyanthis*, *P. maydis*, the *Agropyron* parasite, etc., so far as we can determine they do not serve to multiply the epibiotic stage of the fungus.

Many epibiotic zoospores did, however, fuse in pairs. This occurred with great rapidity between free-swimming (usually not amoeboid) swarmers. They met head-on, and by a few quick twitches reoriented their opposed flagella so that they lay parallel and so close together as almost to appear as one. They then merged their bodies, and resumed motility (Fig. 15). It was sometimes possible to determine that the globule of at least one of the two fusing elements was colored (Fig. 15 left) but as earlier indicated, the intensity of pigmentation in motile spores was frequently so slight as to be undetectable. Biflagellate structures with blunt apex (Fig. 16) were very numerous within a half hour after sporangial discharge was initiated. If further studies confirm both the aforementioned inability of epibiotic swarmers to form new epibiotic sporangia, and the fusions, it probably indicates we are dealing with a species in which gametes cannot function facultatively as zoospores. The fate of the biflagellate swarmers could not be followed and hence, it cannot be said with absolute certainty, whether the endobiotic phase arises from fused or unfused epibiotic swarmers. It should be noted again, however, that the aforementioned repeated introduction of

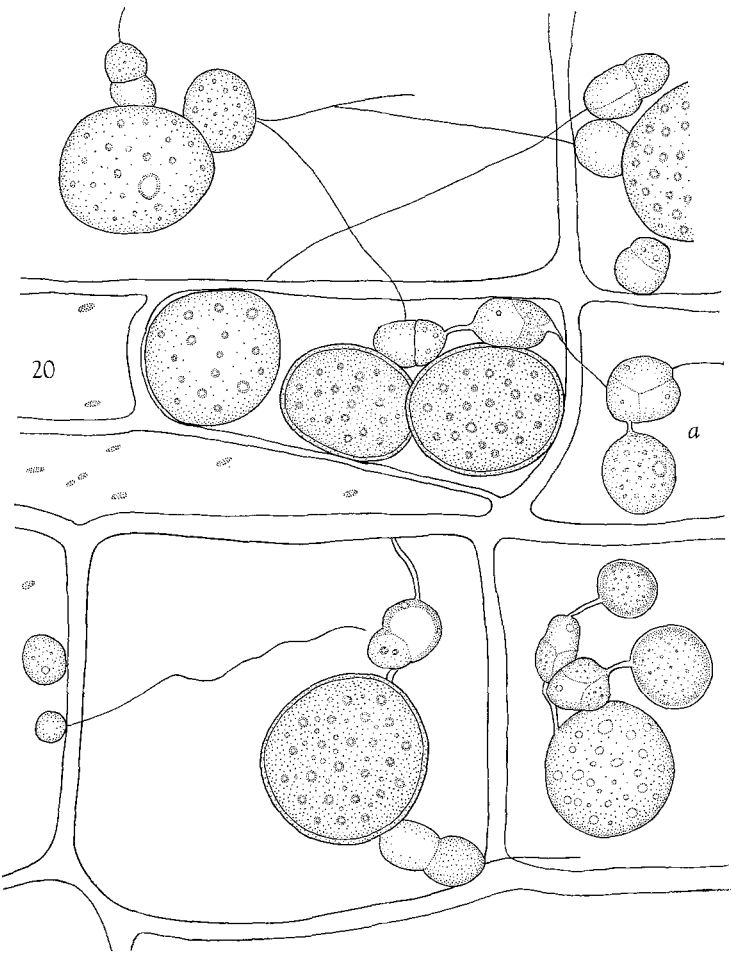


Fig. 20. *Physoderma* sp. on *Asclepias incarnata*. Portion of infected host tissue with turbinate cells and resting spores in varying stages of development. At *a* and below it, young resting spores are forming at tip of short tubes from turbinate cells. In cell to left of *a* a turbinate cell outgrowth has produced another turbinate cell. ×825

uninfected *Asclepias incarnata* seedlings (24) into dishes in which are seedlings bearing epibiotic sporangia and their swarmers does not result in the development of new epibiotic sporangia in the uninfected material. These seedlings (14 survived) did, however, when potted, develop in the greenhouses the endobiotic phase and bore mature resting spores.

Host Range

At the moment we have little data on the host range of the *Asclepias* parasite. At the site of collection Physodermas have been found on *Eleocharis compressa, Lycopus americanus, Mentha arvensis, Thalictrum dasycarpum* and *Ranunculus septentrionalis,* and intensive cross-inoculation studies using these hosts and certain others would be of value in determining the taxonomic disposition of our fungus. A few preliminary tests were attempted. One, using seedlings of a strictly terrestrial species, *Asclepias tuberosa,* immersed in water with resting spore zoospores, indicated quite definitely that the epibiotic stage formed in a normal manner on this host. These seedlings were potted and grown for several months in the greenhouse. Scattered minute, reddish-brown flecks produced by the endobiotic stage were found on them at the end of that period. An examination of the resting spores produced in such infected areas showed that they were nearly all limited to the epidermis, were abnormal morphologically, and probably not viable. This stage of the parasite did produce disintegration of infected host cells. Seedlings of *Apocynum cannabinum* L. likewise exposed to resting spore zoospores were not attacked[2]. Another, less precise series involved placing uninfected *Lobelia cardinalis, Lycopus americanus* and *Mimulus ringens* (three common associates of *Asclepias incarnata* in swamps) in tubs of water along with infected *Asclepias* plants. No infection resulted in these hosts although in one tub four of seven uninfected plants of *A. incarnata* placed in company with an infected one became parasitized. In the latter trial seventeen uninfected control plants placed under similar condition in water from the same source remained uninfected.

Discussion

It is evident that the most interesting feature of this fungus is the behavior of its epibiotic zoospores. Both the fact that such zoospores have been seen to fuse in appreciable numbers and that they cannot apparently function to multiply the epibiotic stage is unlike other species of *Physoderma* studied by us, save, perhaps, *P. lycopi* (Sparrow, 1957). Although the fate of the biflagellate zygotes (?) could not be followed directly, it does seem of significance that the seedlings introduced into the water containing epibiotic zoospores and biflagellate swarmers, which survived (14 of 24) all developed the endobiotic thallus and resting spores. The inference is that these thalli developed from the products of fused swarmers, but it is only an inference. Before we are ready to say unequivocally that in the *Physoderma* on *Asclepias*

2 This is of particular interest since we have found a *Physoderma* on *Apocynum cannabinum* and germinated its resting spores.

we have an organism that fulfills the senior author's contention of many years standing (Sparrow, 1940) that alternation of heteromorphic generations occurs in *Physoderma*, we want considerable confirmation of our present observations.

Similarly, our limited cross-inoculation studies need repetition and amplification. We suspect, however, that our fungus is confined to *Asclepias*, but until this is unquestionably confirmed we will not describe it as a new taxonomic entity.

Finally, we can confirm in the case of the *Asclepias* parasite what we have repeatedly observed in other species of *Physoderma*, namely, its dependence upon the presence of standing water at the site. In the decade during which we have followed the occurrence of this fungus, winters of light snow followed by dry springs and summers, with no standing water present at the site, have resulted in no infected plants, whereas years of heavy snow and wet early growing seasons have always produced them.

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