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Observations on Chytridiaceous Parasites of Phanerogams

XII. Further Studies of Physoderma Claytonianum var. Sparrowii*

By

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With 18 Figures in the Text

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Claytonia virginica L., or "Spring Beauty" is one of the most striking elements of the spring flora of Eastern North America. This colorful member of the *Portulacaceae* makes a spectacular display in wide areas of our woodlots by its gregarious habit of growth. Fortunately, its recorded fungus enemies are few, SEYMOUR's host index (1929) for example, listing only two, *Puccinia mariae-wilsoni* G.M. Clinton, and *Peronospora claytoniae* Farlow. In recent years, however, at least two other parasites have been found on it, namely, *Physoderma claytonianum*[1] H. C. Greene (1944), and *Polymyxa graminis* Ledingham (SPARROW 1947). The former organism is one of a number of phycomycetous fungi which belongs to a genus of the *Chytridiales* which are all obligate parasites of vascular plants.

It has been known for nearly three-quarters of a century that some members of *Physoderma* produce two radically different types of thalli in the course of their development. One, which manifests itself macroscopically at maturity by causing streaks, pustules, etc., on the affected host part, is endobiotic, extensive, polycentric and bears the dark-colored resting spores by which the fungus overwinters. The other, entirely independent thallus, is not visible macroscopically, is epibiotic and is composed of a sessile sporangium and a very limited, bushy, rhizoidal system which is confined to a single host cell. It has been suggested (SPARROW 1940) that this phase is gametangial in nature and is an intregal part of a heteromorphic life cycle. In two instances fusion of its swarmers has been seen (SPARROW 1957; LINGAPPA 1959).

In an earlier paper the senior author (1947) published an account of the external manifestations of *Physoderma claytonianum* on its host, as well as some morphological data on its endobiotic system and statistics on its resting spore size. These data were based upon material collected from

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[1] According to the late Prof. H. H. BARTLETT this name should be *claytonianum*, not *claytoniana* as given by GREENE.

the vicinity of Ann Arbor, Mich., and Amherstburg, Ontario. Since then, SAVILE and PARMELEE (1956) have reported *P. claytonianum* from several sites in Quebec and Ontario and have pointed out that the Michigan and Ontario fungi bear consistently larger resting spores than the type material from Wisconsin and their own from Quebec. Hence, they have segregated our fungus as *P. claytonianum* var. *sparrowii* Savile and Parmelee. It has been suggested by KARLING (1956) that the *Physoderma* collected by K. H. MCKNIGHT on *Oreobroma pygmaea* (A. Gray) Howell (not *Claytonia megarrhiza* Parry as stated in KARLING, *l.c.*) is allied by its spore size to *P. claytonianum*. Subsequently, MCKNIGHT and MUMFORD (1958) have declared that this same fungus most closely approximates var. *sparrowii*. They further report *P. claytonianum* var. *claytonianum* on *C. lanceolatum* and also refer to this variety a *Physoderma* found on *Erocallis triphylla* (S. Wats.) Rydb. These authors give careful descriptions of the symptoms caused by their fungi on the three portulacaceous hosts and the ranges of spore sizes. No resting spores were germinated although MCKNIGHT and MUMFORD were fairly certain from the appearance of these structures on *C. lanceolatum* that in this process a lid would be dehisced.

Efforts had been made since the finding of the *Physoderma* by the senior author in 1946 on *Claytonia virginica* in the vicinity of Ann Arbor to follow completely germination of its resting spores. This process as well as the fate of the resultant swarmers had not been seen by any of the aforementioned investigators. It was also hoped that something could be learned of the nature of the epibiotic stage, which we had every expectation would be formed. Successful germination of the resting spores was for one reason or another thwarted until the spring of 1961.

The previous year we had collected (May 25, June 1) a fair amount of yellowed, infected host plants and had buried them in perforated plastic dishes at the site. In October, 1960, some were brought into laboratory and tested for germination on the chance that autumnal infection of the hosts (already sprouted from their corms) occured. The spores appeared mature and in good condition but could not be induced to germinate. In February, 1961, others were similarly tested and at this time a very few germinated in charcoal water. Because of unseasonably warm weather (March 3, 4—14.5° C; March 4, 1.5—12° C) and prolonged rains, the remainder was dug up on March 4 and brought into the laboratory. Here a portion was immediately frozen and the residue placed in a cold room at 5° C. An examination of both lots 2 days later indicated spore germination had taken place and it seemed safe to assume, at least with the frozen ones that this process had started out in the field. The frozen spores were maintained in that condition whereas those originally stored at 5° C were used in part at once for germination studies and the remainder stored at 2—3° C, which inhibited this process.

In resting spore germination the sequence of changes was essentially like that observed in other species, particularly in the *Physoderma* on *Agropyron repens* recently reported by us (1961). It will for this reason

only briefly be reviewed here. The fully mature spore is very broadly ellipsoidal (Fig. 1) and in some views is seen to be flattened on one face. The outer wall (epispore) is $2^1/_2$—3 μ thick and a clear brown. Within the pigmented epispore there is a discrete, thin, colorless endospore. The contents are disposed in the same manner as in all other species of the genus seen by us, namely, there is a large central vacuole surrounded by a varying number of rows of refringent globules (Fig. 1). The onset of germination is usually signalled by the circumscissle dehiscence of a broad cap of epispore from the less convex surface. We did observe instances, however, in which dehiscence was delayed. Coincident with cap formation the central vacuole becomes more uneven in its contour and smaller (Fig. 2). As germination proceeds the refractive material of the contents becomes aggregated into large and small, irregularly-shaped, clod-like bodies. At this time, too, the endosporangium whose wall is continuous with that of the endospore begins to protrude from the resting spore body (Fig. 3). This stage is superceded by one in which the contents steadily become more homogeneous and a broad papilla forms at the apex or side of the endosporangium (Fig. 4). Somewhat later there begins successively to appear in the contents spherical shells of small, highly refractive globules (Fig. 5). This marks the appearance of the characteristic "ring stage" which we have found to be present in the development of all swarmers in this genus. These shells of refractive bodies are equidistant in the contents and are slightly larger than the globules of the maturing zoospores (Fig. 6). The last-named globules are evidently formed by a fusion of the material of the shells and that of the protoplasmic granules, for during zoospore maturation the contents of the endosporangium become clear (Fig. 7-9). Cleavage of the protoplasm into zoospores cannot be detected in the living endosporangium. At full maturity they are liberated upon the swelling and deliquescence of the broad discharge papilla. The first-emerged zoospores are carried out *en masse* and then assume individual motility. The remainder emerge by their own flagellar activity (Fig. 10 and 11). They are at first spherical, and are 5 μ in diameter, with an eccentric colorless globule and posterior flagellum. Upon assuming motility they become more ovoid.

As indicated earlier, it was presumed that, like other species of *Physoderma*, studied by us, the resting spore zoospores after a period of motility would settle down on the surface of the host, penetrate it, and the epibiotic portion develop into an "ephemeral" or "epibiotic" sporangium. Accordingly, to observe these events shallow watch glasses containing charcoal water and resting spore zoospores were set up. Into these were put young plants of *Claytonia*, for the most part with their corms attached, and the whole placed within a damp chamber. Forty-eight hours after exposure to these conditions, a time sufficient for

ephemeral sporangial development in other species, the host plants were found free of such structures. Within another 24 hours primary and even secondary turbinate organs belonging to the endobiotic stage were observed (Fig. 13 and 14). Four days after original exposure of the host to

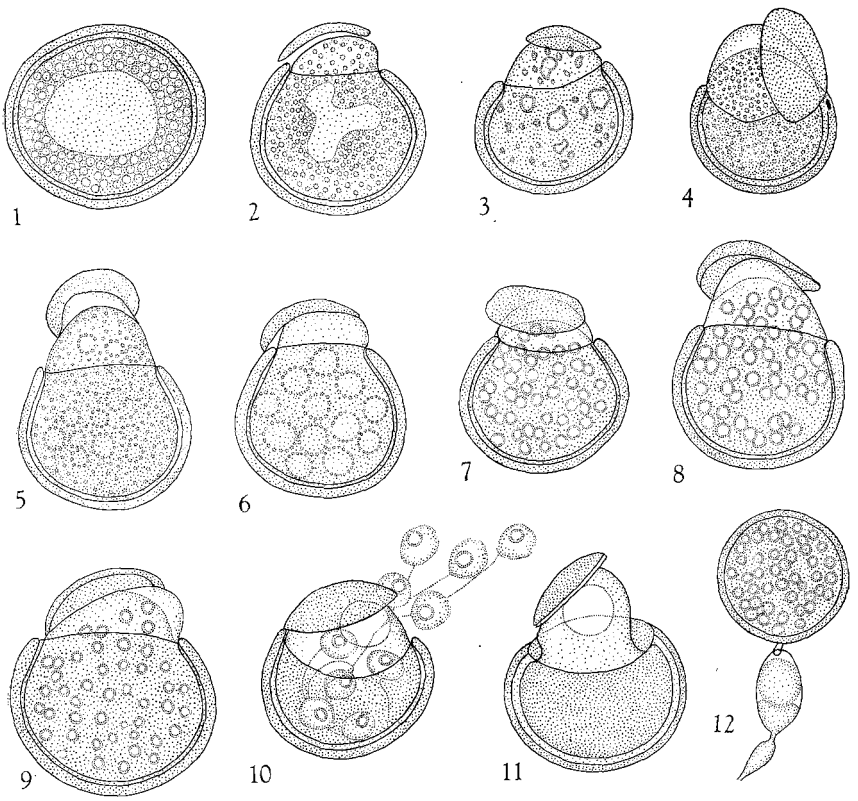

Fig. 1—12

Fig. 1—11. Stages in the germination of the resting spores of *Physoderma claytonianum* var. *sparrowii*. Fig. 1 is the spore prior to germination showing typical disposition of contents. In Fig. 2 the vacuole is disappearing and a lid is dehisced. Fig. 3 shows the large and small granular stage which is followed by a phase of more homogeneous contents, as in Fig. 4. The beginning of the "ring stage" is seen in Fig. 5 as is also the broad discharge papilla on the endosporangium. Fig. 6 shows a late ring stage by which time all the hollow spheres of globules have formed and the protoplasm is clearing. In Fig. 7—9 the globules of the zoospores have been formed and the endosporangium is about to discharge. Fig. 10 shows a final stage in the discharge of the zoospores through a broad pore formed upon the deliquescence of the papilla. In Fig. 11 this pore is well seen in an empty sporangium. Fig. 12 is a nearly mature resting spore found on 9 day old endobiotic thalli. × 825

Fig. 13—18. Various stages in development of endobiotic system in young plants of *Claytonia* after exposure to resting spore zoospores. Fig. 13 indicates primary turbinate organs and Fig. 14 secondary turbinate organs found in plants 3 days after exposure. Fig. 15 shows thalli 4 days, and Fig. 16, five days after exposure. Spherical, young resting spores are seen in Fig. 17, 18, eight days after exposure to resting spore zoospores. × 825

swimming resting spore zoospores numerous, well-developed, polycentric endobiotic systems of the parasite were detected within the young

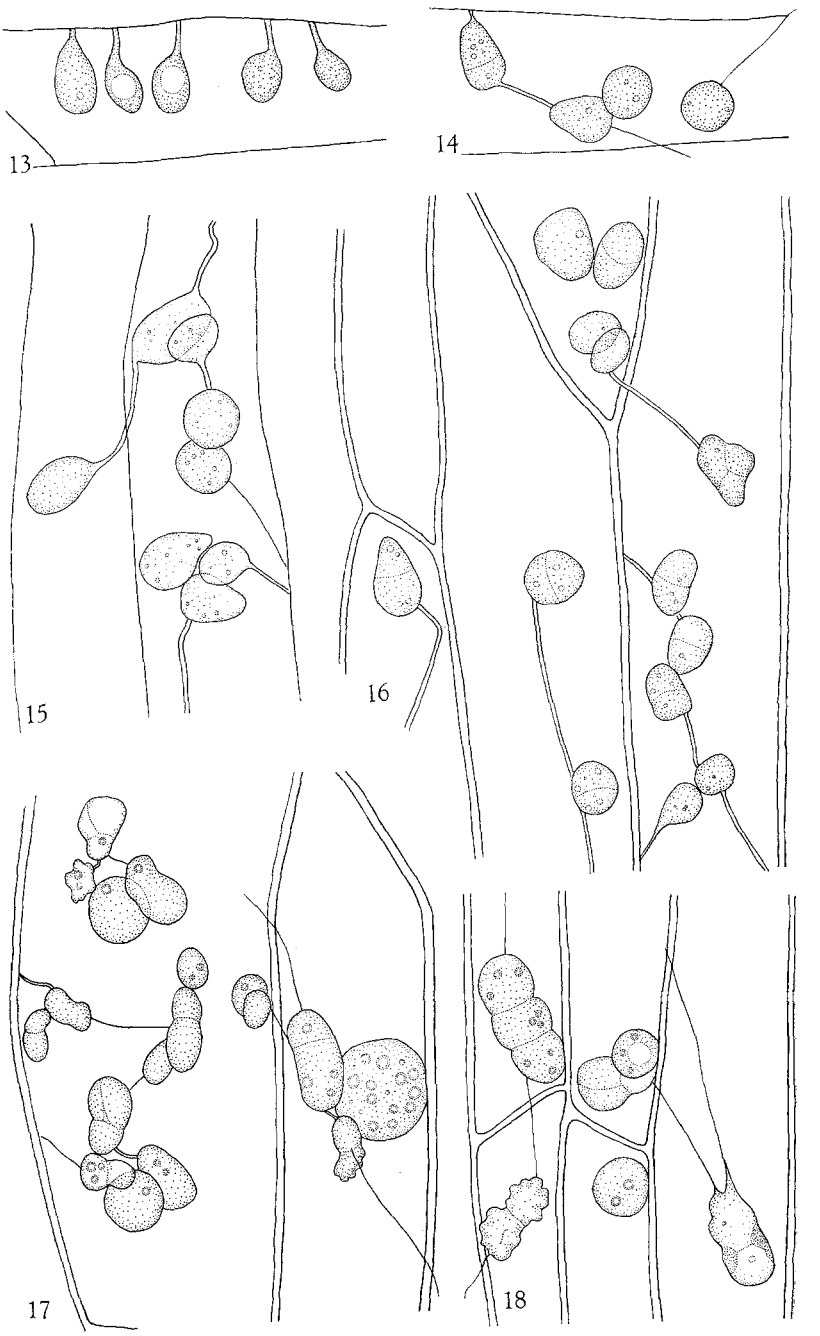

Fig. 13—18

Claytonia plants (Fig. 15 and 16). The speed with which these were established further strengthened the observational evidence that an ephemeral, epibiotic stage was completely lacking.

8 days after host plants had been exposed to resting spore zoospores, developing rudiments of resting spores were found. These were produced as in most, if not all, true species of *Physoderma*, singly, at the tips of short, lateral outgrowths of turbinate organs (Fig. 17 and 18). The latter were spherical, ovoid or oblong, 1—3-celled, 12—20 · 6—8 μ, and frequently were formed in clusters which tended sometimes to obscure their relationship to the developing resting spores (Fig. 17). Nine days after original exposure of the hosts, numerous pale, amber-colored, nearly mature resting spores were observed (Fig. 12). During the succeeding two days these became fully mature; that is, 11 days after original contact of fungus and host. This sequence of development was confirmed in six instances.

One further observation concerning the endobiotic system might be included. It was repeatedly noticed that there was a distinct tendency for the fungus to establish such systems within the upper, anthocyanin-pigmented parts of the leaf petioles as contrasted with the lower, colorless portions. Whether this is a nutritional relationship or is due to other factors, light, for example, awaits investigation.

Discussion

Several features of general biological interest are presented by the parasite of *Claytonia*. Foremost, is the lack in our material of any evidence for an epibiotic, ephemeral sporangial stage. Since, however, it has been shown by us in the *Physoderma* on *Agropyron repens* (1961) that the resting spore zoospore may give rise either to the endobiotic system or to the ephemeral sporangium, it is possible that the latter may under certain conditions be produced by the *Claytonia* parasite.

Spring Beauty, like many components of the Eastern North American vernal flora has a relatively short life in the vegetative and flowering stages, probably no more than 2—3 months in the vicinity of Ann Abor. It is one of the first of the spring flora to emerge, and as early as the last week in February or first week in March may be found above ground. The host is, therefore, subjected to a great range of early Spring temperatures involving alternate freezing and thawing conditions. The low temperatures at which the resting spores germinate probably enable the fungus to take advantage of temporary periods of thawing during which the zoospores accomplish infection. Elimination of the ephemeral sporangial stage might at first be considered an adaptation for quick establishment of the endobiotic system during short periods of favorable weather. Inasmuch as the aforementioned parasite of *Agropyron repens*, a summer

grass, could also omit this same stage and proceed directly to the endobiotic system there seems little support for this idea.

A final observation might be made which is concerned with the endobiotic system. From a study of this and many other species of *Physoderma* it seems to us that the main function of the extensive rhizoidal system within the host is not nutritional but distributional. That is, this system of delicate rhizoids with its occasional turbinate organs wanders from cell to cell invading new areas of host tissue. The few-celled, nucleated turbinate organs left behind act as centers of resting spore formation, for it is from these that the resting spores have their origin. The contents and presumably the nuclei of a cell of a turbinate organ pass out to the enlarging bud, the actual rudiment, after which the empty segment usually collapses. The enormous increase in size subsequently undergone by these rudiments, a number of which may be associated with a single thallus and in a single cell, makes it seem unlikely that they are being nurtured solely by their delicate rhizoidal system. Rather, it is more reasonable to believe that each resting spore rudiment is, in fact, functioning as a holocarpic chytrid and absorbing food over its entire surface. This action probably continues until such time as when the pigmented, rigid wall makes its appearance and final maturation of the resting spore is initiated. According to this viewpoint the chief function of the rhizoidal system is to get the precursors of the resting spores to favorable areas of host tissue.

Summary

Resting spores of the chytrid parasite of *Claytonia virginica* or "Spring Beauty", *Physoderma claytonianum* var. *sparrowii*, were overwintered in plastic containers buried at the site of collection near Ann Arbor, Mich., USA. Germination took place in the field in early March. The process was followed in the laboratory and involved dehiscence of an operculum, protrusion of an endosporangium, zoospore formation and discharge. Resting spore zoospores were repeatedly placed in contact with young *Claytonia* plants but there was no evidence that they ever developed into epibiotic, "ephemeral" sporangia. Rather, in all instances within 36 hours the endobiotic, polycentric thallus was established inside the host. Both observational evidence and the speed with which the endobiotic thallus first appeared pointed to the complete lack of an epibiotic stage in our material.

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