

ANOTHER HYPHOMYCETOUS FUNGUS PARASITIC ON PYTHIUM OOSPORES

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In an earlier paper (3) 2 hyphomycetous fungi, *Trinacrium subtile* Riess and *Dactylella spermatophaga* Drechsl., were reported as having been found subsisting parasitically on mature oospores formed by root-rotting oomycetes in isolation plate cultures prepared by planting sizable fragments of diseased vegetable tissue on maize-meal agar. From their morphological features and developmental traits these 2 fungi could be recognized as closely akin to the series of mucedinaceous forms most familiarly exemplified in *Arthrobotrys oligospora* Fres., the larger number of which, under natural conditions, habitually secure their nourishment by capturing nematodes, amoebae, and testaceous rhizopods.

More recently a third fungus of like biological habit and presumably also of like taxonomic kinship came to light in an old Petri-plate culture of *Pythium graminicolum* Subr., following the addition of a small quantity of leaf mold taken from woods near Haugen, Wis., in September 1939. Throughout a tract of approximately 500 square millimeters bordering the deposit of forest refuse, many thousands of oospores were found reduced to membranous remains. Day after day other oospores could be observed undergoing destruction along the margin of the slowly enlarging tract. The units of sexual apparatus about to be attacked arrested attention by the development of a distended crook-necked structure whose broadly rounded apex was closely appressed to the oogonial membrane; so that an appearance curiously suggestive of fertilization was presented, though true fertilization had everywhere been accomplished fully 2 months earlier. The crook-necked structure, while closely resembling the antheridia of various species of *Pythium*, including *P. graminicolum*, soon revealed its proper character by functioning as an appressorium in thrusting a process, often 1.5 to 2 μ wide, through the oogonial membrane and into the oospore wall. Within this wall the intruded process would sometimes gradually expand for about 2 hours and thus form a conspicuous bulbous enlargement 8 μ or 9 μ in transverse diameter (Fig. 1, A-D) before breaking into the chamber of the spore (Fig. 1, E) and extending throughout the protoplast a rather massive, somewhat branched haustorium (Fig. 1, F, G). Usually perforation of the oospore wall was accomplished with less pronounced inflation, the invading process, in most instances, becoming distended to a width of perhaps 4 μ or 5 μ in passing through the thick envelope (Fig. 1, H, I).

The protoplasmic content of the oospore usually revealed no change in its normal unitary organization (Fig. 1, A) until the invading fungus had almost penetrated the enveloping wall. At that stage the reserve globule often lost its smoothly circular contour, while, simultaneously, the refringent

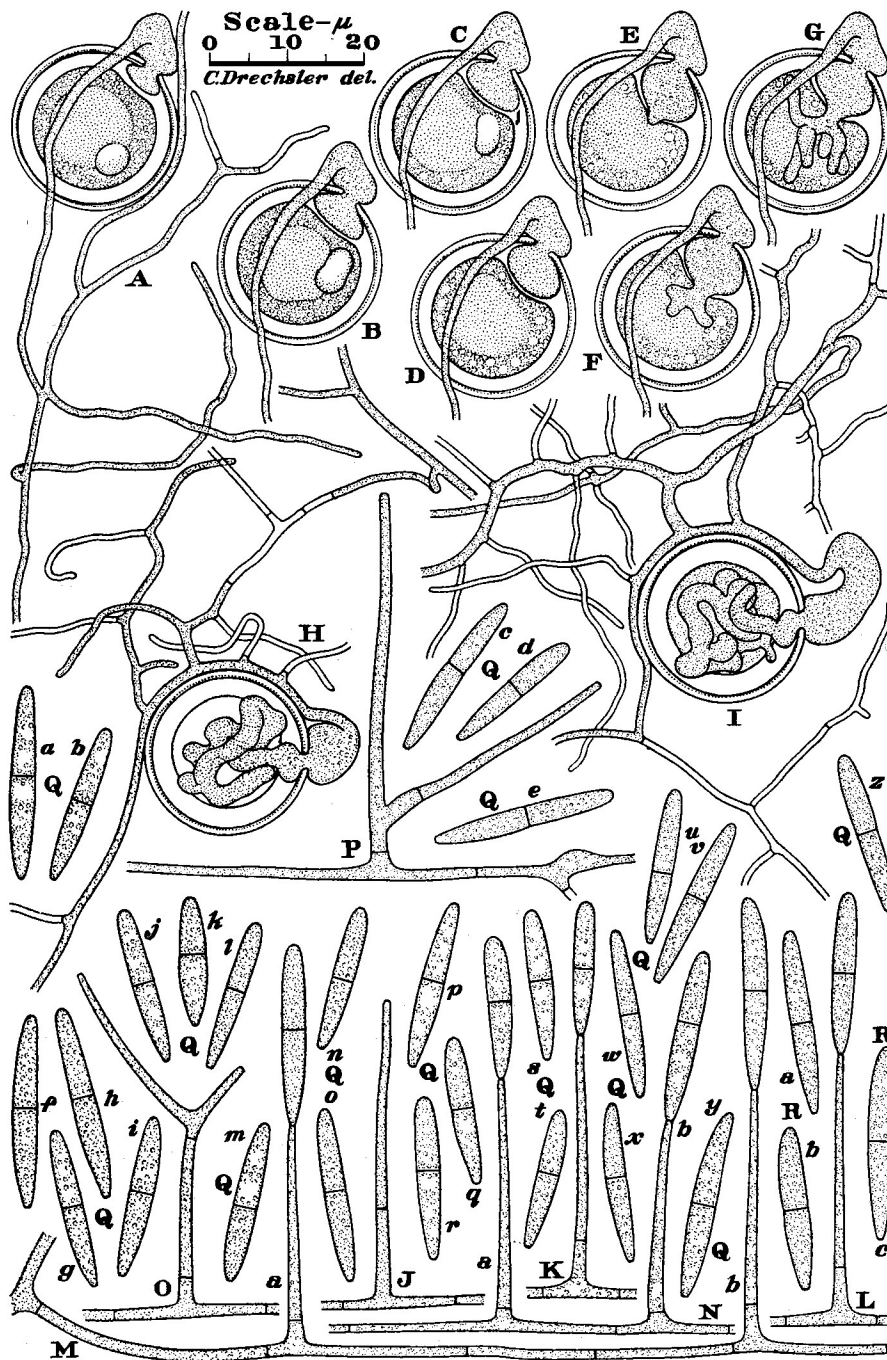


FIG. 1. *Trichothecium arrhenopum* drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$ throughout. A-G. Successive stages in the penetration and invasion of an oospore of *Pythium graminicolum* observed during $2\frac{1}{2}$ hours. H, I. Portions of mycelium, each with an appressorium from which an oospore of *P. graminicolum* has been invaded. J-N. Portions of prostrate hyphae bearing one or two simple erect conidiophores. O, P. Branched conidiophores. Q, a-z; R, a-c. Random assortment of conidia, showing variations in size and shape.

body assumed an elongated (Fig. 1, B) or irregular outline. Soon afterwards the parietal granular layer became interspersed with numerous small vacuoles, and merged more and more with the misshapen reserve globule (Fig. 1, C, D). By the time the invading fungus had irrupted into the chamber of the oospore the refringent body often could no longer be clearly distinguished (Fig. 1, E-G). Gradually the disorganized mass of protoplasm became less dense in texture as the substances composing it were absorbed by the haustorium. The oospore wall became noticeably swollen and evidently underwent some softening (Fig. 1, H, I), though it was not apparent that the softened materials were assimilated. Eventually, after the granular residues of the host had disappeared, the protoplasmic contents of the haustorium itself were withdrawn into the external mycelium of the parasite.

This mycelium consisted of delicate hyphae giving off branches rather promiscuously in all directions (Fig. 1, A, H, I). Many of the finer ramifications were appreciably narrower than $1\ \mu$, while the axial filaments from which they arose often did not exceed $1.5\ \mu$ in width. The delicate vegetative habit appeared to represent an adaptation whereby the parasite is enabled, with obvious economy, to seek out oospores in scattered positions. When an oogonium containing an oospore was encountered, the successful exploratory branch enveloped the oogonial membrane very tightly about half way around its circumference (Fig. 1, H, I) before giving rise terminally to the massive crook-necked appressorium already described. Unsuccessful exploratory branches, on the other hand, were soon evacuated through retraction of their protoplasm into the parent filaments. Septa could not be recognized within any portion of the exploratory mycelium that remained full of protoplasm, though partitions were readily visible within empty portions of hyphae, as well as at the boundaries of living portions. Perhaps intercellular septa, as distinguished from retaining walls, were omitted here in order to promote freer movement of protoplasm, and thus to facilitate the ready production and extension of exploratory branches. However, it is also possible that intercellular septa were present, but owing to the small dimensions of the filaments, were indiscernible to ordinary microscopical inspection. The apparent continuity of its living portions made the submerged parasitic mycelium resemble in some degree the mycelium of a delicate phycomycete.

That the parasite is, nevertheless, not a phycomycete was evident from the indubitably septate condition of some coarser, more straightforward, and only sparingly branched hyphae formed on or near the surface of the agar medium (Fig. 1, J-P). These hyphae were concerned more directly with the asexual reproduction of the fungus than with its vegetative development, since they gave rise at moderate intervals to erect, slender, colorless, frequently uniseptate, aerial conidiophores (Fig. 1, J-L; M, a, b; N, a, b), which usually concluded their development by bearing a single colorless elongated conidium. Sometimes, to be sure, a conidiophore, after declining to the substratum, extended its usefulness by sending up a secondary

conidiophore as a lateral branch (Fig. 1, O, P); the secondary conidiophore, after bearing its single conidium, then occasionally falling over in turn and giving rise to a tertiary fertile branch. With respect to such protracted development, the fungus showed similarity not only to the oospore-destroying *Dactylella spermatophaga*, but also to many of the more robust members of the predaceous series of hyphomycetes subsisting habitually through capture of animals.

The conidia (Fig. 1, Q, a-z; R, a-c), whether produced on simple conidiophores or on branches arising therefrom, were only about two-thirds as wide and less than half as long as those of *Dactylella spermatophaga*, from which they differed further in being divided regularly by a single cross wall rather than by 3 cross walls. Their meager septation, blunt ends, and lack of pronounced curvature, removed them from any close resemblance to the macroconidia of *Fusarium*, though some general similarity to the much less distinctive microconidia produced by members of that genus was evident. Taxonomic disposition of the fungus is, therefore, not disturbed by such perplexities as are attached to the curiously ambiguous conidial morphology of *D. spermatophaga*.

The character of its asexual reproductive apparatus clearly refers the fungus to *Trichothecium* Link in the more restricted sense in which this genus was adopted by Saccardo (8, v. 4, p. 178). *Trichothecium*, throughout the *Sylloge Fungorum*, is held distinct from *Cephalothecium* Corda,—the former being made to include species that bear uniseptate, hyaline or light-colored conidia singly on simple erect conidiophores, while the latter is reserved for species bearing such conidia plurally in more or less capitate arrangement on conidiophores likewise erect, simple and septate. The distinction thus drawn has fallen somewhat into disfavor from a belief that *T. roseum* Link and *C. roseum* Corda, on which, respectively, the 2 genera would seem to have been founded, and by which, certainly, they have long been most familiarly exemplified, represent one and the same fungus. The fungus that presumably is in question here, best known to students of plant diseases from its causal connection with pink rot of apples, may, indeed, be found bearing solitary spores on conidiophores just at the beginning of their reproductiveness, whereas its older conidiophores are commonly found supporting a number of spores in a contorted spicate cluster having the general appearance of a head. Owing to the formation of the conidia one after another, in basipetal order, the clustered condition is necessarily preceded for a brief period in the development of the individual conidiophore by a condition that could be interpreted as expressive of a solitary sporulating habit; though the usual prevalence of a clustered arrangement wherever the fungus is present in quantity should remove virtually all occasion for such error.

The earlier definitions of *Trichothecium* were flagrantly wanting in morphological detail. Link failed to specify any particular manner of spore formation both in his first (5, p. 18) and in his second diagnosis (6 p. 28)

of the genus. Nor is any particular manner of spore formation mentioned in his diagnosis of *T. roseum* (6, p. 28); and the single relevant figure given by him (5, Tab. 1, 27) merely shows detached uniseptate spores scattered promiscuously among a number of septate hyphae. The usage that in early times grew up with regard to the species would seem to have been based more on considerations of color, habitat, and distribution than on considerations of morphology. Confusion of more than ordinary seriousness arose. As late as 1912 Grove (4) held that *T. roseum*, *T. candidum* Wallr., *T. obovatum* (Berk.) Sacc., *C. roseum*, *C. candidum* Bon., *Arthrotrys superba* Corda, *A. oligospora*, and *A. rosea* Mass. might represent varying states of one species.

The citation of *Arthrotrys superba* and of *A. oligospora*, with which *A. rosea* appears identical, among the dubious species of *Trichothecium* and *Cephalothecium* was without much justification, since the distinction between the truly capitate sporulation of *A. oligospora* and the successive, basipetal production of conidia in the pink-rot fungus had been repeatedly pointed out during the preceding half-century. This difference in manner of cluster development seems, indeed, of far greater taxonomic significance than the difference between production of single capitate clusters and successive capitate clusters; wherefore, some years ago, in describing as new 2 nematode-capturing species that give rise only to single capitate clusters, I ventured to assign them under the names *A. musiformis* and *A. dactyloides*, to *Arthrotrys* (3) rather than to *Cephalothecium*, as would have been required in strict accordance with Saccardian usage. Through such extension of *Arthrotrys* at the expense of *Cephalothecium*, the latter genus, of questionable validity because of its erection subsequent to *Trichothecium*, is conveniently excluded from the predaceous series of hyphomycetes. Of the 7 species compiled under *Cephalothecium* in the *Sylloge Fungorum*, *C. macrosporum* Speg. with fertile hyphae "apice minute abrupteque noduloso-sterigmatophoris" (8, v. 10, p. 549), and *C. microsporum* Eichelb. with conidiophores "apice inflatis et minute verruculosus" (8, v. 22, p. 1305) might perhaps belong in the predaceous series. On the other hand, although Zopf's figure of *C. roseum* (9, p. 309, fig. 26, IV) shows uniseptate conidia borne in pronouncedly capitate arrangement on an erect conidiophore, the large, subspherical, terminal, *Oedocephalum*-like enlargement to which the conidia are attached seems scarcely less alien to *Arthrotrys* than to *C. roseum* as originally described and figured by Corda (1, Tab. X, fig. 62).

The hyphomycetous forms intimately akin to *Arthrotrys oligospora*, but producing their uniseptate conidia singly, offer no prominent morphological feature whereby they can be separated readily from the forms that while likewise producing solitary uniseptate conidia on erect sporophores are related to *Trichothecium roseum*. Under natural conditions of development a heavy, somewhat crustose, and rather bright-colored turf of sporiferous hyphae is likely to denote affinity with the pink-rot fungus, while scant development of colorless or only faintly tinged sporiferous hyphae would

more probably betoken affinity in the predaceous series,—the abundant display of conidial apparatus in the former instance being referable to direct utilization of the substratum as food supply, whereas the meager display in the latter instance derives from utilization only of minute animals or fungal structures associated with the substratum. Because of its very delicate aerial development *T. griseum* Speg. (8, v. 4, p. 180), for example, may be suspected of belonging in the predaceous series. The similarly very delicate *T. inaequale* (8, v. 18, p. 539) described by Masee and Salmon (7) from horse dung and rabbit dung in England, almost certainly is a member of this series, and very probably, in view of its close resemblance to the nematode-capturing fungus I described as *T. polybrochum* (2), habitually subsists on eelworms. As the fungus found parasitizing oospores of *Pythium graminicolum* gives rise to conidial apparatus in quantity so small as to be wholly invisible to the naked eye, its presence would ordinarily escape detection on opaque natural substrata. At all events, it cannot be identified with any of the 18 species compiled under *Trichothecium* in the *Sylloge Fungorum*, and has apparently not been described hitherto. It is, accordingly, presented as a new species under a specific name meaning “masculine-looking,” which is intended to be descriptive of its antheridium-like appressoria.

***Trichothecium arrhenopum* sp. nov.**

Mycelium bonam partem valde ramosum, hyphis incoloratis, certe ex parte septatis, plerumque 0.6–2 μ crassis, in oogonium Pythii maturum incasis id partim arte circumplectentibus denique appressorium ei late applicantibus; appressorio plerumque 5–8 μ crasso, 8–18 μ longo, ad instar antheridii curvato, membranas oogonii oosporaeque perforante denique ramos assumentes 1.5–3 μ crassos intrudente. Hyphae fertiles erectae, incoloratae, pauce septatae, plerumque 25–50 μ altae, basi 1.7–2.5 μ crassae, apice 0.7–1.5 μ crassae; conidiis solitariis, incoloratis, clavulatis vel elongato-ellipticis, basi obtusis, sursum rotundatis, medio septatis, plerumque 17–25 μ (saepe circa 21.4 μ) longis, 2.6–3.7 μ (saepe circa 3.1 μ) crassis.

Habitat in humo silvestri prope Haugen, Wisconsin.

Mycelium in large part abundantly branched, and in part, at least, septate; the vegetative hyphae colorless, mostly 0.6 to 2 μ wide, on encountering a mature *Pythium* oogonium enveloping it very closely along approximately one-half of its circumference and then producing an appressorium in broad contact with it; appressorium mostly 5 to 8 μ wide, 8 to 18 μ long, curved or crook-necked after the manner of some antheridia, after penetrating the walls of both oogonium and oospore intruding a haustorium with branches 1.5 to 3 μ wide to appropriate the protoplasmic contents. Conidiophores erect, colorless, usually with a single septum near the base, measuring mostly 25 to 50 μ (average 34.4 μ) in height, 1.7 to 2.5 μ (average 2.2 μ) in basal width, and 0.7 to 1.5 μ (average 0.9 μ) in apical width. Conidia solitary, colorless, somewhat clavate or of elongate-elliptical outline, blunt at the base, rounded at the tip, mostly 17 to 25 μ (average 21.4 μ) long, 2.6 to 3.7 μ (average 3.1 μ) wide, divided by a median septum, the position of which is sometimes marked by a slight constriction.

Occurring in leaf mold near Haugen, Wis.

SUMMARY

A delicate mucedinaceous fungus from Wisconsin leaf mold was found energetically parasitizing oospores of *Pythium graminicolum* in an agar culture. It is described as a new species under the name *Trichothecium arrhenopum*. Like *Trinacrium subtile* and *Dactytella spermatophaga*, which have previously been made known as oospore parasites, it seems closely related to the series of predaceous hyphomycetes exemplified in *Arthrobotrys*

oligospora rather than to the familiar *T. roseum*. It penetrates the oogonial envelope and the oospore wall by means of a massive crook-necked appressorium having a curious resemblance to the antheridia of many species of *Pythium*.

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