A NEW NON-HELICOID BISPOROUS HELICOCEPHALUM PARASITIZING NEMATODE EGGS

CHARLES DRECHSLER

(WITH 1 FIGURE)

More than half a century ago Thaxter (4), then stationed at New Haven, Conn., erected the genus *Helicocephalum* on a curious fungus he described under the name *H. sarcophilum* after discovering it on carrion in a laboratory culture. The fungus was set forth as having the general habit of a large *Mortierella* or *Syncephalis,* its aseptate or rarely septate hyaline mycelium creeping over the substratum and giving rise here and there to tall, erect, continuous, unbranched, gradually tapering, hyaline sporophores supported at the base by rhizoid-like attachments. If the vegetative mycelium and the columnar portion of the sporophore offered little that could be held unusual, the distal portion of the sporophore not only presented remarkable external differentiation in widening out and coiling abruptly two or three times, but also displayed noteworthy internal development by undergoing segmentation into a chain of large, dark brown conidia, which after their disarticulation cohered in a rounded mass.

Spiral coiling in the terminal portion of the sporophore and subsequent segmentation of the helicoid part into a chain of spores which after their disarticulation remain attached in a cohering cluster likewise marked the reproductive development of an obviously congeneric fungus that came to my attention ten years ago in an old agar plate culture originally planted with decaying spinach (*Spinacia oleracea* L.) roots from southeastern Virginia. This species I described as *Helicocephalum oligosporum* (1) since it produced commonly 5 to 10 (rarely 11) spores in a head, whereas the Connecticut form had been found bearing as many as 21 spores, seldom more, on the individual fertile hyphae. Besides being produced in lesser numbers, the conidia of *H. oligosporum* were

smaller, measuring mostly 32 to 45 μ in length and 20 to 25 μ in width as compared with a length of 55 μ (maximum 65 μ) and a width of 30 μ (maximum 35 μ) attributed to the spores of *H. sarcophilum*. The sporophores bearing these smaller spores measured only 350 to 600 μ in height and 13 to 16 μ in basal width, as compared with a height of 1 mm. or more and a basal width of 20 to 25 μ recorded by Thaxter. Commensurate with the less robust proportions of its reproductive apparatus my fungus had a more delicate mycelium, its vegetative hyphae measuring only 1 to 1.3 μ in width, as against a hyphal width of 2 μ in *H. sarcophilum*.

A fungus in many ways closely resembling *Helicocephalum sarcophilum* and *H. oligosporum* came to light in several maizemeal-agar plate cultures that after being permeated with mycelium of *Pythium mamillatum* Meurs had received some little addition of partly decayed bluegrass (*Poa pratensis* L.) leaves removed on May 10, 1941, from a heap of old lawn clippings in Arlington, Va. Its conidiophores, somewhat more slender but evidently no less tall than those of Thaxter's fungus, began to appear about a week after the decaying material had been added. Day after day for a period of more than three weeks, during which the temperature of the laboratory varied between 28° and 32° C., new sporophores came up in scattered positions. Eventually the empty membranous remains of old sporophores could be found sparsely distributed here and there in nearly all portions of each culture.

When examined under a microscope of sufficiently high magnification, preferably at a somewhat early stage of development, the individual sporophore was found anchored to the agar substratum by frequently more than a dozen rhizoidal outgrowths extending away in different directions (FIG. 1, *A; B, b; C, b; D, d*). These outgrowths seemed to function only in giving support, for very soon after attaining definitive length they lost their granular contents to persist as empty tubular membranes. A single filamentous branch, less conspicuous than the rhizoidal outgrowths and often only 25 to 50 μ in length, was always found connecting the slightly swollen base of the sporophore with the vegetative mycelium (FIG. 1, *A; B, c; C, c; D, e*). This unpretentious branch represented, of course, the hyphal element from which the entire unit of reproductive apparatus originated, and through which it necessarily had

to receive all its protoplasmic materials. With respect to width the communicating branch and all other mycelial hyphae that likewise remained visible for a long time were coarser than the corresponding filamentous elements of *Helicocephalum oligosporum*, but more delicate than the sterile hyphae of *H. sarcophilum*. Nowhere in the vegetative mycelium were cross-walls found separating masses of protoplasm, though retaining walls were often observed delimiting a portion of living hypha from a contiguous empty portion. In view of the strictly unseptate character of its living mycelium, the fungus must be reckoned among the Phycomycetes no less unreservedly than *H. oligosporum*.

When fully grown the sporophore tapered upward very gradually, often for a distance of about 1 mm., and then widened rather abruptly to form an elongated head that usually came to show a broad, somewhat gradual constriction midway toward the rounded apex (FIG. 1, *A; B, a*). As a rule the head was entirely straight, its axis prolonging that of the supporting columnar shaft (FIG. 2, *A; B, a*); though occasionally some slight curvature was noticeable (FIG. 1, *C, a*). This meager and infrequent curvature may readily have been due to some chance inequality in the pull eventuating from the surface tension of a droplet of clear watery liquid that at an early stage would begin accumulating around the head, especially below the median constriction (FIG. 1, *A; C, a*). With continued extrusion of watery liquid this adhering droplet grew often to a diameter of 100 μ, while simultaneously the elongate head, through insertion of two partitions, one near the base and the other at the constriction, was converted into two spores in end-to-end arrangement. On becoming disarticulated, these spores, now deep brown in color and prolate ellipsoidal in shape, were coerced by the surrounding droplet into a new positional arrangement making them longitudinally parallel and laterally contiguous with one another as well as with the upper portion of the sporophore (FIG. 1, *D, a, b, c*).

As disarticulation inevitably entailed circular rupture of the peripheral membranous layer that originally constituted the sporophore wall (FIG. 1, *E*), the spore of distal origin was found marked at its proximal end by an annular flange-like thickening which appeared in profile as a slight external irregularity. The spore of proximal origin bore a flange-like thickening of similar compass at

its distal end; while at its basal end it was marked by annular thickening of lesser compass (FIG. 1, *E*). In the side-by-side arrangement of the two spores lengthwise along the distal portion of the sporophore (FIG. 1, *D, a; F, a*) the one of proximal origin (FIG. 1, *D, b; F, b*) was usually, though not invariably, found inverted; while the one of distal origin (FIG. 1, *D, c; F, c*) was commonly found in normal posture, with its distal end—the end distinguished by absence of any irregularity in the outer contour of the wall—directed upward. Generally the two spores of a pair were approximately equal in size, yet now and then instances came under observation where one spore was fully a third larger than its mate (FIG. 1, *F, b, c; G, H*).

After remaining erect for perhaps a week the aging sporophores usually fell over, bringing the twin spores into contact with the moist substratum. Germination then often ensued within a few days, usually as in *Helicocephalum oligosporum* by the production of a single germ hypha (FIG. 1, *I*). This single hypha always emerged from a portion of wall laid down as a delimiting septum, never from the more extensive portion of spore wall representing the membrane that originally enveloped the fertile head of the sporophore. Within the circular area laid bare at the time of disarticulation emergence most often was from a peripheral position close to the flange-like modification. By using up the large supply of finely granular protoplasm in the massive spore the germ hypha soon developed into a rather extensive mycelium consisting for the most part of sparingly branched, submerged or prostrate, colorless filaments, 1.5 to 1.9 μ wide,—of filaments, therefore, quite like those making up the mycelium that gave rise to sporophores. However, the younger mycelium could be seen bearing here and there fairly long branches only .7 μ or .8 μ or .9 μ wide, which had no visible counterpart in any mycelium old enough for reproduction. When one of these branches encountered a nematode egg it would give rise, often laterally, to a swollen appressorium that after penetrating the echinulate egg integument introduced an elaborate haustorium composed of handsomely flexuous hyphal elements mostly 2 to 2.7 μ wide (FIG. 1, *J*). Once the haustorium had completely assimilated the materials within the egg, its own

protoplasmic contents were withdrawn backward into the delicate branch, which, from continued retreat of the protoplasm into the wider parent filament, was in turn evacuated, and as a result became indiscernible to ordinary microscopical examination. The delicate branches appeared to constitute a somewhat specialized apparatus whereby the fungus was enabled, with frugal expenditure of its substance, to seek out nematode eggs widely scattered through an extensive mass of substratum. In their exploratory function and early evanescence they resembled the similarly slender branches by means of which the hyphomycete I recently described as *Trichothecium arrhenopum* seeks out and destroys *Pythium* oöspores (3).

The fungus appears remarkable especially for the dimensional extremes found combined in it. For while its exploratory branches are so narrow that they invite comparison with the vegetative filaments of *Actinomyces,* and with the mycelial hyphae of such minute Zoöpagaceae as *Acaulopage rhaphidospora* Drechsl. (2) and *Stylopage lepte* Drechsl. (2), its unicellular spores, fully twice as voluminous as those of *Helicocephalum sarcophilum,* are perhaps the largest aerial asexual spores produced by any phycomycete now known. Apart from the slenderness of its exploratory branches, and the unseptate character of its mycelium, it offers some little parallelism with *Actinomyces* and with several genera of the Zoöpagaceae—the genera *Zoopage, Cochlonema,* and *Bdellospora*—in its catenulate sporulation. This parallelism reaches fuller expression in *H. sarcophilum* and *H. oligosporum,* since these species produce their spores in longer chains which previous to disarticulation are spirally coiled like the spore chains in numerous species of *Actinomyces.*

Although Thaxter's diagnosis of *Helicocephalum* specifies fertile hyphae "spirally coiled at the apex," the fungus parasitizing nematode eggs resembles the helicoid forms so closely in all essential respects that no reasonable doubt can be entertained as to its intimate kinship with them. I am therefore referring it to the same genus, despite its failure to display the remarkable feature signalized in the generic name.

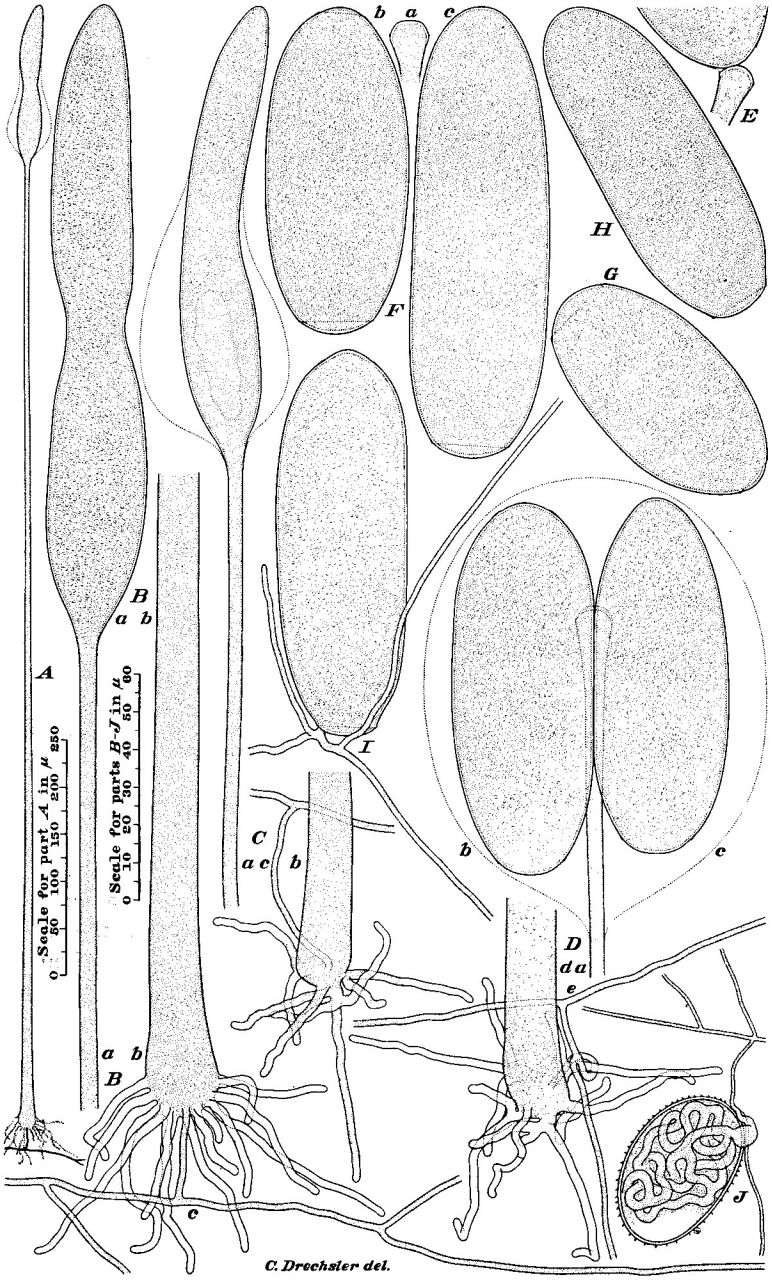

FIG. 1. *Helicocephalum diplosporum.*

Helicocephalum diplosporum sp. nov.

Mycelium parcum, hyalinum, continuum, parve ramosum, in hyphis vivacioribus plerumque 1.5–1.9 μ crassis et ramulis evanidis vulgo .7–.9 μ crassis constans; his tenuibus ramulis in ovum vermiculi nematoidei incasis, appressorium saepius circa 6 μ latum ei applicantibus, putamen eius perforantibus, haustorium intrudentibus; haustorio ramoso, ex filis flexuosis 2–2.7 μ crassis constante. Hyphae fertiles columnares, erectae, continuae, magnam partem hyalinae, 8–20 ramis radiciformibus 10–60 μ longis 2–2.5 μ crassis sustentatae, basi plerumque 14–20 μ crassae, sursum tenuatae, prope apicem 3.5–5.5 μ crassae, capite denuo latescentes etiam fuscescentes, ibi duas sporas in catenulam rectam (numquam in spiram) gignentes, quae postea, catenula diffissa, ad partem superam hyphae fertilis in longitudinem haerent; sporis fulvis, elongato-ellipsoideis, plerumque 70–130 μ longis, 34–39 μ crassis, membrana .5–1 μ (magna parte circa .6 μ) crassa circumdatis.

Ova vermiculi nematoidei interficiens consumensque habitat in foliis *Poae pratensis* putrescentibus in Arlington, Virginia.

Mycelium scanty, colorless, continuous, sparingly branched, consisting of rather long-lived hyphae mostly 1.5 to 1.9 μ wide and of more evanescent branches commonly .7 to .9 μ wide; the narrow branches on encountering a nematode egg producing in contact with it an appressorium, often about 6 μ wide, that perforates the egg integument and intrudes a haustorium composed of flexuous assimilative filaments 2 to 2.7 μ wide. Fertile hyphae columnar, erect, continuous, for the most part colorless, supported below by 8 to 20 rhizoidal branches mostly 10 to 60 μ long and 2 to 2.5 μ wide, above the slightly swollen base 14 to 20 μ in diameter tapering gradually upward to a width of 3.5 to 5.5 μ before widening out into a brownish, elongated, medially constricted, straight or nearly straight, terminal head, which through deposition of two transverse partitions is converted into two spores that after disarticulation adhere lengthwise to the upper portion of the erect column, immersed for some time in a droplet of clear watery liquid; spores brown, elongate ellipsoidal, mostly 70 to 130 μ long, 34 to 39 μ wide, surrounded individually by a wall .5 to 1 μ (mostly about .6 μ) thick.

Destroying and consuming nematode eggs it occurs in decaying leaves of *Poa pratensis* in Arlington, Va.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND

LITERATURE CITED

1. **Drechsler, C.** A new species of *Helicocephalum*. Mycologia **26**: 33–37. 1934.
2. ——. Some non-catenulate conidial Phycomycetes preying on terricolous *Amoebae*. Mycologia **27**: 176–205. 1935.
3. ——. Another hyphomycetous fungus parasitic on *Pythium* oöspores. Phytopathology **33**: 1943.
4. **Thaxter, R.** On certain new or peculiar North American Hyphomycetes. II. Bot. Gaz. **16**: 201–205. 1891.

EXPLANATION OF FIGURE

FIG. 1. *Helicocephalum diplosporum*, drawn with the aid of a camera lucida; all parts except *A* shown at a magnification of 500 diameters. *A*, Full grown, immature sporophore with lower portion of terminal head surrounded by a droplet of watery liquid; × 125. *B*, Portions of the same sporophore shown at a magnification four times greater: *a*, upper portion, showing the slender proximal attachment and median constriction of the terminal head, the outline of the adhering droplet being omitted; *b*, lower portion, showing distribution of the rhizoidal branches about the slightly bulbous base, and connection of the basal part with the mycelial filament *c*. *C*, Portions of a sporophore almost fully grown: *a*, upper portion, showing slender attachment and slight median constriction of terminal head, as well as envelopment of the wider proximal part of the head in a droplet of watery liquid; *b*, lower portion, showing distribution of rhizoidal branches about the slightly expanded base; *c*, mycelial branch connecting sporophore with the mycelium. *D*, Portions of a fully mature sporophore: *a*, terminal portion to which are adhering the inverted proximal spore *b* and the distal spore *c* with its apex directed upward; both spores being surrounded by a droplet of watery liquid; *d*, expanded basal portion of sporophore, showing its supporting rhizoidal branches as well as the hypha connecting it to the mycelial filament *e*. *E*, Basal portion of proximal spore and tip of mature sporophore, showing manner of disarticulation. *F*, Expanded tip of mature sporophore *a*, flanked by the two spores, *b* and *c*, in usual arrangement,—the proximal spore *b* inverted, the exceptionally long terminal spore *c* in normal posture, with apex directed upward. *G*, An exceptionally short spore of proximal origin. *H*, Spore of average dimensions formed terminally in the same head as *G*. *I*, Spore of proximal origin germinating from its distal end. *J*, Slender exploratory branch with an appressorium from which a haustorium has grown into a nematode egg.