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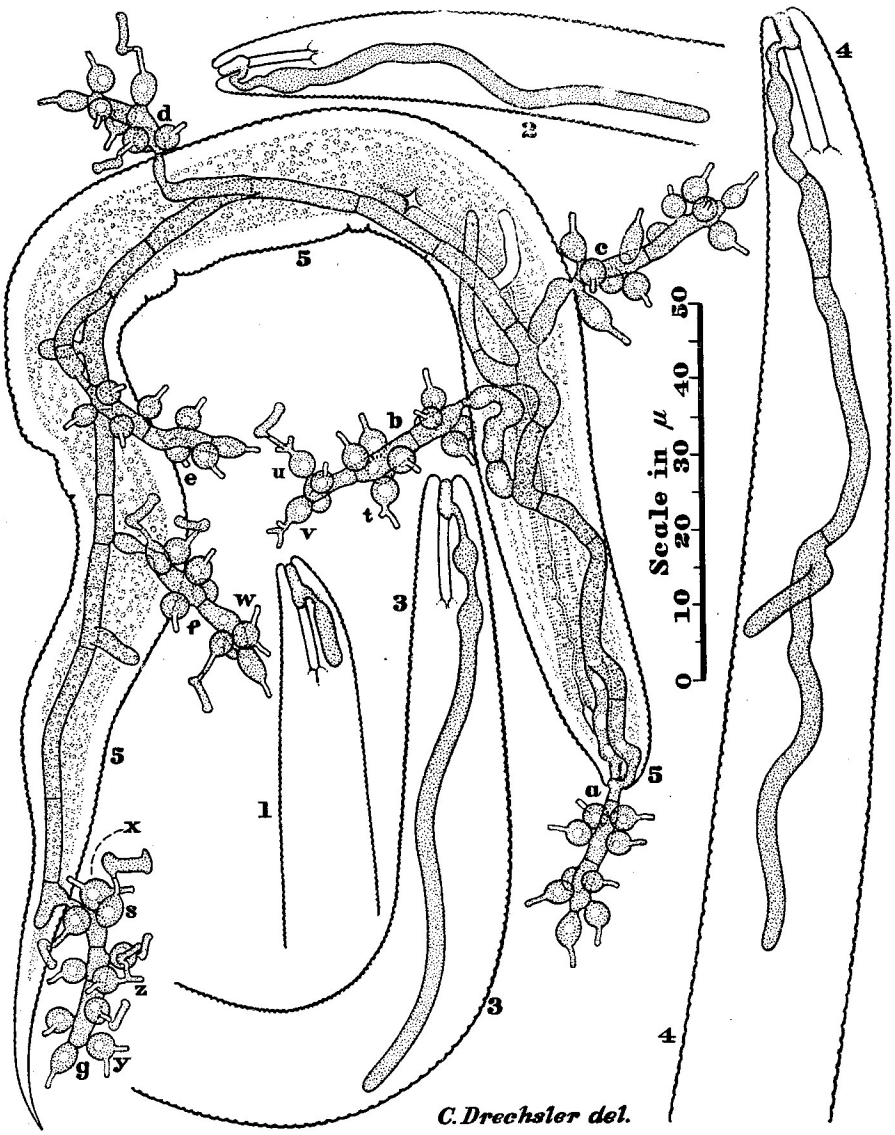
A SPECIES OF HARPOSPORIUM INVADING ITS NEMATODE HOST FROM THE STOMA

CHARLES DRECHSLER[1]

In 1874 Lohde (3) briefly described a hyphomycete that he found growing parasitically on numerous eelworms belonging to a species of *Anguillula*. To this hyphomycete he applied the binomial *Harposporium Anguillulae;* the generic term then presented for the first time having reference to the crescentically curved, sickle-shaped conidia which the fungus produced terminally on delicate sterigmata arising singly from peculiar round protuberances formed laterally on the hyphal branches or hyphal prolongations that were extended from the multicellular mycelial filaments, from 2 to 4 in number, passing through the animal's body. As the report of Lohde's findings gives only few intimate particulars and lacks all mention of measurements, it would seem to serve far better in establishing the genus than in defining a species. Descriptive details were very adequately provided by Zopf (4) 14 years later in a well illustrated account of a nematode parasite he held identical with Lohde's. The globose sporiferous cells, which in conformity with usage prevailing at the time (1, p. 65; 5, p. 314) he designated as basidia, were stated by Zopf to vary in diameter from 2.5 to 3.6 μ; the sterigmata were represented as mostly being shorter than the diameter of the globose parts; the crescentic conidia were set forth as being 1–2 μ thick and as measuring 7–13 μ along the chord connecting the two ends. From correspondence with these structures and with the chlamydospores which Zopf found developing in the assimilative hyphae within the animal, it is clear that this later author dealt with the same fungus that in my cultures has been more abundantly destructive to nematodes than any other hyphomycete attacking these animals parasitically rather than in a predaceous manner. Occasionally I have encountered *Harposporium* material which, though bearing crescentic conidia, and in general conforming to Lohde's account, appears alien to the ubiquitous species so accurately made known by Zopf. Thus, in a maizemeal-agar plate culture that had been planted with deciduous leaf mold collected on Polish Mountain about 10 miles east of Cumberland, Maryland, early in July, 1935, numerous nematode larvae were found undergoing destruction by a minute *Harposporium* whose globose sporiferous cells measured only 2.3–2.6 μ in diameter, while its sickle-shaped conidia,

[1] Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture; Plant Industry Station, Beltsville, Maryland.



FIGS. 1–5. *Harposporium bysmatosporum*, drawn to a uniform magnification with the aid of a camera lucida; × 1000. FIG. 1. Conidium of fungus shown lodged in stoma of living animal host (*Rhabditis* sp.); the spore has pushed a narrow germ tube through the stomatal lining and given rise within the fleshy body wall to a young assimilative hypha. FIGS. 2, 3. Longer assimilative hyphae, both still in unseptate unbranched condition; each shown arising laterally from a conidium lodged in anterior portion of the stoma of the living animal host. FIG. 4. A still longer assimilative hypha shown arising laterally from a conidium lodged in the stoma of the living nematode host; this hypha has laid down 3 crosswalls, and is putting forth a branch. FIG. 5. Dead body of animal host with a long

borne on single sterigmata about 1.3 μ long and 0.6 μ wide, measured only about 0.8 μ in width and 3–4.5 μ along the chord between the pointed ends. To determine whether this form and certain other forms with crescentic conidia belong to *H. Anguillulae* Lohde *emend.* Zopf, or whether they represent species distinct from it, will require further accessions of relevant material. Much less difficulty is offered by various forms which give rise to conidia not typically crescentic, yet which in their parasitism on eelworms, in their sporulating habit, and more especially in the globose shape of their sporiferous lateral cells (phialides) reveal unmistakably a congeneric relationship to *H. Anguillulae*. In an earlier paper (2, p. 793–800) I have described three such forms as separate species under the binominals *H. helicoides*, *H. oxycoracum*, and *H. diceraeum*. To these may now be added a fourth species which likewise produces conidia of a very distinctive shape other than crescentic.

The species in question came to light in a maizemeal-agar plate culture which after being permeated with mycelium of *Pythium arrhenomanes* Drechsl. had been further planted with several pinches of partly decayed, friable barley (*Hordeum vulgare* L.) straw collected near Greeley, Colorado, early in October, 1945. As the fungus was not observed until 47 days after the addition of the decaying straw its earlier development must have taken place more slowly than that of most hyphomycetes subsisting parasitically on eelworms. Its attack was confined evidently to sharp-tailed eelworms which were held referable by Dr. G. Steiner to a single species of *Rhabditis*. However, as the manner of infection displayed by the fungus appears about equally well adapted for other species of *Rhabditis*, the likelihood of a wider host range is not to be dismissed.

Infection is found accomplished always by the germination of a single conidium lodged in the anterior portion of the animal's tubular stoma; the spore having very probably reached this place of lodgement through the sucking action whereby the members of the genus *Rhabditis* ingest their food. The individual nematodes that have become gagged by intake of a spore seem usually to abandon their leisurely manner of locomotion and instead will hurry on at their greatest speed; their energetic deportment then recalling the violent locomotion more usually observed in nematodes of the genus *Wilsonema*. Close observation of gagged animals is, of course, impossible while their hurried movement continues. Microscopical examination was carried out conveniently on preparations made by transferring a sizable

assimilative filament extending lengthwise through it; the filament bears 11 branches, of which 7 have given rise externally to the conidiophorous prolongations a-g; s, phialide bearing a simple sterigma with an unusually large conidium; t, phialide bearing a sterigma with one branch; u, v, phialides bearing single sterigmata with two branches; w-y, phialides, each bearing two separate sterigmata; z, phialide with 2 sterigmata, one of them simple, the other bearing a single branch.

slab of agar from the Petri plate culture to a glass slide and covering it with a cover-glass; the movement of eelworms in such preparations being gradually halted, apparently from suffocation, everywhere except near the periphery of the mount. Among nematodes thus brought to quiescence, gagged specimens are readily detected by noticeable distension of the anterior portion of the stoma, and by the presence of a partition separating the expanded stomatal region from the unmodified posterior region (figs. 1–4). The curious partition represents obviously the spore membrane at the more deeply inserted end of the conidium. As a rule the membrane at the opposite end of the conidium invites less attention, since it usually lies approximately in alignment with the frontal profile of the animal's head and therefore often merges more or less with the outline of the stomatal orifice. The lateral portion of the conidial membrane is generally found so snugly appressed to the lining of the stoma that it no longer can be distinguished as a separate wall, though its presence is betrayed through pronounced thickening in the contour bounding the distended portion of stoma.

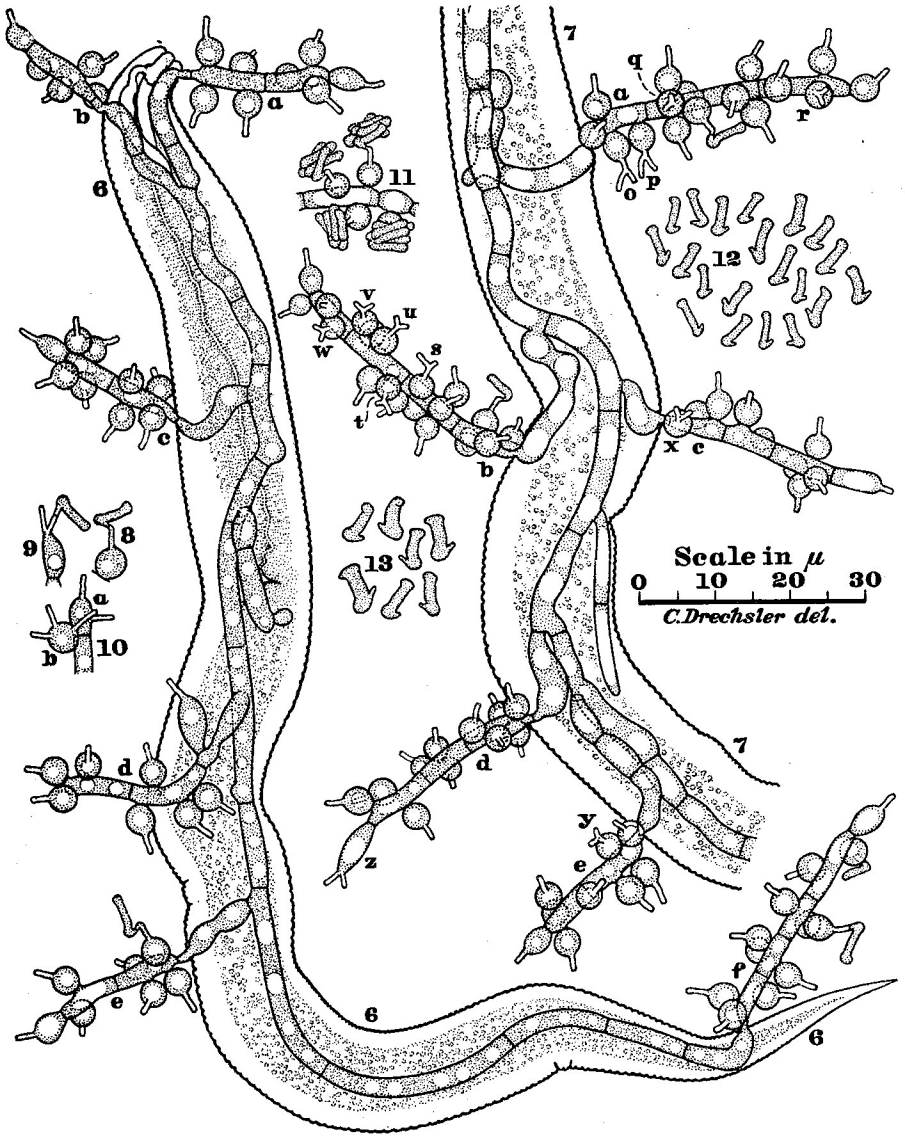
Once the conidium is seated securely in the stoma of the nematode it puts forth a single germ-tube from a position near its more deeply inserted end. This germ-tube, mostly about 1 μ wide, penetrates the stomatal lining to enter the fleshy tissues of the head. Sometimes it widens out almost immediately to a diameter in excess of 2 μ (fig. 1), but rather more often such widening is delayed until a length of about 5 μ has been attained (figs. 2–4). The crooked course frequently taken by the young infective germ-tube, as also the visible accumulation of yellow material around it, gives the impression that at the beginning invasion meets with resistance from the affected tissues. Occasionally after widening out the invading hypha may elongate narrowly for some little distance a second time (fig. 3). Whatever impediment may cause this initial inconstancy of width soon becomes inoperative, and thereupon the hypha elongates at a diameter of about 2.5 μ to make its way lengthwise through the fleshy body into the tapering tail until it stops growth perhaps within 25 or 30 μ from the pointed caudal tip (figs. 5, 6). In most of the smaller eelworm hosts, certainly in most eelworms measuring less than 250 μ in length, only a single main assimilative filament is produced; the total absorptive system being, however, augmented substantially through the development frequently of more than a dozen lateral branches at intervals varying from 5 to 100 μ. While the branches do not usually attain lengths in excess of 25 μ within the animal, most of them participate in further development by narrowly perforating the host integument to extend a conidiophorous prolongation outside. With the continued expropriation of host materials required to sustain development of reproductive apparatus externally, the assimilative hyphal system emerges into view more and more clearly. It is then revealed as mostly varying in width between 2.5 and 3.5 μ,

and as being divided by cross-walls into segments 5 to 25 μ in length (figs. 5–7). As the fleshy host contents approach exhaustion—the firm muscular oesophages and oesphageal bulb remain recognizable longest, but after a time melt away like the softer tissues—the mycelial segments become pronouncedly vacuolate (figs. 6, 7). When at last the host contents have vanished entirely, the assimilative elements undergo complete evacuation in continuing to supply the reproductive apparatus.

The conidiophorous hyphae (figs. 5, a–g; 6, a–f; 7, a–e) of the fungus closely resemble those of the ubiquitous *Harposporium Anguillulae*. They are mostly extended procumbently if the animal host happens to die on the surface of the agar culture. If, on the other hand, the animal dies well under the surface of the agar, they develop readily in submerged positions. They mostly vary in length from 15 to 40 μ, and in width from 2 to 3 μ; the shorter examples often containing only 2 axial segments (fig. 6, b), while the longer ones frequently contain 5 such segments (figs. 6, f; 7, a) and sometimes a greater number. All axial segments except the terminal one are of generally cylindrical shape, and each of them is beset laterally with globose cells in numbers varying commonly from 2 to 6. Most usually the spherical cell bears a single delicate sterigma together with which it makes up a phialide having the shape of a narrow-necked Florence flask (fig. 5, s; fig. 8). The terminal segment of the conidiophorous hypha serves directly as a phialide, being provided most frequently with a single apical sterigma. As the terminal phialide often has a somewhat prolate venter it approaches in outward shape the more familiar gradually tapering phialides that among hyphomycetes parasitic on nematodes occur in members of the genera *Acrostalagmus, Cephalosporium,* and *Spicaria* (2).

Although the larger number of phialides bear individually only a single unbranched sterigma, this single sterigma in more than a few instances gives off a branch and thus comes to be provided with 2 sporiferous tips (figs. 5, t; 7, o–y). Occasionally the sterigma gives off 2 branches, thereby acquiring 3 sporiferous tips (fig. 5, u, v). Sometimes 2 sterigmata arise from the same venter, either close together (figs. 7, z; 9) or from well separated positions (figs. 5, x, y; 10, a); and more rarely 3 sterigmata are borne separately (fig. 10, b). Three sporiferous tips are found present also in scattered instances where 2 separate sterigmata have been produced, one remaining simple while the other has given off a branch (fig. 5, z).

It seems probable that phialides with plural sterigmatic tips may bear a larger number of conidia than those provided with only one tip. In undisturbed condition, strongly vacuolated phialides with single unbranched sterigmata are commonly found bearing individually a cluster of from 4 to 6 conidia in more or less parallel arrangement (fig. 11). The conidia are of bizarre shape in some degree reminiscent of the conidia of *Harposporium*



FIGS. 6–13. *Harposporium bysmatosporum*, drawn to a uniform magnification with the aid of a camera lucida; ×1000. FIG. 6. Dead body of nematode host (*Rhabditis* sp.) with a long assimilative hypha extending lengthwise through it; 6 of the 7 lateral hyphal branches present have given rise externally to the conidiophorous prolongations a-f, which bear phialides furnished uniformly with single unbranched sterigmata. FIG. 7. Median portion of dead animal host, showing 6 branches coming from the main assimilative hypha; five of the branches have given rise externally to the conidiophorous prolongations a-e; o-y, phialides furnished with single sterigmata, each of which bears one branch; z, elongate terminal phialide with two sterigmata arising from its apex. FIG. 8. Globose

diceraeum. A narrow, recurved, minute basal pedicel is directed at an acute angle to the main cylindrical portion of the spore. Proximally the spore bulges out rather markedly toward the side opposite the pedicel. At the distal end it often widens perceptibly, the contour here usually bulging slightly less toward the pedicel side than toward the opposite side. On the whole the conidia appear to be somewhat larger than those of *H. diceraeum*, their length varying mostly from 4.5 to 6.5 μ, and their width, measured in the unmodified median region, varying from 0.8 to 1.4 μ (fig. 12). Here and there relatively massive conidia are found measuring 1.5 to 2.1 μ in median width (figs. 5, s; 13).

It may be presumed that the curious modifications in the form of conidia represent features adapting them for lodgment in the stoma of the host animal. Such lodgment obviously affords a much more secure foothold on the eelworm than could ordinarily be provided through adhesion to the integument, being wholly unaffected by any brushing action brought about by the movement of the nematode through materials of harsh texture. Among congeneric forms *Harposporium diceraeum* would seem most likely to be similarly adapted for stomatal infection, as its conidia are of size and shape whereby they might fit snugly in the stoma of its usual nematode host, *Plectus parvus* Bastian. The possibility of stomatal infection in *H. diceraeum* is hardly to be considered disproved by the single instance of infection from an externally adhering conidium which I happened to observe and which I took occasion to illustrate (2, p. 798, fig. 7, A). In any case the curious adaptational features developed by the Colorado fungus in relation to its manner of attack may be appropriately signalized in a specific epithet compounded in part of a term meaning "plug."

Harposporium bysmatosporum Drechsler, sp. nov. Mycelium nutritum incoloratum, septatum, intra vermiculos nematoideos viventes evolutum, saepe ex unica hypha filiformi 150–300 μ longa (quandoque in pluribus hyphis ejusmodi) et pluribus (vulgo 5–15) ramis constans; hypha et ramis in cellulis 5–25 μ longis 2.5–3.5 μ crassis consistentibus. Hyphae fertiles post mortem animalis extra corpus evolutae; submersae vel repentes, plerumque simplices, rarius parum ramosae, septatae, in axe ex 2–6 cellulis constantes, saepius 15–40 μ longae, 2–3 μ crassae, hic illic ramulos conidiferos (phialas) globosos 3–4 μ crassos ferentes; his ramulis conidiferis saepius unico sterigmate quandoque 2–3 sterigmatibus praeditis; sterigmatibus plerumque sim-

phialide with a single unbranched sterigma to which is attached a full-grown conidium. FIG. 9. Terminal phialide with two simple sterigmata arising from its apex. FIG. 10. Terminal portion of a conidiophorous hypha, showing a terminal phialide, a, with 2 sterigmata, and a lateral phialide, b, with 3 separate sterigmata. FIG. 11. Median portion of a conidiophorous hypha, showing 4 lateral phialides which have produced clusters of conidia; the number of spores in a group varying from 4 to 6. FIG. 12. Random assortment of conidia, showing usual variations in size and shape. FIG. 13. Conidia of more than usual size.

plicibus, 2–4 μ longis, circa 0.8 μ crassis, subinde 1 vel 2 ramusculis instructis, vulgo 4–6 conidia apice deinceps gignentibus; conidiis continuis, incoloratis, ad formam ossi lacerti (umero) paulum similibus, deorsum in minutum pedicellum oblique recurvis, sursum rotundatis et saepius plus minusve dilatatis, plerumque 4.5–6.5 μ longis, 0.8–2.1 μ crassis, quandocunque ab vermiculo nematoideo singulatim sumptis stoma animalis obturantibus et inde hypham germinationis in corpus ejus intrudentibus.

Vermiculos nematodeos specie Rhabditis necans habitat in stramento (foliis acere caulibusque) Hordei vulgaris putrescenti prope Greeley, Colorado.

Assimilative mycelium colorless, septate, growing within living nematodes, frequently consisting of a single main filamentous hypha 150 to 300 μ long (sometimes of several such hyphae) together with lateral branches numbering commonly from 5 to 15 and measuring from 5 to 25 μ in length; the hyphae and branches being composed of segments mostly 5 to 25 μ long and 2.5 to 3.5 μ wide. Conidiophorous hyphae, after death of animal host, formed in the surrounding or on the underlying material, with respect to their main axis mostly simple, more rarely somewhat meagerly branched, divided mostly by 1 to 5 cross-walls, measuring usually 15 to 40 μ in length and 2 to 3 μ in width, bearing laterally here and there globose conidiiferous cells (phialides) mostly 3 to 4 μ in diameter; these cells commonly provided with a single sterigma, though occasionally provided with 2 or 3 separate sterigmata; the sterigmata usually simple and usually measuring 2 to 4 μ in length and 0.8 μ in width, but occasionally bearing 1 or 2 branches. Conidia commonly formed in clusters of 4 to 6, continuous, colorless, somewhat resembling the human upper-arm-bone in shape, at the base recurved obliquely into a minute pedicel, at the distal end rounded and often noticeably widened, usually measuring 4.5 to 6.5 μ in length and .8 to 2.1 μ in median width, wherever sucked in by a host nematode stopping up the animal's stoma, and then intruding a germ tube into its fleshy body.

Destroying nematodes referable to *Rhabditis* sp., it occurs in decaying straw (stems, leaves, and chaff) of *Hordeum vulgare* near Greeley, Colorado.

U. S. DEPARTMENT OF AGRICULTURE
BELTSVILLE, MARYLAND

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