MYCOLOGY.—*Two new species of* Harposporium *parasitic on nematodes.*

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In Petri-plate cultures prepared with decaying plant detritus, eelworms were observed undergoing destruction by two mucedinaceous fungi markedly different from any hyphomycetous parasite hitherto recorded as attacking nematodes. The two fungi are herein described as new species of *Harposporium* Lohde (1874) though neither of them produces conidia of the distinctive crescentic shape signalized in the name of that genus. However, both fungi resemble *H. anguillulae* Lohde emend. Zopf (1888), the widespread species on which the genus was erected, in forming their conidia for the most part on minute slender sterigmata arising from globose cells borne laterally on hyphal elements extended from parasitized animals.

1. **Harposporium baculiforme** sp. nev.

Hyphae assumentes incoloratae, intra vermiculos nematoideos viventes evolutae, simplices vel ramosae, plerumque 2–4.5μ crassae, primo parce septatae sed postea ex magna parte in cellulis 3–20μ longis constantes; genitabiles rami externi, incolorati, clavati, saepius 4–15μ longi, basi 0.6–1.0μ lati, apice 1.5–2.5μ lati, ibi aliquot (fere 2–12) cellulas conidiferas (phialas) ferentes; cellulae conidiferae vulgo globosae sed interdum elongato-ellipsoidae vel obovoideae, 2.5–6μ longae, 2.5–4μ crassae, 1–2 sterigmatibus praeditae; sterigmata vulgo 1–3μ longa, circa 0.6μ crassa; conidia incolorata, baculiformia, aliquando sursum leviter attenuata, basi et apice rotundata, plerumque 2.5–5μ longa, 0.7–1.5μ crassa.

Vermiculos nematoideos interficiens habitat in foliis arborum putrescentibus prope Durango et Steamboat Springs in Colorado.

Assimilative hyphae colorless, growing within living nematodes, in small host animals simple or only sparingly ramified but in larger animals forming moderately branched mycelia, mostly 2 to 4.5μ wide, at first rather scantily septate though later becoming divided into cells mostly 3 to 20μ long, from some of which lateral branches are extended that narrowly perforate the host integument and elongate externally into conidiophores; conidiophores colorless, somewhat club-shaped, 4 to 15μ long, 0.6 to 1.0μ wide at the base, 1.5 to 2.5μ wide at the tip, whereon are borne several (mostly 2 to 12) conidiiferous cells in loosely capitate and sometimes partly in catenulate arrangement; conidiiferous cells commonly globose though sometimes elongate-ellipsoidal or inverted egg-shaped, 2.5 to 4μ wide and 2.5 to 6μ long exclusive of the 1 or 2 sterigmata; sterigmata commonly 1 to 3μ long, about 0.6μ wide; conidia colorless, cylindrical or tapering very slightly toward the apex, always with rounded ends, 2.5 to 5μ long and 0.7 to 1.5μ wide.

Type of species: Figs. 1–14.

Harposporium baculiforme came to light in several maize-meal-agar plate cultures which after being permeated with mycelium of *Pythium ultimum* Trow had been further planted with small quantities of leaf mold collected in woods near Durango in southern Colorado early in July 1958. Later it appeared also in some maize-meal-agar plate cultures that after being overgrown by *Pythium debaryanum* Hesse were further planted with leaf mold gathered in woods near Steamboat Springs in northern Colorado on July 23, 1958. In both sets of cultures it subsisted by parasitizing eelworms referable to a species of *Plectus*. Mostly it attacked relatively young individuals, in each instance extending through the small animal host a single assimilative hypha with scarcely any vegetative branches (Figs. 1–5). Moderate ramification was usual when a more robust animal was invaded (Fig. 6). An assimilative hypha could in some instances be seen to have originated from a conidium lodged in the forward region of the stoma (Figs. 1, a; 5, a). A manner of initiating attack corresponding to that found usual in my *H. bysmatosporum* (Drechsler, 1946, 1954; Aschner and Kohn, 1958) was thus disclosed. Many infected eelworms, however, did not show any recognizable conidium within the stoma, and in these animals the avenue of attack remained conjectural.

The conidiophores of *Harposporium baculiforme* differ markedly in their small dimensions from the robust filamentous conidiophores found in all known congeneric forms as well as in all known nematode-destroying members of the related *Cephalosporium-Verticillium-Acrostalagmus* series. Soon after they have been extended from an infected animal they commonly give rise directly from the slightly expanded tip to globose conidiiferous cells in numbers varying from 1 to 5 (Figs. 1, b, c; 2, a–f; 3, a–g; 4, a, b; 5, b; 6, a–c; 7–11). Some of the globose cells may give rise to 1 or 2 others, so that usually 5 to 12 conidiiferous cells, interspersed with a few sterile cells of similar subspherical shape, become assembled in a loose irregular cluster. Although the globose cells in relatively young clusters most often bear only a single sterigma, many cells in older clusters are provided with two sterigmata (Fig. 11).

If left undisturbed the conidia produced on individual sterigmata tend to cohere side by side in compact sheaves (Fig. 12). Since usually a large proportion of them are moved short distances in different directions by roving eelworms and protozoans they commonly are seen lying separately in haphazard disorder (Figs. 13, 14) around infected eelworms. On careful scrutiny some appear to have a very slightly tapering shape—a spore 1μ wide at its base, for example, diminishing to a width of 0.9μ at its rounded tip.

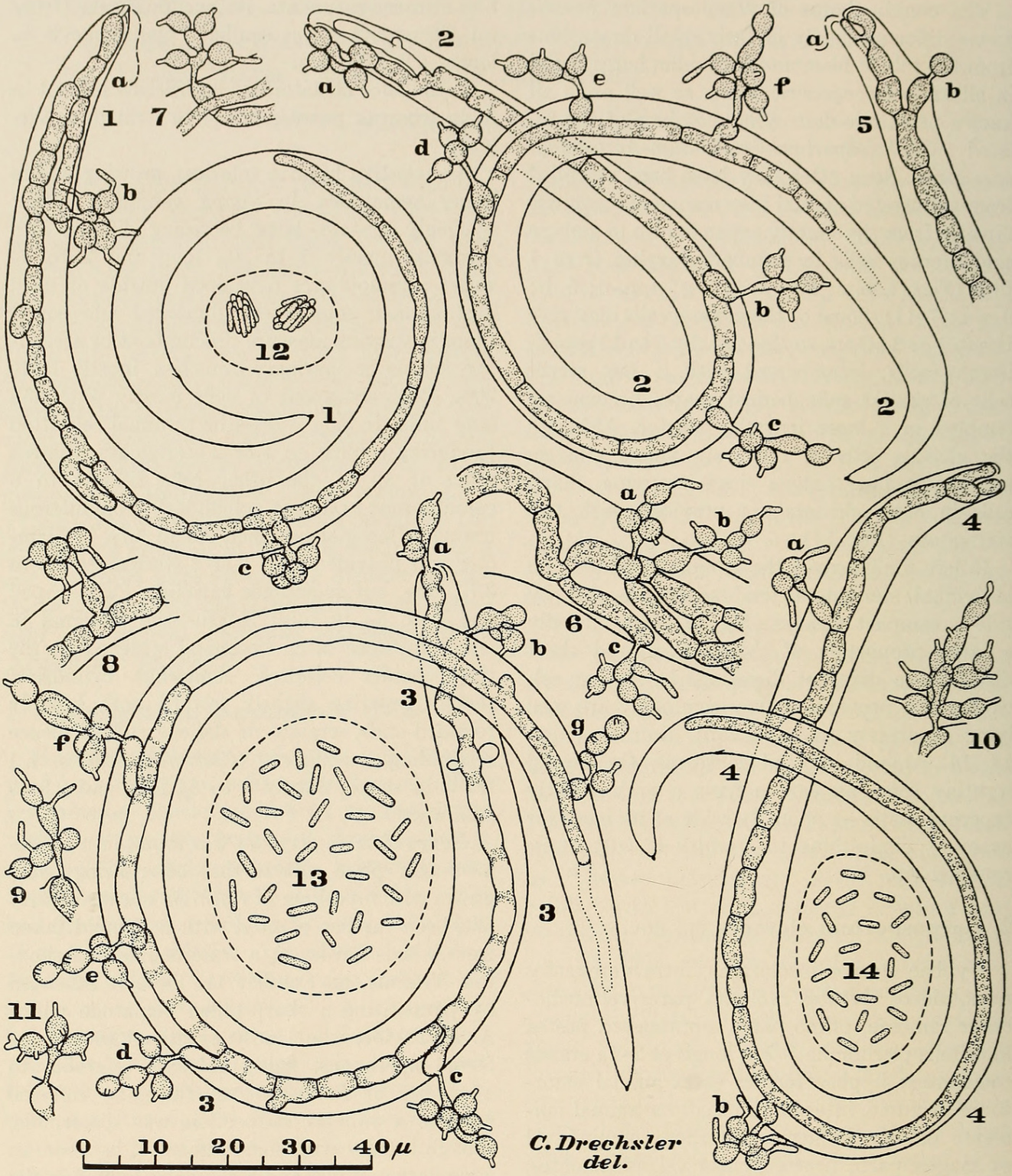
2. **Harposporium sicyodes** sp. nov.

Hyphae steriles incoloratae, intra vermiculos nematoideos viventes evolutae, parce vel mediocriter ramosae, primo parce septatae sed postea in cellulis plerumque 5–20μ longis et 2–4μ crassis constantes; hyphae fertiles extra animal emortuum evolutae, interdum in materia animal ambiente omnino immersae interdum omnino vel ex magna parte procumbentes vel ascendentes, in axe simplices vel ramosae, saepius 10–200μ longae, in cellulis plerumque 4–25μ longis et 2–3.5μ latis constantes, cellula terminalis vulgo conidia ex 1–2 sterigmatibus gignens, aliae cellulae saepius 1–6 ramulos conidiferos ferentes; ramuli conidiferi saepissime globosi sed interdum lageniformes, plerumque 2.8–3.7μ crassi, 1–2 sterigmatibus 1.2–3.7μ longis et 0.6–0.8μ latis praediti, in toto vulgo 4–8μ longi; conidia incolorata, cylindrata, recta vel leviter curvata, basi et apice rotundata, ita cucumiformia (fructui *Cucumeris sativi* similia), plerumque 3–5μ longa, 0.9–1.2μ lata.

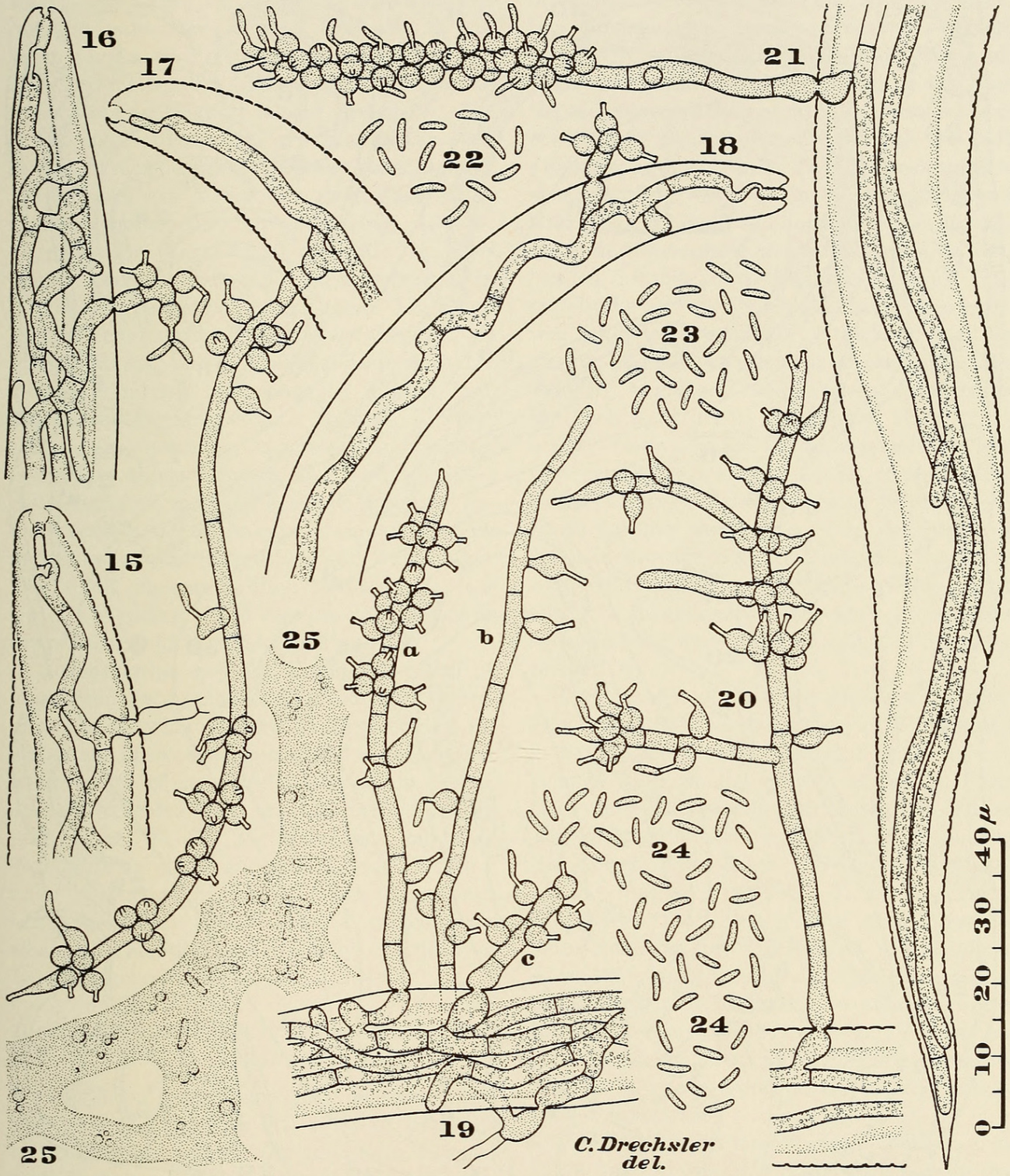
Vermiculos nematoideos interficiens habitat in foliis arborum putrescentibus in Arlington, Virginia.

Assimilative hyphae colorless, growing within living nematodes, in young condition rather sparingly septate, later becoming divided into segments mostly 5 to 20μ long and 2 to 4μ wide; conidiophores developed outside of dead host animal, sometimes submerged and sometimes in varying measure procumbent or ascending, simple or sparingly branched, mostly 10 to 200μ long, composed of cells mostly 4 to 25μ long and 2 to 3.5μ wide—the terminal cell often producing conidia on 1 or 2 sterigmata whereas some or all of the other cells bear 1 to 6 conidiiferous branches (phialides); conidiiferous branch often globose, then 2.8 to 3.7μ in diameter and provided with 1 or 2 sterigmata 1.2 to 3.7μ long, but sometimes variously flask-shaped and 4 to 8μ in total length—the sterigma in either instance often becoming widened at the tip; conidia colorless, somewhat cylindrical though tapering slightly toward both broadly rounded ends, straight or slightly curved, hence resembling cucumber (*Cucumis sativus* L.) fruits in shape, mostly 3 to 5μ long and 0.9 to 1.2μ wide.

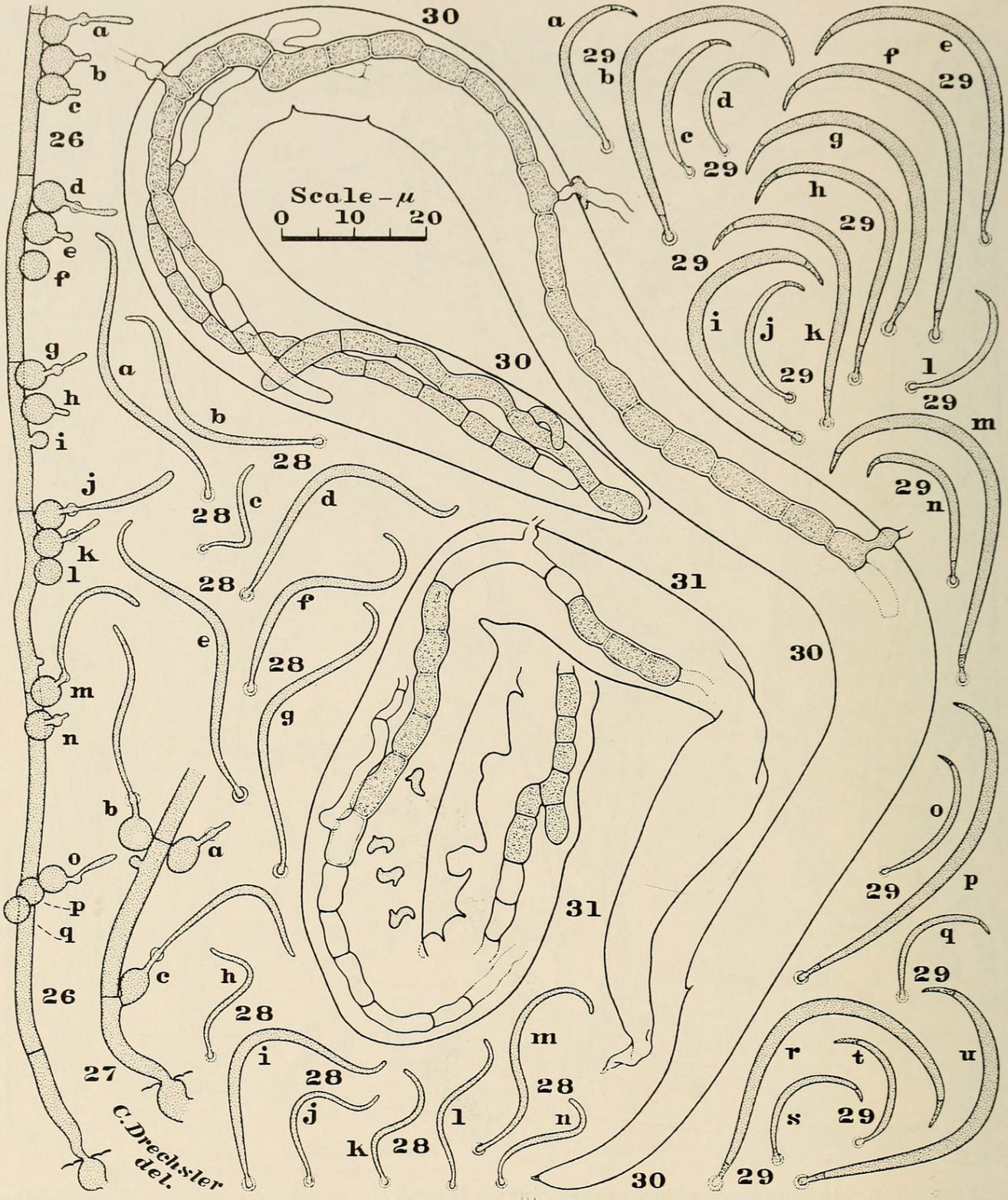
Harposporium sicyodes was found in a maize-meal-agar plate culture that after being overgrown with mycelium of *Pythium vexans* deBary had been further planted with leaf mold taken from woods bordering a watercourse in Arlington, Virginia, on October 11, 1958. It subsisted by parasitizing a sharp-tailed nematode which A. L. Taylor, who kindly examined several infected specimens, held probably referable to a species of *Panagrobelus*. In many infected animals a tubular connection was discernible, though usually with some difficulty, between an assimilative hypha and a conidium lodged in the stoma (Figs. 15–18). Some parasitized eelworms showed one or two ellipsoidal bodies lodged within the oesophagus 20 to 50μ from the anterior end. These bodies may have been swollen infective condia but owing to their deeply imbedded positions the presence of hyphal connections with any assimilative hypha could be neither established nor disproved. Invasion of the eelworm was accompanied often by pronounced withdrawal of the musculature from the



FIGS. 1–14.—*Harposporium baculiforme* from leaf mold gathered in Colorado, partly near Steamboat Springs (1, 4, 5, 8, 13) and partly near Durango (2, 3, 6, 7, 9–12, 14), drawn to a uniform magnification with the aid of a camera lucida, × 1000: 1, eelworm (*Plectus* sp.) with infecting conidium, a, in its stoma, and with 2 conidiophores, a and b; 2, infected eelworm (*Plectus* sp.) with 6 conidiophores, a–f; 3, infected eelworm (*Plectus* sp.) with 7 conidiophores, a–g; 4, infected eelworm (*Plectus* sp.) with 2 conidiophores, a and b; 5, forward portion of eelworm (*Plectus* sp.) showing origin of assimilative hypha from conidium, a, lodged in stoma, and a young conidiophore, b; 6, middle portion of infected eelworm (*Plectus* sp.) with branched assimilative mycelium and 3 conidiophores, a–c; 7–11, conidiophores; 12, conidia cohering in sheaves; 13, 14, two assortments of conidia, each assortment being from a separate infected eelworm.



Figs. 15–25.—*Harposporium sicyodes* developing parasitically on an eelworm (probably *Panagrobelus* sp.), drawn to a uniform magnification with the aid of a camera lucida, × 1000: 15, forward portion of infected eelworm showing origin of assimilative mycelium from conidium lodged in stoma; 16–18, forward portions of 3 infected eelworms, each showing an assimilative hypha connected with a conidiophore and with a conidium lodged in the stoma; 19, median portion of infected eelworm with 3 conidiophores, a–c; 20, middle portion of infected eelworm with a branched conidiophore; 21, posterior portion of a large eelworm showing a simple conidiophore; 22–24, three assortments of conidia, each assortment being taken from a separate eelworm; 25, eight conidia ingested by a proteomyxan rhizopod (probably *Leptomyxa reticulata*).



FIGS. 26, 27.—Proximal portions of two conidiophores of *Harposporium helicoides* showing, respectively, 17 conidiiferous cells, a–q, and 3 conidiiferous cells, a–c, at different stages of development, × 1,000. FIG. 28.—Detached conidia of *H. helicoides*, a–n, × 1,000. FIG. 29.—Detached conidia of *H. oxycoracum*, a–u, × 1000. FIG. 30.—Indurated hyphae of *H. diceraeum* within integument of an eelworm (*Plectus* sp.) in a culture 65 days old, × 1,000. FIG. 31. Portions of indurated hyphae of *H. diceraeum* that have given rise within integument of an eelworm to 4 new conidia about 45 days after production of conidia on external conidiophores had ceased in the 65-day-old Petri-plate culture, × 1,000.

integument (Figs. 15, 16, 19, 20, 21). Development of assimilative hyphae, especially in posterior regions of large eelworm hosts (Fig. 21), appeared rather less abundant than in nematodes attacked by most related parasites.

The conidiophores of *Harposporium sicyodes*, like those of all congeneric species other than *H. baculiforme*, are moderately stout filamentous hyphae whether they are short (Figs. 16; 18; 19, c) or long (Figs. 17; 19, a, b; 20; 21). They usually remain simple, yet axial branching (Fig. 20) is not exceptional among them. Their unicellular conidiiferous branches, or phialides, are mostly shaped like a Florence flask, with the globose main part being abruptly distinct from the narrow sterigma arising from it. In more than a few instances, however, the distended main part tapers upward and merges with a distal sterigma, so that the conidiiferous cell appears transitional to the type of phialide familiar in species of *Cephalosporium* and *Verticillium*. The slight distension often noticeable at the tip of a sterigma would seem to come about in the abscission of the first conidium.

Harposporium sicyodes, like nearly all of its known congeners, conveniently continues to produce conidia while exposed to microscopical examination in an agar slab under a cover glass. Assortments of its conidia formed on conidiophores extended from separate individual animals (Figs. 22, 23, 24) show only moderate variability in shape and size. In my culture a large proportion of the detached spores were being constantly ingested by a proteomyxan rhizopod (Fig. 25) provisionally identified as *Leptomyxa reticulata* Goodey (1914).

SUPPLEMENTARY OBSERVATIONS ON THREE OTHER SPECIES OF HARPOSPORIUM

Several maize-meal-agar plate cultures which had been planted with leaf mold of the same collection that yielded *Harposporium sicyodes* permitted abundant development of *H. helicoides* Drechsler (1941). Occasion was taken to study more closely the manner in which the phialides of the latter species give rise to conidia (Figs. 26, a–q; 27, a–c). The individual phialide originates as a wart-like protuberance (Fig. 26, i) that continues to grow until it reaches a diameter of 3.5 to 4.5μ (Fig. 26, f, l, p, q). It then puts forth a sterigma usually 0.7 or 0.8μ wide (Fig. 26, c, h), which soon appears to swell distally in forming a terminal globular knob often about 1.5μ in width (Fig. 26, b, e). From this globular knob is now extended a slender outgrowth, approximately 0.5μ wide, whose tubular membrane is clearly continuous not with the globular contour but with a somewhat narrowing tube passing centrally through the knob (Figs. 26, a, d, g, k, n, o; 27, a). The outgrowth elongates first with gradually increasing and later with gradually diminishing width, at the same time describing a helicoid spiral of left-handed rotation (Figs. 26, j, m; 27, b, c). Ultimately the helicoid filament is cut off by a cross-wall at the lower boundary of the knob and then readily becomes detached as a conidium (Fig. 28, a–n). Manifestly the peripheral substance of the knob is of plastic consistency, since in detached spores it covers the basal membrane, and thus persists as a minute deposit of mucus that clothes the very slightly widening proximal end. In all cultures the conidia of *H. helicoides* are given to pronounced variations with respect to size, some measuring as much as 48μ in length and 1.7μ in greatest width, while others have corresponding dimensions of only 20μ and 0.7μ, respectively.

The conidia of *Harposporium oxycoracum* Drechsler (1941) resemble those of *H. helicoides* in having the slightly expanded basal end enveloped in a small quantity of mucus. They often show considerably greater dimensions than were assigned to them in the original account of the species. Thus, among the conidia produced by the fungus (Fig. 29, a–u) in Petri plate cultures that had been planted with forest detritus collected near Beltsville, Maryland, in April 1958, some measured no less than 60μ in total length and fully 2.3μ in greatest width. Moreover, in many of the larger conidia not only the tip but also a proximal portion, usually 2 to 5μ in length, appeared to be filled solidly with wall material.

The lot of forest detritus from southern Colorado that yielded *Harposporium baculiforme* supplied also some development of *H. diceraeum* Drechsler (1941) in several Petri plate cultures. Destruction of the nematode (*Plectus* sp.) parasitized by *H. diceraeum* was apparently completed about 20 days after the cultures had been prepared. When the cultures were 65 days old all external conidiophores of the fungus, together with all the conidia they had borne, had disappeared, but within the integuments of many parasitized animals could be seen variable por-

tions of somewhat indurated living mycelium (Fig. 30). Some of the integuments loosely inclosed a few living conidia of *H. diceraeum* (Fig. 31), which in each instance must have been recently formed on a small conidiophore extended from a single indurated segment. As the hyphal segments here had undergone only slight thickening of their walls and showed only faint yellowish coloration they seemed less strongly modified for endurance than the chlamydospores always produced within eelworms destroyed by *H. anguillulae*.

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GRAPHICAL DIAGNOSIS OF INTERLABORATORY TESTS

A simple way to analyze the discrepancies between different testing laboratories that presumably use the same test procedure was recently worked out at the National Bureau of Standards.[1] Devised by W. J. Youden of the NBS applied mathematics laboratory, the method employs a graphical presentation of the test data which allows each laboratory to tell at a glance how its performance compares with that of others. The graph can point the way to corrective action to eliminate the bias, if any, in the technique used by a particular laboratory; or it may indicate the need for an improved test procedure—one that lends itself better to uniform application by all laboratories. In addition, it provides an estimate of the precision of the test-procedure results.

DISCREPANCIES, NORMAL AND ABNORMAL

Duplication of tests by two or more laboratories is constantly being undertaken in science and industry in order to verify results, to detect systematic errors, and in general to monitor the techniques of measurement. Sooner or later all important results in the physical sciences are checked by other laboratories. In industry, the same quality-control tests may be used by the various plants of a single company and—perhaps alternatively—the same acceptance tests are performed by laboratories at different depots of the purchaser.

In all such cases, discrepancies in the results from different laboratories are not only expected, but inevitable. It is basic to all measurement processes (except those of the crudest sort or those involving only simple counting) that when the same procedure is repeatedly carried out on the same specimen with the same equipment and personnel, the results are not all identical but are scattered over a certain range of values. When the same measurements are made by a number of different laboratories, using nominally identical equipment, the scatter is even greater. In any case, the more precise the procedure, the narrower the range of scatter.

However, when the scatter is unusually large or when a particular laboratory differs markedly from most of the others, something must be wrong. The problem—which the present method of analysis is intended to help solve—is to determine just where the difficulty lies. The difficulty may be due to a number of factors, some of the most important being (1) intrinsic lack of precision in the procedure; (2) faulty technique in carring out the procedure; (3) ambiguity or vagueness in the formulation of the procedure,

[1] For further technical details see the following articles by W. J. Youden: *Presentation for action,* Ind. and Eng. *Chem.* **50:** 91A. Oct. 1958; *Circumstances alter cases, ibid.* **50:** 77a. Dec. 1958; *What is a measurement? ibid.* **51:** Feb. 1959.