Conidial propulsion in Meristacrum asterospermum.

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With Plate XV.

In the original account of *Meristacrum asterospermum* Drechsler (1940) the conidia were set forth as becoming detached spontaneously soon after they attain full development, but it was left uncertain whether their detachment comes about, as usually in the more familiar insectivorous *Entomophthoraceae,* through some kind of forcible propulsion. In trying to determine whether forcible propulsion occurs in the fungus, I had placed vaseline-coated glass slides horizontally at a height of 1 millimeter over areas where groups of its conidiophores arose from the agar in nematode-infested Petri-plate cultures. After the slides had remained in such position for 6—8 hours they were closely examined under a microscope. Despite diligent search conidia of *M. asterospermum* were never seen adhering to the slides. That my negative findings might have little significance later became evident when experience with various other *Entomophthoraceae,* especially with readily culturable members of the family, showed that the procedure used was unsuitable for detecting the relatively feeble propulsion accompanying release of the smaller asexual spores originating from conidia either through multiplicative reproduction or through prolonged repetitional development.

As *Meristacrum asterospermum* has been encountered only rarely, occasion was taken to study anew its manner of conidial release when the fungus came to light in a Petri plate of maize-meal agar that after being overgrown with *Pythium* mycelium had been planted with a pinch of leaf mold gathered under elm (*Ulmus* sp.) trees near Denison, Iowa, on August 20, 1959. In this culture it developed on only one individual eelworm, which had an unusually wide stoma (fig. 1, a; 2) and a slender tail (fig. 1, e; 6). When first seen the fungus had extended from the dead animal 4 conidiophores (fig. 1, a—d) readily recognizable by their helically contorted, noticeably widened, slopingly ascending terminations. Spore development had not begun, as none of the conidiophores showed either cross-walls or lateral protrusions. In order that sporulation might proceed without exposure to air movements or excessively strong light, the culture, after brief microscopical examination under a dry objective, was placed in a secluded nook and tightly covered with a small bell jar.

After 5 hours the culture was again examined. Spores were now being produced on 3 conidiophores, which, as a result, presented a decrepit appearance. Fully a dozed conidia were observed on the substratum, lying in haphazardly scattered positions rather than in positions directly under the individual conidiophores. This scattered distribution gave satisfactory evidence that conidial adjunction here had come about through forcible propulsion, not in passive response to gravity. Several of the more remote detached conidia lay at horizontal distances of 50—65 μ from the nearest decrepit-looking conidiophore. Since in the sporogenous distal portion each conidiophore was elevated 20—30 μ above the substratum the propulsion operative here must be considered very weak in comparison with the powerful discharge manifest in the wide halo surrounding a house fly that has succumbed to attack by ***Entomophthora* (*Empusa*) *muscae*** (Cohn) Fresenius (1856).

To permit microscopical examination at higher magnification a cover-glass was carefully laid over the scanty material of ***Meristacrum asterospermum.*** Of the 4 conidiophores (shown separately in fig. 2—5 but corresponding, respectively, to parts a—d in fig. 1) one (fig. 3) had undergone rather little development during the 5 hours; its distended distal portion still showed helicoid convolvement with rotation like that of a left-handed screw, though at the tip this spiral modification was becoming obliterated by the budding forth of 3 young spores. Despite the disorderly appearance which the other 3 conidiophores (fig. 2, 4, 5) had acquired in their production of spores, they displayed an adequate sequence of developmental stages. One conidiophore segment showed a young conidium being budded forth laterally (fig. 4, a). Several conidia (fig. 4, b, c; 5, a) were being produced on primary conidia that apparently had aborted while still attached. A newly delimited conidium of such virtually secondary origin (fig. 4, d) clearly showed the centrally up-curved basal septum protruding into its interior. A septum similarly arched upward was also found proximally delimiting a primary conidium (fig. 4, e) newly cut off from an axial segment of the conidiophore. Both of the basal septa became gradually everted within 20 minutes after the cover glass had been placed on the material, so that in each instance the conidium (fig. 4, f) became rounded at its proximal end. When afterwards close scrutiny could be given to several other full-grown conidia (fig. 2, a, b; 4, g, h; 5, b—g) that were still attached to the conidiophore the basal end in each was likewise found convexly rounded. A rounded proximal end was observed in all detached conidia (fig. 7; 8, a) lying scattered on the substratum. At least one of these newly abjointed spores had extended an outgrowth on whose very slender tip a secondary conidium (fig. 8, b) was being formed.

As the secondary conidia that develop singly on slender pedicels are delimited proximally by an ordinary partition, they presumably are adapted to become detached passively. However, the more numerous primary conidia that are borne on conidiophores and become delimited by a dome-shaped septum, whether from an axial segment or from an abortive conidium or a distended lateral cell (fig. 2, c; 4, i), are obviously adapted for springing off, under normal conditions, through sudden eversion of the basal septum. In their basal delimitation and somewhat feeble self-propulsion, and also, indeed, in their shape and dimensions, they rather closely resemble the microconidia whose development on detached conidia of *Delacroxia coronata* (Cost.) Sacc. & Sydow emend. Gallaud (1905) through plural simultaneous budding was first described by Costantin (1897). By developing in fairly close array and in numbers varying commonly from 5 to 15 — their numbers, rather than their dimensions, varying with the size of the mycelial segment below — they further resemble the microconidia of *D. coronata* in providing a display of multiplicative reproduction approximately equivalent, it is believed, to that accompanying the development of a sporangium. The correspondence between the conidia of *Meristacrum asterospermum* and the microconidia of *D. coronata* extends to the microconidia produced by *Conidiobolus brefeldianus* Couch (1939) as well as to the microconidia of the congeneric species I described (Drechsler 1955 b, 1956) under the binomials *C. polytocus, C. chlamydosporus* and *C. megalotocus.*

The discovery that the conidia of *Meristacrum asterospermum* are forcibly abjointed through sudden eversion of their basal membrane diminishes somewhat the considerable parallelism of this fungus with *Ballocephala sphaerospora* Drechsler (1951), an entomophthoraceous form destructive to a species of tardigrade (*Macrobiotus* sp.). Since it was first encountered, *B. sphaerospora* has come to light several times, having developed in 3 maize-meal-agar plate cultures planted with leaf mold taken from woods near Beltsville, Maryland, early in March 1951, and also in a Petri plate culture to which had been added a small quantity of leaf mold collected near Park Falls, Wisconsin, on November 18, 1954. The strains from Maryland and Wisconsin agreed well with the strain obtained from Pennsylvania material in 1950, though in instances where relatively large host animals had succumbed to attack, the conidiophores of the parasite were found erupting not only from the lateral portions of the cuticle but also from the wide dorsal regions. Measurements of especially well-developed reproductive parts disclosed some maximum dimensional values that exceed the corresponding values given in the diagnosis of the species. Among the more robust propulsive branches were found a few that had attained a length of 7.5 μ or a greatest width of 5 μ. Some globose conidia were found that reached a diameter of 5 μ, and some large zygospores had a length of 22 μ or a greatest width of 17.5 μ. No further knowledge was gained concerning the forcible discharge of the conidia, for the meager membranous shreds clinging to some detached spores gave no clues indicating how the bursting of the propulsive cell may have begun or may have progressed. Since the globose conidia, as might be expected from their delimitation by a flat partition, underwent no noticeable change in shape during their disjunction and flight, the conclusion that they are wholly passive projectiles was abundantly confirmed. In being propelled without change of shape — or, more particularly, without sudden extension of a basal papilla — through violent bursting of the supporting cell, they resemble the campaniform conidia of *Entomophthora* (*Empusa*) *muscae* and *Entomophthora* (*Empusa*) *culicis* (A. Braun) Fresenius (1858). It seems probable, besides, that the conidia of *B. sphaerospora,* like those of the 2 insectivorous fungi, acquire their manifestly strong adhesiveness in becoming coated with the colorless liquid by which they are forcibly propelled. In contrast, when a conidium of *M. asterospermum* springs off, the membrane of the parent cell is not ruptured and no liquid cellular contents are squirted out to envelop the spore. With respect to conidial disjunction *M. asterospermum* finds considerable parallelism, among related insectivorous forms, with *Entomophthora* (Empusa) *sphaerosperma* Fresenius (1856, 1858) as set forth by Sawyer (1931), and would seem in general agreement also with *Completoria complens* Lohde (1874), judging from the account Leitgeb (1881) gives of that parasite on fern prothallia.

Interpretation of the conidia in *Meristacrum asterospermum* as being equivalent to sporangiospores is encouraged by their resemblance to the microconidia produced plurally in related genera through multiplicative development of large conidia. Although in their origin on propulsive cells the conidia of *Ballocephala sphaerospora* differ markedly from any microconidia hitherto reported, they appear interpretable as sporangiospores partly from parallelism with the conidia of *M. asterospermum* and partly by virtue of their small size and their multiple production on hyphal shafts arising singly from assimilative segments of only moderate volume. Under ordinary microscopical inspection the asexual spores of *M. asterospermum* and *B. sphaerospora,* as well as all known microconidia, appear surrounded by single walls. Recognition of only one surrounding wall was earlier (Olive, 1906; Sawyer, 1931) held sufficient to dismiss any likelihood that a conidium of an entomophthoraceous fungus might represent a one-spored sporangium. However, in more recent treatments of the *Mucorales* (Hesseltine, 1955; Benjamin, 1959) conidia with only one wall are accepted as equivalent to one-spored sporangiola in all members of ihe *Cunninghamellaceae,* and it seems probable that similar tolerance may be required in construing the conidia formed in the *Helicocephalidaceae,* a family recently established by Boedijn (1958). Moreover, in sporangia of *Basidiobolus,* which result from internal division of globose conidia or elongated conidia or hyphal segments (Drechsler, 1955 a, 1958), the sporangial envelope and the portions of spore wall laid down in contact with it are not distinguishable as separate membranes as long as they remain in contact.

References

Benjamin, R. K., 1959. The merosporangiferous *Mucorales*. Aliso *4*: 321—433.

Boedijn. K. B., 1958. Notes on the *Mucorales* of Indonesia. Sydowia *12*: 321—362.

Costantin, J., 1897. Sur une Entomophthorée nouvelle. Bull. Soc. Mycol. France *13*: 38—43, pl. 4, 5.

Couch, J. N., 1939. A new *Conidiobolus* with sexual reproduction. Amer. Journ. Bot. *26*: 119—130.

Drechsler, C., 1940. Three fungi destructive to free-living terricolous nematodes. Journ. Washington (D.C.) Acad. Sci. *30*: 240—254.

— 1951. An entomophthoraceous tardigrade parasite producing small conidia on propulsive cells in spicate heads. Bull. Torrey Bot. Club *78*: 183—200.

— 1955 a. A southern *Basidiobolus* forming many sporangia from globose and from elongated adhesive conidia. Journ. Washington (D.C.) Acad. Sci. *45*: 49—56.

— 1955 b. Two new species of *Conidiobolus* that produce microconidia. Amer. Journ. Bot. *42*: 793—802.

— 1956. Two new species of *Conidiobolus*. Amer. Journ. Bot. *43*: 778—786.

— 1958. Formation of sporangia from conidia and hyphal segments in an Indonesian *Basidiobolus*. Amer. Journ. Bot. *45*: 632—638.

Fresenius, G., 1856. Notiz, Insekten-Pilze betreffend. Bot. Ztg. *14*: 882—883.

— 1858. Über die Pilzgattung *Entomophthora*. Abhandl. Senckenb. naturf. Ges. *2*: 201—210, pl. IX.

Gallaud, I., 1905. Études sur une Entomophthorée saprophyte. Ann. Sci. Nat. Bot. IX. *1*: 101—134.

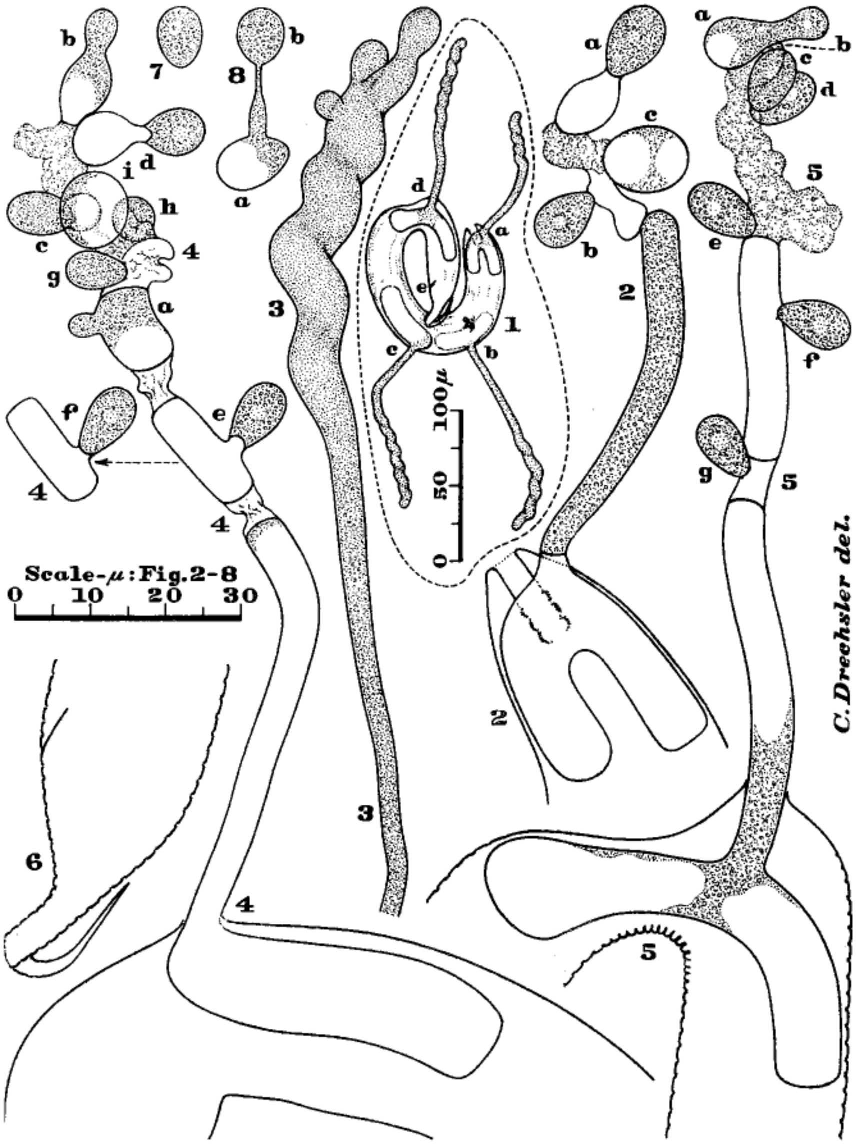
Hesseltine, C. W., 1955. Genera of *Mucorales* with notes on their synonymy. Mycologia *47*: 344—363.

Leitgeb, H., 1881. *Completoria complens* Lohde, ein in Farnprothallien schmarotzender Pilz. Sitzungsb. K. Akad. Wiss. Wien *84*: 288—324, 1 pl.

Lohde, G., 1874. Über einige neue parasitische Pilze. Tagebl. Versamml. deutsch. Naturf. u. Aerzte *47*: 203—206.

Olive, E. W., 1906. Cytological studies on the *Entomophthoreae* II. Nuclear and cell division of *Empusa*. Bot. Gaz. *41*: 229—261, pl. XVI.

Sawyer, W. H., 1931. Studies on the morphology and development of an insect-destroying fungus, *Entomophthora sphaerosperma*. Mycologia *23*: 411—432, pl. 30, 31.



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Explanation of Plate XV

Fig. 1. Dead nematode containing hyphal segments of *Meristacrum asterospermum* which have extended 4 conidiophores, a—d, with helicoid terminations; e, posterior end of animal; × 200. Fig. 2. Same segment and conidiophore as in Fig. 1, a, but drawn 6½ hours later; a, conidium produced on an abortive conidium; b, full-grown primary conidium; c, distended cell; × 1000. Fig. 3. Same conidiophore as in Fig. 1, b, but drawn 6¼ hours later; × 1000. Fig. 4. Same segment and conidiophore as in Fig. 1, c, but drawn 5 hours later; a—c, conidia in successively later stages of growth; d, e, newly delimited conidia; f, same as e but drawn 20 minutes later; g, h, full-grown conidia; i, distended cell; × 1000. Fig. 5. Same segment and conidiophore as in Fig. 1, d, but drawn 7½ hours later; a, abortive conidium budding forth a new conidium; b—g, full-grown conidia; × 1000. Fig. 6. Same as Fig. 1, e; × 1000. Fig. 7. Normally abjointed conidium; × 1000. Fig. 8. Normally abjointed conidium, a, producing a secondary, conidium, b; × 1000.