TWO SPECIES OF CONIDIOBOLUS WITH MINUTELY RIDGED ZYGOSPORES.

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Through a procedure set forth in an earlier paper (Drechsler, 1952a) many isolations of entomophthoraceous fungi growing well on maize-meal agar were obtained from several samples of decaying plant detritus collected in southern Louisiana on December 20, 1952. About one-fourth of the isolations appear referable to the ubiquitous Delacroixia coronata (Cost.) Saccardo and Sydow (1899). A somewhat smaller number manifestly belong to the species of Basidiobolus (probably my B. haptoporus) with zygospores of undulate profile that occurs widely and abundantly in leaf mold (Drechsler, 1952b). Among the remaining isolations more than a dozen clearly belong to two species of Conidiobolus distinguished from all the congenic forms by their ready production of handsomely ridged zygospores. Although the figures of sexual spores given by Brefeld (1884, pl. 5, fig. 30–33, 38–44) in the account of his C. utriculosus likewise show a serrate profile the sculpturing in that fungus is set forth as being minutely verrucose (Brefeld, 1884, p. 58; pl. 5, fig. 38b) rather than ridged. In any case as the sexual spores of C. utriculosus vary from 60 to 100μ in diameter their linear dimensions would seem about three times greater than those of the ridged zygospores produced in my cultures. The two Louisiana species accordingly are described herein as new members of the genus, one under an epithet derived in part from a word (μρηζογεμ) meaning “wrinkled” and the other under an epithet (επιμελια) meaning “odorous.”

1. Conidiobolus rhysosphorus Drechsler sp. nov.
Mycelium incoloratum; hyphis sterilibus medioriter ramosis, 1.8–12μ (plerumque 4–11μ) crassatis, mox septatis, hic illic disjunctis, cellulis eorum protoplasmati disperse granulosis et saepius valde vacuolati repletis; hyphis fertilibus primiformibus incoloratis, simplicibus, erectis vel aecidi-bus, in aerem vulgo 40–100μ ad lucem prodedentibus, basi 5–8μ crassis, sursum saepe aliquid latescentibus, apice vulgo 7–12μ crassis, ibi unum conidium formae globosae ferentibus; conidis formae globosae violenter prolihintibus, incoloratis, basi papilla vulgo 3–7μ alta et 7–12μ lata praeditis, plerumque ex toto 22–36μ longis, 18–30μ latis, in parte parietem juxta vulgo hyalinis sed in parte mediae saepe granulosi; conidis formae elongato-ellipsoidae incoloratis, saepius 12–32μ longis, 6.5–16μ latis, in apice hyphae fertilibis gracilibis singulatim oriundis; hyphis fertilibus gracilibus incoloratis, 100–250μ albis, basi 2–4.5μ crassis, sursum leniter attenuatis, apice 1.2–2.2μ crassis; zygospora interdum e copulatione cellularum aliqua atque aliqua hyphae ortis interdum e copulatione durum cellularum ejusdem hyphae oriundis, saepe flavidulis, globosis, 13–31μ (vulgo 20–25μ) crassis, levibus vel speciosae rugosis, muro simplici vel duplici plerumque 1.2–2.5μ crasso circumdatae.
Habitat in materiis plantarum putrescentibus prope Laplace, Louisiana.

Mycelium colorless; assimilative hyphae moderately branched, 1.8–12μ (mostly 4–11μ) wide, soon

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Fig. 1-25. *Conidiobolus rhizosporus* as found in maize-mal agar cultures; ×1000.—Fig. 1-3. Stout conidiophores bearing globose conidia.—Fig. 4-17. Detached globose conidia.—Fig. 18-20. Conidia in two stages of receptacular development.—Fig. 21, 22. Conidia germinating on agar already permeated with mycelium of fungus.—Fig. 23. Hypha with 2 non-adjacent segments, a and b, that are ready to conjugate by union of their terminally swollen branches, c and d.—Fig. 24. Same, 45 min. later; the 2 segments, a and b, now nearly empty from migration of contents into the enlargement c.—Fig. 25. Same, after another interval of 45 min.; young zygospore now full-grown.
becoming septate, in some places becoming disjunct, the delimited segments usually containing dispersedly granular and rather strongly vacuolated protoplasm. Primary conidiophores colorless, unbranched, erect or inclined, extending 40–100μ into the air and toward the main source of light, 5–6μ thick at the base, widening gradually upward, commonly 7–12μ thick near the tip, forming terminally a single globose conidium; globose conidia springing off forcibly, colorless, measuring 18–30μ in width and 22–36μ in total length inclusive of a basal papilla 3–7μ high and 7–12μ wide, containing usually a central granular protoplasmic mass that is surrounded by a nearly clear parietal layer; ellipsoidal conidia colorless, commonly 12–32μ long, 6.5–16μ wide, formed solitary on slender conidiophores from which they become detached passively; these slender conidiophores colorless, usually unbranched, 100–250μ high, 2–4μ wide at the base, tapering gradually upward, 1.2–2.2μ wide at the tip. Zygospores arising sometimes from conjugation between 2 segments of separate hyphae and sometimes from conjugation between 2 segments of the same hypha, in most instances distinctly yellowish, usually subspherical, 13–31μ (commonly 20–25μ) in diameter; the zygospore wall usually 1.2–2.5μ thick, in some instances smooth but rather generally bearing ridges nearly parallel to the equator, commonly consisting of 2 layers that are usually separated only in a small area but in some few instances are everywhere separated.

Occurring in decaying plant materials near LaPlace, Louisiana, Specimens (microscope mounts and dried agar cultures) have been deposited in the Mycological Collections, Plant Industry Station, Beltsville, Maryland.

When a mycelium of Conidiobolus rhysosporus grows unimpeded in a Petri plate of maize-meal agar the hyphae at the advancing margin commonly are 8–12μ wide. In these hyphae the terminal segment, which usually shows most active growth, is ordinarily 300–750μ long. At its tip for a distance of about 100μ it is rather generally filled with protoplasm through which small granules are scattered singly or in small groups. Vacuoles appear farther back in the terminal segment, and extensive vacuolation occurs in segments toward the rear, which commonly are 50–300μ long. A branch given off near the tip of a main hypha may make its way to the forefront of the mycelium and then may develop as a robust filament parallel to its parent. Branches given off at some distance from the advancing margin are usually soon halted in their growth. They generally remain narrower than the parent hyphae, their definitive width, as a rule, varying between 4 and 8μ.

The conidiophores (fig. 1–3) produced in young cultures of Conidiobolus rhysosporus would seem of moderate dimensions when compared with the corresponding structures of the several other members of the genus. The conidia they hold aloft commonly appear slightly flattened into an oblate ellipsoidal shape (fig. 1–3). This flattening is in many instances still recognizable after the conidia, through sudden eversion of the upper layer of the arched basal septum, have sprung off forcibly. Rather commonly in detached conidia of the present species (fig. 4–17) the protruding basal papilla formed by the everted membrane gradually becomes more and more distended, so that in one or two hours the general shape of the spore may change almost beyond recognition. On a fresh agar substrate a newly detached conidium usually puts forth a germ hypha that develops into a mycelium. If a conidium falls on an agar substrate already overgrown and permeated by the fungus it may give rise to a stout conidiophore (fig. 18) soon surmounted by a secondary globose conidium (fig. 19, 20), or it may extend a rather narrow germ hypha (fig. 21, 22) between the filaments of the occupying mycelium.

Sexual reproduction takes place freely in maize-meal agar cultures. In instances where two well separated segments (fig. 23, a, b) of a mycelial hypha are preparing to conjugate, each segment gives off a branch. The two branches grow toward one another and on meeting each swells out abruptly at its tip (fig. 23, c, d). Through solution of the walls in the broad region of contact the 2 swellings merge into a single globose enlargement (fig. 24, c) which continues to expand as it receives protoplasmic materials from both sides (fig. 24, a, b). When the 2 segments and their branches have contributed all their living contents the globose enlargement is delimited as a young zygospore (fig. 25). Conjugation between two adjacent segments presents an appearance hardly more distinctive than chlamydospore formation, though internally the same events occur here—evacuation of protoplasm from both segments into a globose enlargement, deposition of successive retaining walls (fig. 26, 27), and delimitation of the enlargement as a young zygospore (fig. 28). Conjugation between segments (fig. 29, a, b) of separate hyphae is accomplished through production of paired branches with terminal knobs (fig. 29, c, d), fusion of the juxtaposed knobs (fig. 30), progressive evacuation of the segments (fig. 31, a, b) as their contents migrate into the enlargement (fig. 31, c) at the place of union, and delimitation of the enlargement as a young zygospore (fig. 32). The hyphal membranes of the two segments and their branches vanish from sight piecemeal, successive portions commonly evanescent within 15 or 20 minutes after they have been emptied. Although in many instances the conjugating segments have branches of varying length, formation of the young zygospore is usually completed in 2 hours.

The internal reorganization concerned in the process of ripening commonly requires a period several times as long. In cultures prepared with maize-meal agar, a medium apparently no less favorable for sexual than for asexual reproduction, the mature zygospore as a rule is surrounded by a
Fig. 26-52. Conidiobolus rhysosporus; ×1000.—Fig. 26-28. Successive late stages in conjugation between 2 adjacent hyphal segments.—Fig. 29. Two segments, a and b, of separate hyphae, which are about to conjugate by union of their terminally swollen branches, c and d.—Fig. 30. Portion of same about 10 min. later, showing terminal swellings fused.—Fig. 31. Same after additional 40 min.; segments a and b now nearly empty from migration of contents into enlargement c.—Fig. 32. Same after additional 15 min.; young zygospore now walled off.—Fig. 33-50. Zygospores showing variations in size, shape, and wall structure.—Fig. 51. Narrow hyphal segments, a and b, in agar slant culture 85 days old.—Fig. 52. Small chlamydospores, a-q, in agar slant culture 85 days old.
distinctly yellowish wall with minute ridges which for the most part follow circular courses nearly parallel to the equator (fig. 33-43). It seems possible that the handsomely rippled condition here results from puckering of a thin outer layer when this layer becomes shrunk on a thicker inner layer of smaller compass, for in some few zygospores where two layers are everywhere spatially separated (fig. 44) the outer contour is always smooth. A smooth outer contour is found likewise in some zygospores where the two wall layers are only partially separated (fig. 45-48). Partial separation is observable more especially in the polar regions, near the protuberances marking the places where the zygospore was attached. Undersized zygospores of irregular shape (fig. 49, 50) commonly appear surrounded by a smooth, relatively thin wall consisting of a single layer. As in related species the fully mature sexual spore shows internally a large spherical reserve globule within a parietal layer of nearly homogeneous protoplasm. The presence of several reserve globules would seem indicative usually of a slightly immature state (fig. 33, 34).

Long-continued repetitional development of conidia results naturally in reduced dimensions. After 2 or 3 months maize-meal agar cultures of Conidiobolus rhysosporus usually show numerous percurrent or submerged hyphal segments only 1.6-2.μ wide (fig. 51, a, b). Associated with these narrow filaments are thick-walled, ovoid or plate elliptoidal bodies (fig. 52, a-q), generally 8-15μ long and 5-12μ wide, which may conveniently be designated as chlamydospores since they appear to be formed both in intercalary and terminal positions. Like the chlamydospores of Conidiobolus adiaeucus Drechsler (193a) they are produced more abundantly under the surface of the substratum than on the surface.

In maize-meal-agar plate cultures 2-3 months old the zygospores of Conidiobolus rhysosporus germinate freely by giving rise to globose conidia. The reserve globule in the sexual spore gradually decreases in size as its substance becomes intermingled with the parietal protoplasm. Almost at the same time the inner layer of the zygospore wall disappears (fig. 53). The protoplasm now pushes against a small area of the outer layer (fig. 54). As the outer layer gives way a protrusion of the protoplasm emerges. This protrusion thereupon elongates externally as a germ hypha (fig. 55-57) which in some instances widens out (fig. 57) to develop distally into a stout conidiophore and in others ramifies in the substratum before extending a conidiophorous branch into the air (fig. 55). After the single globose conidium produced from each zygospore has sprung off, all the emptied tubular membranes soon evanesce. However, the yellow outer layer of the zygospore wall (fig. 58-61), which now always shows a smooth unrippled outline, remains visible for many days.

Old cultures in which many zygospores have germinated commonly show elongate ellipsoidal conidia being held aloft singly on slender conidiophores arising from empty subspherical envelopes (fig. 62-71). These envelopes represent presumably the membranes of globose conidia, though in general the protuberance modifying their outward contour seems considerably smaller and narrower than might be expected from the usual dimensions of basal papillae. Sometimes a slender conidiophore is found to have originated from the empty envelope of an elongate ellipsoidal conidium (fig. 72). The elongate spores here (fig. 73-83), much like those of Conidiobolus heterosporus Drechsler (1935b), become detached passively. As the elongate spores of C. heterosporus are formed in young cultures of that species soon after globose primary conidia have been produced in some quantity, it seems curious that elongated secondary conidia have not appeared likewise in young cultures of C. rhysosporus. Perhaps their production in C. rhysosporus depends on special environmental conditions more usually present in old than in young cultures. On the other hand it is also possible that in C. rhysosporus the development of elongate conidia may have a relationship to zygospore germination much like the relationship apparent in the two species of Basidiobolus widely occurring in leaf mold (Drechsler 1932b). In those two species elongated secondary conidia, each provided with an adhesive apical beak, are formed in old cultures soon after zygospores have germinated by the production of globose primary conidia. Although the hyphal segments of the two fungi show only a single nucleus except when preparing to divide, the elongated adhesive conidia very commonly show two nuclei in positions near the middle; and they seem to maintain their binucleate state—a state recognizable in unstained living specimens—through numerous generations of repetitional development, despite pronounced reduction in size. An analogous connection between zygospores, globose conidia, and elongate conidia could obviously exist in C. rhysosporus where all three spore types are present. The absence of zygospores in C. heterosporus might well be expected to imply a somewhat different relation between globose and elongate conidia in that species.

2. CONIDIOBOLUS OSMODES Drechsler sp. nov. Mycelium incolaratum, saepe benzeni hexachloridum redolens itaque édore multis speciebus Streptomyces etiam ligamentum speciebus Basidiobolii simile; hyphis sterilibus ramosis, plurunque 4-12μ crassis, oxo septatis, hie illic disjunctis, protoplasmatis crasse granulosis repletis; hyphis fertilibus incolaritis, simplicibus, erectis vel acclivibus, in aerem vulgo 30-60μ ad lucem protrudentibus, interdum medio aliqual infatis, ibi 9-15μ crassis, apice vulgo 7-10μ crassis, unum conidium fereidentis; conidii violenter abollentibus, incolaritis, aliqual obovoides, hae papilla 2.5μ alta et 7-10μ lata praeditis, plurumque ex toto 25-35μ longis, 22-30μ latis, in parte parietem juxta vulgo hyaliniis,
Fig. 53-88. *Conidiobolus rhizophorus* as found in maize-meal-agar plate cultures 80–90 days old; ×1000.—Fig. 53, 54. Zygospores in early stages of germination. Fig. 55–57. Zygospores whose germ hyphae are developing into stout conidiophores.—Fig. 58–61. Empty zygospore envelopes visible after germination.—Fig. 62–71. Empty globose envelopes, each of which has put forth a slender conidiophore bearing an elongated ellipsoidal conidium.—Fig. 72. Empty envelope of elongated conidium that has put forth a slender conidiophore bearing a secondary elongated conidium.—Fig. 73–88. Detached elongated ellipsoidal conidia showing variations in size and shape.
in parte media saepe glebarum protoplasmatis conglutinatarum repleta; zygosporis interdum e copulatione cellularum aliae atque aliae hyphae ortis interdum e copulatione durorum cellularum ejusdem hyphae oriundis, flavidulis, plerumque globosis vel elongato-ellipsoidis, 13–37 μ (saepe 25–30 μ) crassis, quonque levis sed saepius speciosae rugosis, muro simplici vel duplici plerumque 2–6.5 μ crasso circumdata.

Habitat in materiis plantarum putrescentibus prope Laplace, Louisiana.

Mycelium colorless, commonly giving off the smell associated with benzene hexachloride and thereby resembling with respect to odor many species of Streptomyces and some species of Basidiobolus; assimilative hyphae branched, mostly 4–12 μ wide, soon becoming divided at moderate intervals by crosswalls, here and there becoming disjointed or disconnected, filled mainly with coarsely granular protoplasm. Conidiophores, colorless, unbranched, the aerial part erect or inclined, commonly projecting 30–60 μ into the air and toward the main source of light, sometimes noticeably distended near the middle, usually 9–15 μ in greatest width, 7–10 μ wide at the tip on which a single conidium is borne; conidia springing off forcibly, colorless, somewhat obvoid, provided at the base with a papilla 2–5 μ high and 7–10 μ wide proximally, usually 25–37 μ in total length and 22–30 μ in width, showing internally a parietal layer of clear protoplasm and an irregular central mass of conglutinated lumps. Zygospores resulting from conjugation between two segments of separate hyphae or between two segments of the same hypha, yellowish, mostly globose or elongate ellipsoid, 13–37 μ (commonly 25–30 μ) in diameter; the zygospore wall in some instances smooth but much more usually ornamented externally with minute ridges nearly parallel to the equator, mostly 2–6.5 μ thick, commonly composed of two layers which may be wholly adnate or partially separated or wholly separated—the interstitial space in many instances being partly occupied by granular deposits.

Fig. 89–122. Conidiobolus osmades as found in maize-veal-agar plate culture 3–6 days old; drawn at uniform magnification with the aid of a camera lucida; X500 throughout.—Fig. 89. Young conidiophore.—Fig. 90–92. Conidiophores in late stages of development.—Fig. 93. Conidiophore with conidium, a, in late stage of formation; b, same conidium 4 min. later, after its release.—Fig. 94. Conidiophore with conidium: a, conidium ready for release; b, conidium after release in a moist covered mount.—Fig. 95–112. Conidia soon after springing off, showing variations in size and shape.—Fig. 113. Conidium germinating by production of a germ hypha.—Fig. 114–122. Conidia in different stages of repetitional development.
Occurring in decaying plant detritus near Laplace, Louisiana. Specimens (microscope mounts and dried agar cultures) have been deposited in Mycological Collections, Plant Industry Station, Beltsville, Maryland.

The odor given off strongly when Conidiobolus osmodes is actively growing on maize-meal agar seems indistinguishable from the "musty" or "earthy" odor very familiar to plant pathologists and microbiologists dealing with species of Streptomycetes. It is likewise indistinguishable from the odor emitted from cultures of the widespread leaf mold Basidiobolus producing zygospores of undulate profile (Drechsler, 1952b, 1953c) and from cultures of the congeneric fungus distributed as Basidiobolus ranarum Eiland by the Centraalbureau voor Schimmelcultures at Baarn, Netherlands. Further, it resembles very closely the odor that is characteristic of commercial benzene hexachloride and that appears to be associated with the unpleasant flavor detectable in some plant products originating from fields where the insecticide has been used injudiciously. For the present it is not known to what extent C. osmodes and the two species of Basidiobolus may, like some species of Streptomycetes (Waksman, 1950, p. 148, 190), be responsible for imparting disagreeable flavors and odors to foodstuffs.

In Conidiobolus osmodes as in all known congeneric species except C. adiaceus the individual conidiophores originate from separate hyphal segments. They differ perceptibly from the conidiophores of C. rhyosporus in usually being widest near the middle of the aerial termination (fig. 89-93) rather than near the tip. The globose spores they bear aloft show some tendency toward a prolate ellipsoidal shape rather than toward an oblate ellipsoidal shape as in C. rhyosporus. Consequently when its upcurved basal membrane (fig. 93, a; 94, a) is suddenly everted (fig. 93, b; 94, b) the

Fig. 123-150. Conidiobolus osmodes as found in maize-meal-agar plate cultures 4-12 days old; drawn at a uniform magnification with the aid of a camera lucida; ×500 throughout.—Fig. 123. Two paired segments, a and b, of different hyphae; broad contact of lateral spore showing readiness for conjugation.—Fig. 124, 125. Same, 60 min. and 80 min., later, respectively; showing progressive emptying of the segments, a and b, owing to continued transfer of contents into the fusion enlargement, c.—Fig. 126, 127. Two sexual reproductive units in each of which the paired segments, a and b, in different hyphae have supplied most of their contents to the enlargement, c.—Fig. 128. Same reproductive unit as in fig. 127, but 35 min. later; the paired segments, a and b, now empty; the enlargement, c, now walled off as a young zygospore.—Fig. 129. Adjacent hyphal segments in late stage of conjugation.—Fig. 130. Young zygospore resulting from union of 2 adjacent segments.—Fig. 131. Adjacent hyphal segments from whose union a zygospore is being formed laterally.—Fig. 132-150. Mature zygospores, showing differences in size, shape, and wall structure.
propelled conidium (fig. 95–112) acquires a papilla that usually merges more gradually with the general contour of the spore than the papilla in *C. rhyosporus*. A moist substratum a conidium of *C. osmodes* readily puts forth a germ hypha (fig. 113), which may develop into a mycelium or after some prominent growth may elongate ascendingly to produce aloft a secondary globose conidium (fig. 114). More usually, however, secondary conidia are formed individually on a short stout outgrowth extended from the upper surface of a detached primary conidium (fig. 115–121). In instances where the proximal portion of the stout outgrowth is found extended procumbently (fig. 122) the primary conidium may have suffered a change of posture after germination was well advanced.

Initial stages in the sexual reproduction of *Conidiobolus osmodes* are easily recognized where conjugation takes place between segments of separate hyphae (fig. 123–128: a, b). Each of the two segments puts forth a lateral spur or branch. The two spurs grow toward one another until their tips are in broad contact (fig. 123). Soon the apposed portions of membrane disappear and at the place of union a globose enlargement is formed as protoplasmic materials are received from both segments (fig. 124). Progressive evagination of the segments is marked by deposition of successive retaining walls (fig. 125–128). When the globose enlargement (fig. 124–127: c) has received all the contents of the paired segments it is delimited at both ends as a young zygospore (fig. 128c). Conjugation between adjacent segments of a hypha presents a less distinctive appearance. The young zygospore resulting from such conjugation usually is formed in axial alignment with the supporting hypha (fig. 129, 130), but in some instances it develops in a lateral position (fig. 131).

The mature zygospore in *Conidiobolus osmodes*, as in related species, shows a relatively large reserve globule somewhat eccentrically placed within a parietal protoplasmic layer of nearly homogenous consistency. It is distinguished more especially by the unusual thickness of its wall. Zygospores formed in a rich culture medium like lima-bean agar are commonly surrounded by an envelope 5–6μ thick, which may be smooth or nearly smooth externally, and may be wholly devoid of markings suggestive of a layered structure. In maize-meal agar cultures the thick wall is rather commonly ornamented with many minute ridges (fig. 132–138). A faint line of demarcation is usually noticeable whereby an outer layer is delimited from a thicker inner layer. Near the polar protruberances where the zygospore was attached the 2 layers are in many instances found separated. In some instances the 2 layers are separate virtually over the entire circumference. Extensive and rather wide separation of the layers is generally found only in large zygospores with a smooth unripped surface. Undersized and misshappen zygospores (fig. 139–150) are considerably more variable than globose full-sized ones with respect both to wall structures and to internal organization.

The zygospores of *Conidiobolus osmodes* have so far not been observed to germinate. In maize-meal agar cultures they remain in an unchanged resting state for months after the sexual spores of *C. rhyosporus* have given rise spontaneously to conidiophores and conidia. They seem to endure desiccation well, for when a little water is added to an agar culture that has been in an air-dry condition for several months their internal structure rarely shows evidence of injury or degeneration.

**SUMMARY**

Two readily culturable entomophthoraceous fungi found in decaying plant detritus from southern Louisiana are newly described under the binomials *Conidiobolus rhyosporus* and *C. osmodes*. In addition to globose conidia that spring forcibly from stout conidiophores, both species produce minutely ridged zygospores. The zygospores of *C. rhyosporus* germinate spontaneously in aging cultures by putting forth stout conidiophores with globose conidia. In aging cultures *C. rhyosporus* may give rise, besides, to elongate ellipsoidal secondary conidia on slender conidiophores. Like a species of *Basidiobolus* found in leaf mold *C. osmodes* gives off a strong musty or "earthy" odor similar to the odor associated with the unpleasant flavors sometimes imparted to foodstuffs by species of *Streptomycetes* and by the commercial insecticide benzene hexachloride.

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**LITERATURE CITED**


