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# THREE SPECIES OF PYTHIUM WITH PROLIFEROUS SPORANGIA

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#### INTRODUCTION

Some years ago I (6) published diagnoses of 15 new species of Pythium that had been isolated from diseased roots and other decaying plant structures. More recently (9, 10, 11) the diagnoses of 9 of these species have been supplemented with figures and discussion setting forth various details of morphology in a somewhat comparative manner. In the present paper supplementary comment will be given to 2 of the remaining species, namely P. oedochilum and P. palingenes, both of which were made known as proliferous forms closely related to P. helicoides Drechsl. To bring the distinctiveness of these forms into appropriate relief, occasion is taken to describe as new a congeneric species, which, though showing sporangial proliferation, is manifestly alien to the *helicoides* series. The figures illustrating sexual reproduction in the 3 fungi are given at a magnification (*i.e.*,  $\times$  1000) uniform with that of similar figures in previous papers. Owing to spatial necessities the zoosporangia and zoospores are shown in drawings reproduced at the lesser magnification  $(i.e., \times 500)$ , previously employed in illustrations of similarly rangy asexual apparatus.

#### PYTHIUM OEDOCHILUM

The description of *Pythium oedochilum* was based on 4 cultures isolated from separate portions of decaying tissue excised from the stem and larger roots of a dahlia (*Dahlia rosea* Cav.) plant found seriously affected with crown rot in the District of Columbia, late in August 1926. Two weeks of continuous wet weather had led to unusual prevalence of root rot in many cultivated and many wild herbaceous plants, much of the decay being due apparently to oomycetous parasites not frequently encountered under more normal conditions. The fungus came to light subsequently from another source, on being isolated in pure culture from discolored roots of 3 specimens of *Bidens aristosa* (Michx.) Britton, collected near Broad Run, Virginia, in September 1931.

Growing on maize-meal agar in plate cultures, *Pythium oedochilum* shows a rate of mycelial extension about median between that of the slowergrowing members of the genus, as, for example, *P. complens* Fischer, and that of the faster-growing members such as *P. ultimum* Trow and *P. butleri* Subr. Its hyphal branches, on coming in contact with the wall of a glass container, expand terminally to form bulbous or clavate appressoria (Fig. 1, A), which in most instances elongate into curved or slightly branched structures (Fig. 1, B, C). As the result, presumably, of functional frustration inevitable under the circumstances, the curved appressoria may further give rise to irregularly sinuous hyphal outgrowths (Fig. 1, D, E).

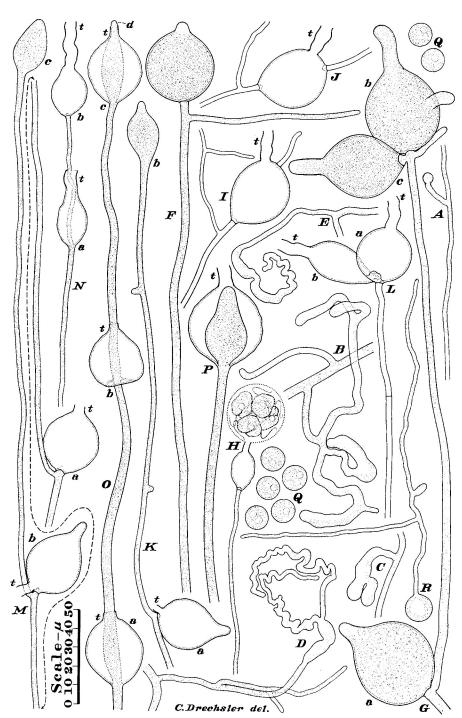


FIG. 1. Strain of *Pythium oedochilum* isolated from dahlia root, drawn with the aid of a camera lucida;  $\times 500$  throughout. A-E. Appressoria. F-Q. Asexual reproductive stage; t, evacuation tube. From lack of space M is shown in parts connected by a broken line.

Only rather scanty aerial mycelium is ordinarily formed on maize-meal agar in Petri dishes, though on the same medium in tubes the fungus produces a substantial cottony mat, which, like the cottony mat of *P. helicoides*, often persists for years without collapsing to the substratum.

Asexual reproduction may conveniently be induced in Pythium oedochilum by excising from a thinly poured Lima-bean-agar plate culture small slabs of medium well permeated with young mycelium, and transferring them to a shallow layer of water in a sterile Petri dish. Each tract of substratum thus placed under aquatic conditions soon extends into the surrounding water a fringe of filaments several millimeters in length. These submerged filaments show in their distal portions little of the racemose or corymbose branching characteristic of the submerse hyphae formed in irrigated preparations of P. helicoides. However, the sporangia which they produce terminally resemble those of P. helicoides rather closely, since, for the most part, they are subspherical, ovoid, or obovoid, and bear a papillate protrusion at the apex. In a stale preparation such a papillate sporangium may undergo no marked change for weeks, except that a central vacuole appears after a few days, and gradually increases in size with the passage of time. If staling is prevented by changing the water at intervals, the sporangial contents are discharged into a vesicle (Fig. 2, A, a), and in 20 to 25 minutes are transformed into biciliate zoospores wholly after the manner usual in the genus Pythium (Fig. 1, H; Fig. 2, A, b). In the meantime, the supporting hypha frequently resumes development to form a second sporangium within the empty envelope of the first (Fig. 2, A, a-d). Apart from instances of proliferation that result in a nested arrangement (Fig. 1, O, d; P; Fig. 2, E, b), uniaxial replication is accomplished also when the supporting hypha continues growth through the empty envelope as a filament and produces a subsequent sporangium externally, whether in a position sessile on the empty evacuation tube (Fig. 2, B, b) or in a more forward position (Fig. 1, N, b; O, b, c; Fig. 2, D, b, c). Sometimes, especially when dehiscence of a sporangium is delayed so that uniaxial prolongation of the supporting hypha cannot take place, this hypha grows out laterally below the sporangial septum and gives rise to a subsequent sporangium, either close by (Fig. 1, G, c; L, b) or farther on (Fig. 1, G, b; K, b; M, b, c; Fig. 2, C, b; D, b; E, c). Filaments bearing 3 or more sporangia may reveal only uniaxial prolongation (Fig. 1, O), or only subsporangial branching (Fig. 1, G; M), or they may show both types of proliferation (Fig. 2, D; E). Replicative development of either kind is found much less frequently associated with the sporangia that come to be produced, often rather abundantly, in intercalary (Fig. 1, J) or subterminal (Fig. 1, I; Fig. 2, F, a, b; G, b) as well as in terminal (Fig. 2, G, a) positions on the tracts of mycelium present in the agar medium before its transfer to water.

The relatively small apical protrusion, which, as has been stated, is usually put forth by the sporangium when it reaches definitive size (Fig. 1, F; Fig. 2, C, b; E, c), sometimes serves in dehiscence without much outward

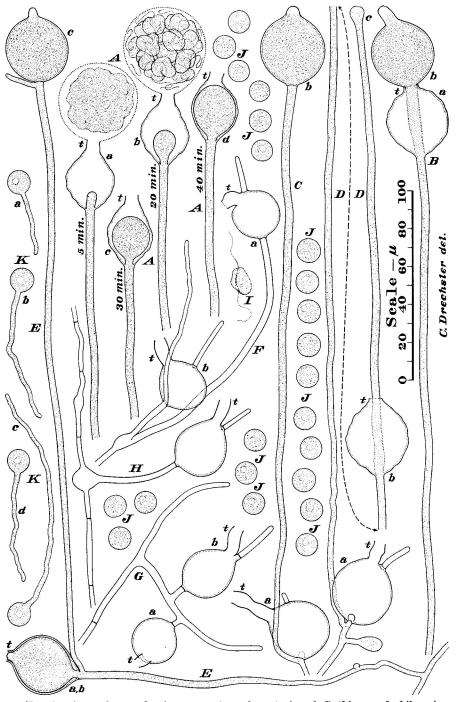


FIG. 2. As exual reproductive apparatus of a strain of Pythium oedochilum isolated from Bidens aristosa, drawn with the aid of a camera lucida;  $\times$  500 throughout; t, evacuation tube. From want of space D is shown in parts connected by a broken line.

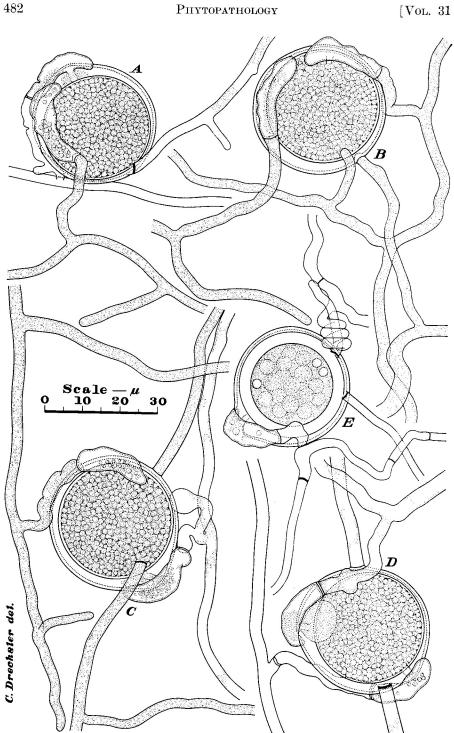


FIG. 3. Sexual reproductive apparatus of a strain of Pythium oedochilum isolated from dahlia root, drawn with aid of a camera lucida;  $\times 1000$  throughout.

change; the empty sporangial envelope in such instances showing only a rudimentary collar (Fig. 2, B, a; D, b). More often, however, the protrusion grows out into a cylindrical process (Fig. 1, G, a, c; Fig. 2, B, b) before it yields at its tip to permit escape of the protoplasmic contents; the emptied sporangial envelope in these more numerous instances bearing an evacuation tube of appreciable length (Fig. 1, H; L, a, b; N, a, b; O, a, b, c; P). When an apical papilla or apical process suffers functional frustration, a tube put forth from some other position (Fig. 1, G, b) operates in the discharge of the sporangia more often discharge through an evacuation tube arising from a forward position than through one arising from an equatorial or proximal position (Fig. 1, I; J; Fig. 2, C, a; D, a; F, a, b; G, a, b; H).

After being liberated by disintegration of the vesicle wherein they are formed, the laterally biciliate zoospores (Fig. 2, I) swim about for some time, and then round up into subspherical cysts (Fig. 1, Q; Fig. 2, J). These cysts germinate by producing usually a single germ tube (Fig. 1, R; Fig. 2, K, a-d).

Sexual reproduction of Pythium oedochilum takes place abundantly in maize-meal agar cultures and with very little degeneration. The oogonia. spherical in shape, often are formed terminally on hyphae of variable lengths (Fig. 3, A; B; Fig. 4, A-C; E-H; I; Fig. 5, A; B; D; F), though, often again, they are found in intercalary (Fig. 3, D; C; Fig. 5, C) or subterminal (Fig. 3, E; Fig. 4, D) positions. They are supplied mostly with 1, 2, or 3 elongated, somewhat expanded antheridia that are borne terminally on rather sturdy hyphal branches. Ordinarily, the mycelial connection between male and female organs, while necessarily present in cultures containing only a single thallus, is too remote to be traceable; yet monoclinous sexual units occur here and there (Fig. 4, E; Fig. 5, E; F), especially in irrigated preparations, where some extramatrical hyphal filaments may be so widely separated from their fellows that interaction is appreciably reduced. Spiral involvement of the oogonial hypha by an antheridial branch belonging to the same sexual unit-a most consistent feature of P. helicoides-occurs also in this species (8, Fig. 4, G), but only very rarely. Now and then an oogonial filament may be found enwrapped spirally by a hyphal branch not closely connected with any male organ (Fig. 3, E; Fig. 5, A); and, occasionally, helicoid involvement is observable where neither of the elements concerned has any close connection with a sexual structure (Fig. 5, G).

In Pythium oedochilum, as in P. helicoides, the elongated antheridium is applied lengthwise to the oogonium. Often, especially when only one male organ is present, the oogonium protrudes rather markedly along the line of contact, the protrusion being indented longitudinally to provide a groove into which the antheridium is partly countersunk. The fertilization tube comes from a median or slightly forward position on the antheridium, where this organ is set most deeply in the groove; so that the indented protuberance and the tube together frequently present a thick-lipped profile

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# $P{\rm hytopathology}$

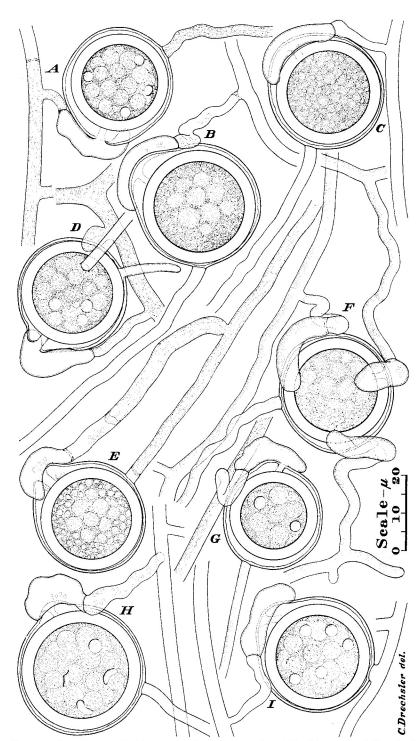


FIG. 4. Sexual reproductive apparatus of a strain of Pythium oedochilum isolated from dahlia root, drawn with aid of a camera lucida;  $\times 1000$  throughout.

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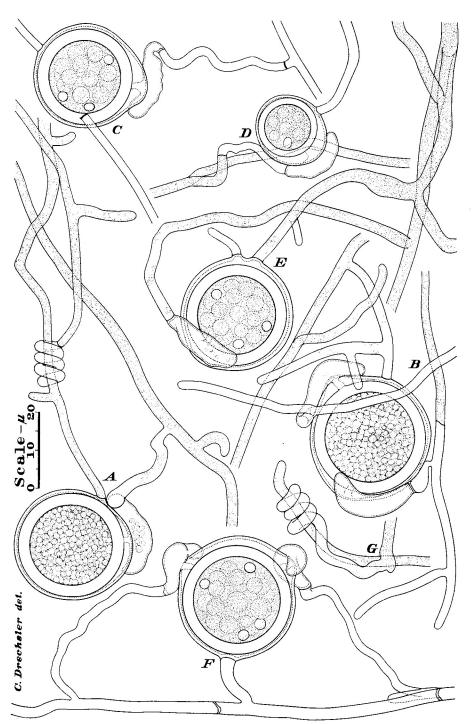


FIG. 5. Sexual reproductive apparatus of a strain of Pythium oedochilum isolated from *Bidens aristosa*, drawn with aid of a camera lucida;  $\times 1000$  throughout.

(Fig. 3, E; Fig. 4, D; E; H), which indeed suggested the specific name given to the fungus, a term compounded from two words meaning "to swell," and "lip," respectively. When fertilization has taken place the oogonial contents contract into a spherical body filled throughout with numerous globules of rather uniformly small size (Fig. 3, A–D). Soon this body becomes surrounded by a thick wall of its own (Fig. 5, A, B). As its globulose contents undergo further gradual conversion (Fig. 4, B; C; E; F) an oospore is brought into being that at early maturity contains 5 to 20 reserve globules and 3 to 4 smaller refringent bodies (Fig. 3, E; Fig. 4, A; D; G; H; I; Fig. 5, C–F). With additional maturation the refringent bodies often show some increase in number. The multiplicate internal structure of its oospores clearly marks the fungus as being closely related to *P. helicoides*.

The sexual apparatus of *Pythium oedochilum*, produced abundantly in maize-meal agar, is not prone to excessive variation with respect to its main dimensions. Thus the 200 measurements for diameter of oogonium, from which was computed the average of  $31.5 \,\mu$  given for this dimension in the diagnosis of the species, showed a distribution of values (expressed to the nearest integral number of microns) as follows: 19 μ, 1; 24 μ, 2; 25 μ, 2; 26 μ, 3;  $27 \mu$ , 9;  $28 \mu$ , 7;  $29 \mu$ , 7;  $30 \mu$ , 24;  $31 \mu$ , 34;  $32 \mu$ , 31;  $33 \mu$ , 26;  $34 \mu$ , 29;  $35 \mu$ , 13; 36  $\mu,$  8; 37  $\mu,$  3; 39  $\mu,$  1. And the 200 measurements for diameter of oospore, from which was computed the average of  $28.1 \,\mu$ , likewise given in the diagnosis, showed the following distribution of values:  $16 \mu$ , 1:  $21 \mu$ , 1:  $22 \ \mu, \ 2 \ ; \ 23 \ \mu, \ 5 \ ; \ 24 \ \mu, \ 9 \ ; \ 25 \ \mu, \ 9 \ ; \ 26 \ \mu, \ 12 \ ; \ 27 \ \mu, \ 29 \ ; \ 28 \ \mu, \ 35 \ ; \ 29 \ \mu, \ 40 \ ; \ 30 \ \mu, \ 40 \ ; \$ 24;  $31 \mu$ , 17;  $32 \mu$ , 9;  $33 \mu$ , 5;  $34 \mu$ , 2. As the strain from dahlia roots was the only one known when the species was described, the metric data then given were derived from cultures of that strain. Closely comparable distributions are shown in the sets of values obtained by measuring the oogonia and oospores in 200 units of sexual apparatus selected at random in a maizemeal agar culture of the strain isolated from Bidens aristosa. Here the values for diameter of oogonium, which gave an average of 31.9  $\mu$ , were distributed as follows:  $24 \mu$ , 1;  $26 \mu$ , 1;  $27 \mu$ , 1;  $28 \mu$ , 2;  $29 \mu$ , 5;  $30 \mu$ , 28;  $31 \mu$ , 42;  $32 \mu$ , 46;  $33 \mu$ , 34;  $34 \mu$ , 25;  $35 \mu$ , 9;  $36 \mu$ , 5;  $37 \mu$ , 1; while the values for diameter of oospore, from which an average of 28.1 u was computed, had the following distribution :  $19 \mu$ , 1;  $24 \mu$ , 4;  $25 \mu$ , 3;  $26 \mu$ , 14;  $27 \mu$ , 45;  $28 \mu$ , 53;  $29 \mu$ , 41;  $30 \mu$ , 26;  $31 \mu$ , 11;  $32 \mu$  1;  $33 \mu$ , 1. The 200 values for thickness of oospore wall obtained in the measurements made on the strain from B. aristosa gave an average of  $2.9 \,\mu$ , as compared with the average of  $2.5 \,\mu$ earlier computed from corresponding measurements of the dahlia strain. Apparently this rather marked dimensional difference is traceable to differences in cultural conditions rather than to morphological dissimilarity between the two strains.

## PYTHIUM PALINGENES

The description of *Pythium palingenes* was based on 2 cultures isolated from darkly discolored roots of a giant ragweed (*Ambrosia trifida* L.) plant

that was taken up from a moist roadside near Delaplane, Virginia, late in August 1926. Cultures of the fungus were obtained also from discolored roots of giant ragweed plants collected 3 weeks later near a watercourse in the District of Columbia. In both localities development of root-rotting parasites had been encouraged by a period of wet weather preceding the collection of the infected material.

Growing free of contaminating organisms, at a temperature of  $24^{\circ}$  C., Pythium palingenes extends its mycelium more rapidly than P. oedochilum, though somewhat less rapidly than P. helicoides. Its submerged mycelium in maize-meal agar cultures presents to the naked eye a diffuse appearance without noticeable luster; and, accordingly, reveals under the microscope promiscuous rather than parallel arrangement of hyphal parts. Since the vegetative filaments vary in width between 2 and 7  $\mu$ , the fungus is of approximately the same texture as the widely familiar P. debaryanum Hesse. On encountering the glass wall of a Petri dish the more delicate branches often give rise to terminal appressoria, clavate or knob-like in shape. Tracts of aerial mycelium often partly clothe the surface of the substratum in plate cultures, while in tube cultures, as a rule, the medium is wholly covered with a weft of cottony growth, which, under ordinarily favorable conditions for storage, persists for years without collapsing.

Material suitable for inducing abundant asexual reproduction in Pythium palingenes is readily obtained by growing the fungus on Lima-bean agar in thinly poured plate cultures. When slabs, well permeated with vigorously growing mycelium, are excised from such cultures and transferred aseptically to a sterile Petri dish containing a layer of water deep enough just to cover the medium sparingly, filaments often more than 1 or 2 mm. in length grow out into the water to produce terminally a subspherical or ovoid sporangium provided with an apical papilla. As the long filaments are largely devoid of branches in their distal portions, the primary sporangia here, like those of P. oedochilum, lack the corymbose or racemose arrangement frequent in P. helicoides. Accordingly, proliferous development here, much as in *P. oedochilum*, eventuates mainly in unilinear series. A sporangiferous filament, after growing through the empty envelope of one sporangium, produces another, either some distance onward (Fig. 6, A, a, b, c, d; B, a, b, f), or directly superimposed on the empty evacuation tube (Fig. 6, C, a, b). Very often, too, a new sporangium is formed within the empty envelope of an old one, so that a nested arrangement is brought about (Fig. 6, A, e; B, c, e, g; C, c; E, a, b, c; G, a, b). Sometimes, again, where dehiscence of a sporangium is delayed, the supporting hypha grows out below the delimiting septum to produce another sporangium, whether at a distance (Fig. 6, B, d) or close by (Fig. 6, D, a, b).

The dome-shape apical papilla, usually present on a sporangium when it attains definitive size, may function directly in dehiscence (Fig. 6, A, b; B. f; C, b; E, a), though often it grows out into an evacuation tube of some length before the sporangial contents are expelled into a vesicle formed at

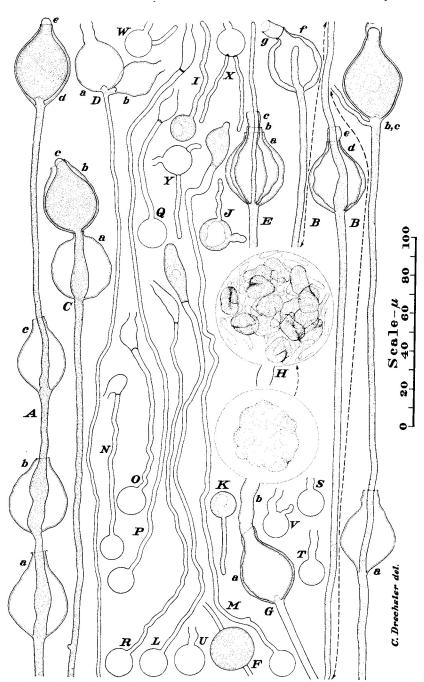


FIG. 6. As exual reproductive stage of *Pythium palingenes*, drawn to a uniform magnification from an irrigated Lima-bean-agar preparation with the aid of a camera lucida;  $\times 500$  throughout. From want of space B is shown in 3 parts connected by broken lines. H represents a more advanced stage of the vesicle shown in G.

its tip (Fig. 6, G, b). Within this vesicle the undifferentiated mass of protoplasm is fashioned into laterally biciliate zoospores wholly after the manner typical of Pythium (Fig. 6, H). On escaping from the vesicle the zoospores, among the largest known in the genus, swim about for a time before they come to rest and round up into cysts having an average diameter of 14 µ. The cysts may germinate vegetatively by 1 or 2 germ tubes (Fig. 6, I, J, K). However, in irrigated preparations, which through repeated renewal of water have been kept fresh as well as moderately free of dissolved nutrients, germination of zoospores often takes place by production of longish sporangiferous filaments whereon are borne miniature sporangia (Fig. 6, L, M) that give rise to single secondary zoospores, morphologically similar to the primary ones, though of smaller size. Besides the numerous empty units consisting individually of the membranous remains of zoospore, sporangiophore, and sporangium (Fig. 6, N-R), irrigated preparations often show numerous empty zoospore envelopes, each provided with a broad empty evacuation tube open at its tip (Fig. 6, S, T, U). Frequently these envelopes, which obviously testify to the development of an iterated swarming stage by emission of protoplasmic contents into a vesicle directly from the encysted zoospore, bear, in addition, 1 or 2 narrow empty processes, in some instances continuous with the cyst (Fig. 6, V), in other instances set off by a cross-wall (Fig. 6, W, X, Y). Presumably these processes represent membranous remains of short germ sporangiophores that became abortive when superseded by the successfully functional evacuation tubes. From the repeated emergence and renewed animation so freely displayed by its zoospores, the fungus was given a specific name defined in the familiar phrase "born again."

Subspherical bodies apparently homologous with zoosporangia are produced in rather large numbers on aerial mycelium overlying agar cultures of *Pythium palingenes*. For the most part they are borne terminally on delicate filaments or branches, though intercalary specimens are not rare (Fig. 6, F). When borne terminally they are usually provided with a small apical papilla. Despite this modification for emission of contents, the bodies on being placed in water develop much more frequently by putting forth germ tubes than by discharging their protoplasmic materials into a vesicle for transformation into zoospores.

Sexual reproduction takes place abundantly and with very little degeneration when *Pythium palingenes* is grown in pure culture on maize-meal agar containing a small quantity of finely divided maizemeal. The subspherical oogonia are often borne terminally, either on relatively long hyphae (Fig. 7, A; Fig. 8, A, C, E) or on shorter branches (Fig. 7, B; Fig. 8, D). Often, too, they arise as intercalary bodies; in many instances of such origin coming to have, when fully grown, a tangential or laterally sessile attachment (Fig. 7, C; Fig. 8, B). Lateral, as distinguished from mesial, attachment is similarly frequent with subterminal oogonia (Fig. 7, D). The antheridia are for the most part borne terminally on branches.

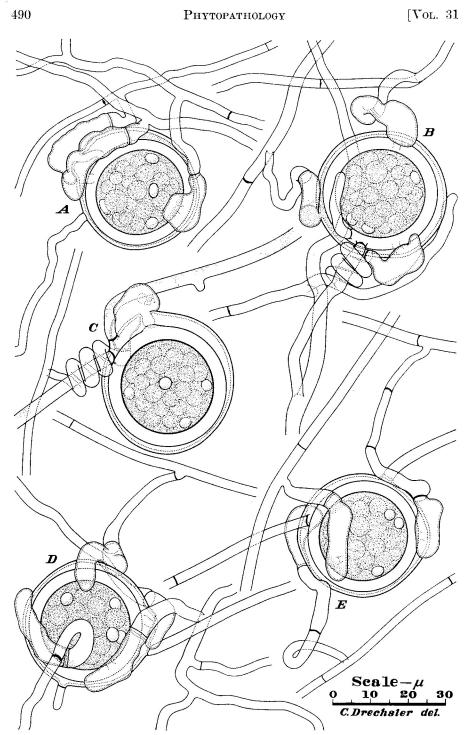


FIG. 7. Sexual reproductive apparatus of  $Pythium \ palingenes$  produced in maizemeal agar, and drawn with aid of a camera lucida;  $\times 1000$ .

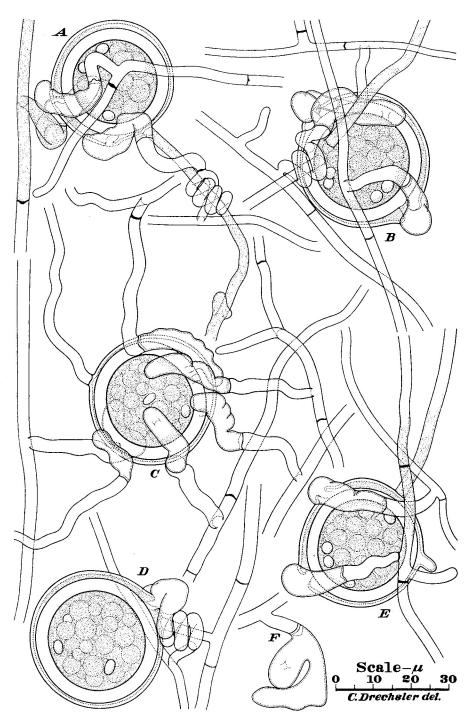


FIG. 8. Sexual reproductive apparatus of Pythium palingenes produced in maizemeal agar, and drawn with the aid of a camera lucida;  $\times 1000$ .

of variable lengths arising from hyphae having no close connection with the oogonial hypha. Although occasionally 6 (Fig. 8, C) and even 7 or 8 male organs have been found supplying an oogonium, the number of antheridia in units of sexual apparatus varies commonly from 1 to 4. Usually a proportion of sexual units varying from  $\frac{1}{4}$  to  $\frac{1}{2}$  show helicoid involvement of the oogonial hypha by an antheridial branch (Fig. 7, B, C; Fig. 8, B, D), or by a hyphal element closely connected with an antheridial branch (Fig. 8, A); the individual helical coil consisting ordinarily of 2 or 3 turns. As a rule the antheridia, which, like those of P. helicoides and P. oedochilum. are of an elongated cylindrical shape, though modified rather often by prominent dorsal undulation (Fig. 7, A; Fig. 8, C) or lobate branching (Fig. 8, F), are applied lengthwise to the oogonium. The fertilization tube usually arises from a position closer to the apex than to the base of the antheridium, and in occasional instances, where through irregularity of development the antheridium is applied only by its anterior end, may arise from a virtually apical position (Fig. 7, B).

The thick-walled, frequently yellowish oospores of Pythium palingenes have a multiplicate internal organization, since they reveal usually from 15 to 30 reserve globules and from 2 to 5 refringent bodies imbedded in a matrix of murkily granular protoplasm. Often the space between the oogonial membrane and the oospore wall is in large part filled with yellowish material, which sometimes appears to be of granular texture, or, again, may be of nearly homogeneous consistency (Fig. 7, A, B; Fig. 8, A, B, D, E). On the whole the variations in the main dimensions of oogonium and oospore may be considered moderate rather than extreme. As the values given in the original diagnosis for the diameters of these structures were based on sets of only 100 measurements, an equal number of additional measurements have since been made to make possible a metrical statement better comparable with metrical statements submitted for other species. An average of  $33.6\,\mu$  was computed from the combined 200 values for organial diameter, which, expressed to the nearest integral number of microns, show a distribution as follows:  $19 \mu$ , 1;  $20 \mu$ , 1;  $21 \mu$ , 2;  $23 \mu$ , 3;  $24 \mu$ , 5;  $25 \mu$ , 2;  $26 \ \mu, \ 1; \ 27 \ \mu, \ 1; \ 28 \ \mu, \ 1; \ 29 \ \mu, \ 4; \ 30 \ \mu, \ 6; \ 31 \ \mu, \ 10; \ 32 \ \mu, \ 15; \ 33 \ \mu, \ 24;$  $34 \mu$ , 30;  $35 \mu$ , 25;  $36 \mu$ , 28;  $37 \mu$ , 15;  $38 \mu$ , 14;  $39 \mu$ , 7;  $40 \mu$ , 4;  $41 \mu$ , 1. And the combined 200 values for diameter of oospore, from which an average of  $30.7 \,\mu$  was computed, show a distribution as follows:  $18 \,\mu$ , 2;  $19 \,\mu$ , 2; 20 μ, 1; 21 μ, 4; 22 μ, 3; 23 μ, 3; 24 μ, 2; 26 μ, 3; 27 μ, 5; 28 μ, 17; 29 μ, 17;  $30 \ \mu, \ 17; \ 31 \ \mu, \ 24; \ 32 \ \mu, \ 30; \ 33 \ \mu, \ 24; \ 34 \ \mu, \ 20; \ 35 \ \mu, \ 17; \ 36 \ \mu, \ 7; \ 37 \ \mu, \ 2.$ Through inclusion of the additional measurements the range in thickness of oospore wall was extended upward to a maximum of 3.8 u, while the arithmetical mean of this dimension was increased to  $2.8 \,\mu$ .

## A PROLIFEROUS SPECIES OF PYTHIUM WITH ASYMMETRICAL UTRIFORM SPORANGIA

Representatives of the genus Pythium were apparently first recorded

as occurring naturally on materials of the water-lily family by Petersen (19) in statements relating to the habitats of P. proliferum de Bary and P. undu-Working in Denmark, he observed the former species (or what latum Pet. he considered to be the former species) 3 times on the leaves and stems of old Nymphaea plants and on the decayed flower of a Nuphar plant; while the latter species of *Pythium* was encountered frequently on decaying leaves and stems of Nuphar luteum Sibth. and Smith and of Nymphaea alba Presl., though, occasionally, it was found also on old iris fruits and on submerged branches of trees. It seems not to have been Petersen's intention to attribute any pathological relationship to the 2 oomycetes, for, in discussing the biological circumstances attending the occurrence of submerse fungi, he expressed the view that aquatic plants—presumably aquatic types of the higher plants—are not attacked as long as they remain alive and are left undisturbed in their original site. Attack on plants partly dead he believed extended only to the dead parts.

From observations carried out during 2 seasons Dissmann (5) found that volunteer water-lily (Nymphaea candida Presl.) plants, growing abundantly in an artificial pond in marshy terrain near Hirschberg, Bohemia, showed yellowing of their leaves as early as May and June, although, ordinarily, foliar yellowing was not wont to occur until near the end of the growing season in autumn. The affected leaves soon turned brown and then became detached from the rhizome. On examining them with the naked eve they were found covered uniformly with a delicate filamentous nap, about 0.5 mm. in thickness, slimy to the touch. Microscopic inspection of this slimy nap disclosed a luxuriant hyphal growth and concomitant zoosporangia, which, from their manner of discharge, could for the most part be referred to the Sections of affected leaves showed the tissues to be thoroughly Pythiaceae. permeated with mycelial hyphae. Of the several oomycetes present in this material, 2 were consistently predominant. Studied in pure culture under varying conditions these 2 forms were identified with the 2 species previously recorded from water-lily materials by Peterson, namely, Pythium proliferum and P. undulatum. A relatively small proportion of affected leaves harbored, besides, 2 additional species of *Pythium*,—one of them from its production of spiny oogonia being held referable to P. artotrogus (Mont.) de Bary, whereas the other, with very minute oogonia, could not be identified with any species then described. The oldest portions of affected leaves, moreover, were regularly occupied by a saprolegniaceous form identified as Achyla americana Humph.

In our Northern States the foliage of some white water-lilies, including Nymphaea odorata Ait. and N. tuberosa Paine, often undergo premature decline from multiplication and extension of water-soaked or discolored areas. When portions of affected tissue are excised from such areas, pressed between sheets of absorbent paper to remove excessive water, and planted on agar media, Pythium mycelium almost always grows out very promptly. Mycelial growths thus obtained need only be freed of contaminating micro-

organisms to yield pure cultures. Among several hundred cultures derived through such procedure from material collected in Massachusetts, New York, and Wisconsin, a species closely similar to *P. helicoides*, and probably identical with Dissmann's *P. proliferum*, was abundantly represented; as was also a species that, judging from the large globose reproductive bodies produced by it in maize-meal agar, must almost certainly be the same as Dissmann's *P. undulatum*. Of less frequent occurrence, though present in more than a dozen cultures isolated from leaves of *N. tuberosa* collected near Butternut, Wis., in August 1938, was a species having an unusually distinctive asexual reproductive phase.

When grown on maize-meal agar the species in question extends its mycelium with a degree of rapidity moderate for members of the genus Pythium. The submerged mycelium produced in plate cultures is of a feebly lustrous, somewhat pronouncedly radiating appearance (Fig. 9, A). On microscopic examination it is seen to consist of relatively coarse axial hyphae, and relatively coarse branching systems; the more delicate ramifications in comparison with those of *P. periplocum*, for example, attaining only meager development. Swollen terminations, interpretable as appressoria, are produced very sparingly where the hyphal branches encounter the glass wall of a culture dish. Aerial mycelium ordinarily does not develop on maize-meal agar in Petri dishes, and often is likewise suppressed even in tube cultures.

As a general rule when the fungus is grown in pure culture on maizemeal agar or Lima-bean agar it gives rise to no zoosporangia whatever. Like other species of *Pythium*, it can be induced to reproduce asexually by transferring thin slabs of Lima-bean agar, well permeated with its mycelium, to a shallow layer of water. This treatment, however, usually results in only rather meager production of sporangia and in correspondingly meager development of zoospores. Yet, extraordinarily copious production of sporangia ensues without irrigation if several small pinches of leaf mold, or small quantities of other decaying vegetable detritus, are added to maizemeal agar cultures of the fungus; the efficacy of such amendment being especially marked if the medium employed is soft enough to afford a thin superficial film of free water. Often, within a few hours after the material has been added, zoosporangia will be found developing on the surface of the substratum in immediate proximity to the deposits of refuse. Their development and multiplication proceed actively for 5 to 10 days, spreading into more distant portions of the culture. However, the reproductive bodies are not distributed uniformly through the medium, being laid down for the most part in crowded arrangement along certain favored radials of the mycelium, mainly on the surface of the substratum, where they become visible collectively as flesh-colored radial streaks (Fig. 9, B).

On microscopic examination the more substantial of the streaky deposits are seen to consist of sporangia piled on one another several layers deep. Where the accumulations are somewhat less dense the individual sporangia 1941] DRECHSLER: THREE SPECIES OF PYTHIUM

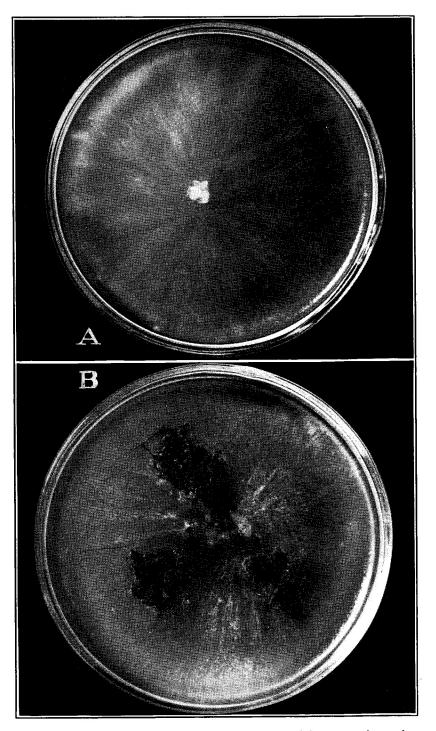


FIG. 9. Ten-day-old plate cultures of *Pythium marsipium* on maize-meal agar; natural size. A. No decaying plant material added,—sporangia absent. B. Leaf mold added after 4 days of growth,—innumerable sporangia present mainly in radial streaks.

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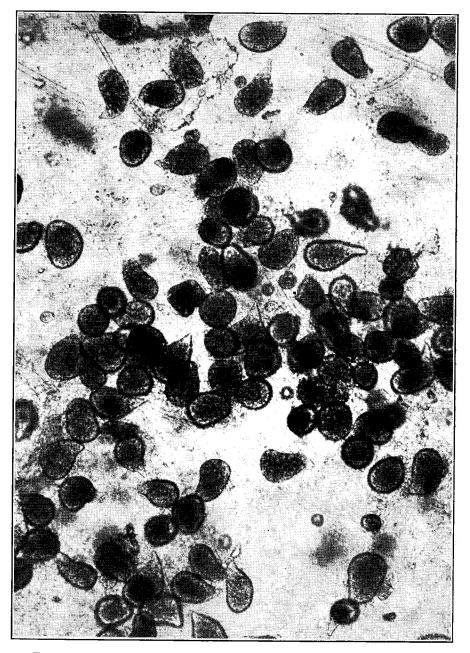


FIG. 10. Sporangia of Pythium marsipium in the plate culture shown in Fig. 9, B; about  $\times 200$ .

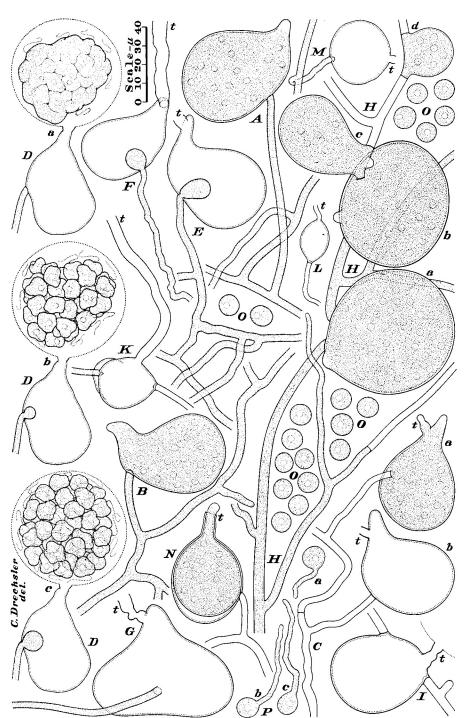


FIG. 11. As exual reproductive apparatus of *Pythium marsipium* originating in a maize-meal agar culture to which leaf mold had been added; drawn with aid of a camera lucida;  $\times$  500.

## Phytopathology

may be discerned as bodies of subspherical, ovoid, and saccate shapes (Fig. 10). A large proportion of the ovoid and saccate specimens are found to be attached laterally, so that their axes are directed athwart the axes of their supporting hyphae; the beak, moreover, being skewed rather sharply from the sporangial axis as if to prolong the supporting filament (Fig. 11, A. B). Comparable unsymmetry of shape, together with lateral attachment and transverse or oblique orientation, has long been known as a remarkable feature pertaining to sporangia of *Pythiogeton*, having been recorded for all 3 of the species—P. utriforme, P. transversum, and P. ramosum—described by von Minden (18) as initial members of the genus; though, it is true, the German investigator wisely decided not to recognize asymmetrical conformation of sporangia as a generic characteristic, after he had noted the presence in his material of spherical sporangia, as well as of sporangia intergrading between the spherical and the elongated asymmetrical types. Elongated asymmetrical sporangia I found to be produced also in my P. autossytum (7), again in association with spherical and intergrading specimens; while only subspherical and slightly ellipsoidal sporangia have been ascribed to P. uniforme Lund (16) and P. dichotomum Tokunaga (14), not to mention some unidentified congeneric species figured by Lund (16) and by Sparrow (22).Sporangia of regular ellipsoidal rather than utriform shape, yet with medio-lateral attachment and transverse orientation, have been set forth by Höhnk (12) in the description of his Diasporangium Jonesianum.

Although formation of zoospores in the fungus follows a course broadly similar to that usual for other species of Pythium, departures with respect to various details of development are observable. During the period immediately preceding discharge, the sporangium here does not reveal any vacuole comparable with the large lacuna-like vacuole that, in most congeneric forms, increases in size until it abruptly disappears very shortly before the protoplasmic materials are expelled. Internally the numerous sporangia in moist agar cultures amended with leaf mold often maintain without noticeable change for several days a granular structure the uniformity of which is relieved only by a more nearly homogeneous texture in the beak, and by the presence of numerous, more or less evenly distributed, subspherical hyaline bodies, approximately 3 µ in diameter. Perhaps more because of some lumping of protoplasm about these bodies than because of any definite demarcation, the sporangial contents present an appearance vaguely suggestive of cleavage, a little like that presented, for example, in sporangia of Phytophthora ready to discharge their zoospores. When water is added discharge of the sporangial contents ensues more promptly than in most allied species. Often within 30 minutes an evacuation tube is pushed out in proximity to the beak (Fig. 11, C, a), or the beak itself becomes elongated; a cap of dehiscence being formed in either case, which, on yielding, receives the escaping protoplasmic materials, and is thereby inflated into a spherical vesicle (Fig. 11, D, a). Almost immediately after its reception in the vesicle the discharged mass begins to show faint lines of cleavage, and to display waving cilia at its periphery. Manifestly, at the time of their discharge, the sporangial contents here have already undergone the initial stages of reorganization that elsewhere are accomplished during the first 5 minutes of their sojourn within the vesicle. Since the further stages of transformation follow in the sequence usual for species of *Pythium*, the laterally biciliate zoospores are ready to be liberated after 15 to 20 minutes, rather than after 20 to 25 minutes as in most congeneric forms. The peculiarities attending asexual reproduction in the genus *Pythiogeton*—discharge of sporangial• contents into a conspicuously elongated vesicle, deliquescense of the vesicular envelope either in place or after violent projection of the extruded mass, and fashioning of zoospores from the unprotected protoplasm—have not been observed in the fungus.

The distinctive asymmetrically beaked sporangia (Fig. 11, C, b; E; F), as also the occasional bilobed specimens (Fig. 11, G) of apparently binate origin, are for the most part borne terminally on mycelial branches. In very prolific agar cultures a large proportion of the sporangia arise as intercalary bodies, often close together, on a main hypha (Fig. 11, H, a–d). These, more usually, are subspherical or ellipsoidal, and as a rule bear a sessile papilla (Fig. 11, H, a, b, c). The papilla, though sometimes functioning in dehiscence without furthering elongation, more frequently grows out into an evacuation tube of varying length (Fig. 11, I, t; K, t). Among the globose intercalary sporangia may be found specimens as much as 60 or 70  $\mu$  in diameter, which, on discharging, give rise to more than a hundred zoospores. Of course not all globose or ellipsoidal sporangia are intercalary, some being borne terminally (Fig. 11, L, M) much like asymmetrical specimens.

After a sporangium has been evacuated, the supporting hypha may resume growth by giving rise, usually within the emptied envelope, to a second sporangium (Fig. 11, D, b, c; E; F; N). Such proliferous development would appear, on the whole, somewhat more frequent in rather wellirrigated material than in material only sparingly irrigated. Deficiency in supply of water may often fail to inhibit sporangial discharge, even while it is causing premature disintegration of vesicles, with the result that numerous discharged protoplasmic masses, deprived of their protective coverings, undergo degeneration on a relatively enormous scale. Under proper conditions, however, irrigation of slabs excised from cultures on maize-meal agar, amended with leaf mold, leads within 1 or 1.5 hours to liberation of active zoospores in extraordinary abundance. After swimming about for some time the zoospores round up (Fig. 11, O), to germinate later by the production usually of a single germ tube (Fig. 11, P, a-c).

In its sexual reproduction the fungus is somewhat capricious. Most cultures on maize-meal agar show no evidence of sexual development, and, apparently, such development cannot be induced by increasing the amount of finely divided maizemeal in the medium, or by adding either leaf mold or yeast extract. Yet, now and then a culture on the same substratum will afford prompt production of oospores wholly correct in structure, without deiete.

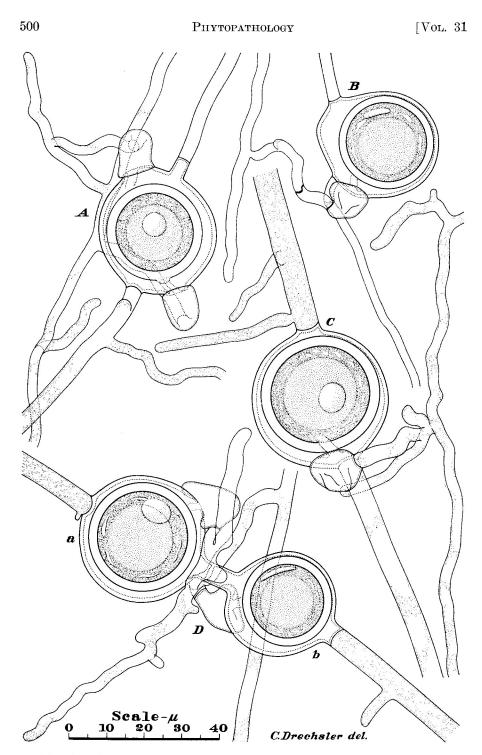


FIG. 12. Sexual apparatus of  $Pythium\ marsipium\ produced\ in\ maize-meal\ agar,$  and drawn with the aid of a camera lucida;  $\times 1000$ .



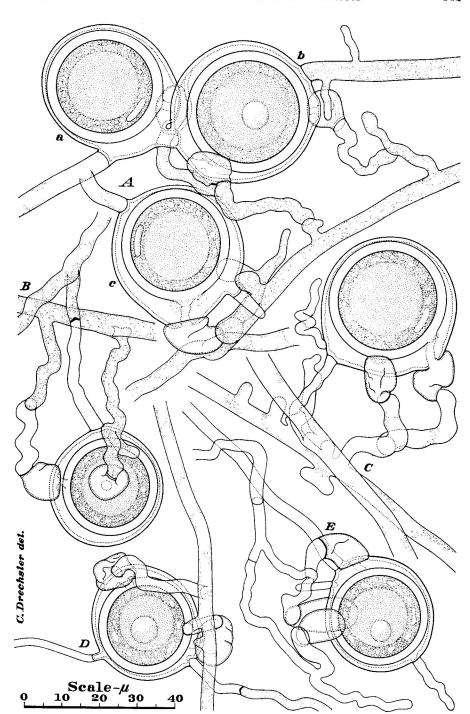


FIG. 13. Sexual apparatus of  $Pythium\ marsipium\ produced\ in\ maize-meal\ agar,\ and\ drawn\ with\ the\ aid\ of\ a\ camera\ lucida\ ;\ \times 1000.$ 

any addition of alien substances. Like the sporangia the units of sexual apparatus usually are found in crowded arrangement along favored hyphae, and thus collectively become visible to the naked eye in radial streaks.

The oogonia are rather large subspherical bodies, frequently intercalary in the wider mycelial filaments, either singly (Fig. 12, A; B; C; Fig. 13, D) or in pairs (Fig. 12, D, a, b; Fig. 13, A, a, b). Often, again, they are borne terminally (Fig. 13, B) or somewhat subterminally (Fig. 13, A, c; C; E) on branches of variable lengths. Fertilization is accomplished usually by 1, 2 or 3 antheridia, borne terminally on rather stout lateral branches arising from hyphae having no close connection with the oogonial filaments. Diclinism is conspicuous in the numerous aggregations of sexual apparatus where all the female elements clearly come from one hyphal system, and all male elements from another. In some instances an antheridium is brought into a lateral position when the supporting hypha continues growth from below the delimiting septum to produce a second antheridium farther onward. As a rule the antheridia are broadly applied to the organium, which often protrudes noticeably in the region of contact, besides showing here marked attenuation of its relatively thick envelope. The fertilization tube is often rather wide, and frequently may be seen attached by its distal end to a wartlike excrescence on the oospore wall (Fig. 12, A; Fig. 13, A, c; C). Internally, the mature oospore always reveals the unitary structure common to most species of *Pythium*, never the multiplicate structure distinctive of the helicoides series. Its single reserve globule is of comparatively large size, as is evident from the relevant metrical data given in the diagnosis, which were derived from measurements of 200 units of sexual apparatus selected at random in maize-meal agar cultures. Expressed to the nearest integral number of microns, the values for diameter of oogonium obtained from these measurements are distributable as follows:  $23 \mu$ , 1;  $24 \mu$ , 2;  $25 \mu$ , 3;  $26 \mu$ , 8; 27 μ, 13; 28 μ, 20; 29 μ, 17; 30 μ, 27; 31 μ, 23; 32 μ, 22; 33 μ, 18; 34 μ, 14;  $35 \mu$ , 10; 36  $\mu$ , 13; 37  $\mu$ , 7; 38  $\mu$ , 2; while the values for diameter of oospore are distributed thus:  $19 \mu$ , 1;  $21 \mu$ , 4;  $22 \mu$ , 9;  $23 \mu$ , 19;  $24 \mu$ , 20;  $25 \mu$ , 25; 26 µ, 33; 27 µ, 28; 28 µ, 13; 29 µ, 23; 30 µ, 11; 31 µ, 10; 32 µ, 2; 33 µ, 2.

Although the fungus produces asymmetrical utriform sporangia conforming to a type held characteristic more especially of several species of Pythiogeton, it clearly does not belong in that genus. As has been noted, the vesicle wherein the sporangial contents are fashioned into zoospores is consistently spherical, and not only fails to deliquesce soon after its inflation, but often shows more than ordinary durability when sizeable portions of its membrane persist in visible state for an hour or two after escape of the zoospores (Fig. 11, I). Despite some aberrant features—prolonged maintenance of the sporangia in a condition of readiness for prompt discharge, initiation of zoospore development previous to discharge—asexual reproduction here is unmistakably that of a species of Pythium. While pronounced vacuolization is not apparent in the sporangium shortly before dehiscence, it remains possible that the protoplasmic reorganization associated with such vacuolization may be accomplished here much less obtrusively just before the sporangium enters on its prolonged condition of readiness for discharge, that is, at a stage often long preceding discharge as regards time, yet, corresponding approximately to the developmental stage immediately preceding discharge in the generality of congeneric forms. On aging, naturally, the sporangia here, like those of other oomycetes, sooner or later show a vacuole of senescence, which often increases in size until the granular protoplasm is crowded into a thin parietal layer.

The literature on Pythium is not wholly free of comment in which fungi with unsymmetrical zoosporangia have been designated as species of that genus. Cornu (4), in 1872, made reference, under the binomial Pythium utriforme, to a new species whose zoosporangia he described as being always irregular, utriform, and elongate-reniform in shape; his further characterization of them as being occasionally intercalary presumably implying that like the spherical sporangia of another new species, Pythium imperfectum, they were borne for the most part terminally on delicate filaments similar to the sporangiferous filaments of Pythium proliferum. In the 2 new species, both proliferous like Pythium proliferum, the protoplasm issued from the tip of a long evacuation tube, to be received at the beginning in a vesicle, which, however, normally burst after attaining very meager development. Von Minden properly recognized the external shape ascribed to the sporangium of Pythium utriforme, and the early disintegration, in that species, of the vesicular membrane, as features indicating probable identity with one or another of the 3 species he described in the genus Pythiogeton. Though he believed, not without justification, that Pythium imperfectum was precluded from such identity by reason of its spherical sporangia, this inadequately described species, with its early disintegration of vesicular envelope, would now seem referable to Pythiogeton no less properly than Pythiogeton uniforme, Pythiogeton dichotomum, and the several unnamed congeneric forms figured by Lund and Sparrow, for all of which likewise only globose sporangia have become known.

Soon after Cornu's insufficient characterization of *Pythium utriforme*, Pringsheim (20) referred somewhat incidentally to a closely allied species, which, from the varied, mostly lateral origin of its evacuation tube, he designated as *Pythium laterale*. This species, though inviting von Minden's speculations relative to its possible affinities in *Pythiogeton*, could only be dismissed from consideration, since the relevant descriptive comment, again, gave no clear picture of the fungus observed.

From the several remaining proliferous forms that have been described as species of Pythium the fungus would seem to differ primarily in its production of utriform sporangia. Apart from this general difference, the habitual declinism of its sexual phase offers a marked contrast with P. proliferum, in which, according to de Bary (1), the antheridium as a rule arises from the oogonial hypha in close proximity to the oogonium, and only occasionally is contributed by a neighboring filament. Later writers that dealt at first hand

with some proliferous form to which they applied de Bary's familiar binomial have not always, it must be admitted, insisted on predominance of the monoclinous relationship. Of the 3 antheridia shown in Ward's figures (23, pl. 35, fig. 20, 21) one is clearly monoclinous, one is diclinous, and the other is of ambiguous origin. Butler (3) found male organs coming from neighboring hyphae more frequently than from the oogonial hypha. In Matthews' (17) account the antheridia are figured and described as coming rather impartially from neighboring filaments or from the oogonial filament, in the latter instance arising either adjacent to the oogonium or at some distance from it.

In his original account of Pythium ferax, de Bary (2) characterized this form as being very similar to P. proliferum, though differing in various details. Among the details representative of difference was mentioned the absence of a large vacuole in the sporangium. As stalk antheridia, that is, antheridia made up individually of a segment of oogonial hypha adjacent to the oogonium, were stated to predominate over branch antheridia the sexual apparatus of P. ferax presents an arrangement of parts wholly alien to the present fungus.

Dissimilarity with respect to origin of antheridia likewise sets the fungus apart from *Pythium nagai* Ito et Tokunaga (13), a proliferous species associated with decay of rice (*Oryza sativa* L.) seedlings in Japan. Here, oogonia measuring only 14 to  $22 \mu$  in diameter are fertilized usually by a single male organ, ovoid, globose, or clavate in shape, that is borne terminally on a slender branch arising from the oogonial stalk.

Ito (14) in his diagnosis of *Pythium iwayamai*, a species found by Iwayama (15) to attack barley (*Hordeum sativum* Jess.) and wheat (*Triticum vulgare* L.) in Japan, makes no mention of a proliferous habit, though the terminal attachment of sporangia described in part as being spherical, or more rarely ellipsoid, ovoid, or lemon-shaped, offers some obvious parallelism with proliferous forms. The oospores of this parasite, which measure 19 to  $24 \mu$  in diameter, are not only smaller than those of the fungus under consideration but differ further in that they completely fill the oogonial chamber.

Parallelism with proliferous forms is suggested also in the subspherical or lemon-shaped conformation attributed by Sideris (21) to the sporangia of 2 fungi he described as new species under the names *Pythium polycladon* and *P. euthyhyphon*, after he had isolated them in Hawaii from castor bean (*Ricinus communis* L.) roots and pineapple (*Ananas sativus* Schult.) roots, respectively. To these species were ascribed, somewhat misgivingly, oogonia and oospores imperfectly seen within host tissue. The measurements given for the diameters of the bodies observed—about 20  $\mu$  for the bodies presumed to belong to *P. polycladon*, and about 18  $\mu$  for those referred to *P. euthyhyphon*—are well below the usual values for any analogous dimension in my sporangia, which often show proliferous development. In an old culture on fungus.

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Pythium carolinianum Matthews (17), described as a parasite on a species of Spirogyra in North Carolina, gives rise to subspherical or elongated 7 carrots, some of its sporangia were observed to form bodies resembling a young oospore in appearance but never developing typical oospore structure. These bodies, dubiously designated as resting spores, were figured with a lumpy internal organization slightly suggestive of the multiplicate organization in normal, mature oospores of *P. helicoides* and its allies. As its hyphae were stated to be only 1 to  $4 \mu$  wide, *P. carolinianum* would seem appreciably more delicate than any proliferous congeneric form I have encountered.

In fine, the fungus from water-lily leaves appears not to have been previously described, and is, therefore, presented as a new species; the specific name applied to it—a term meaning "small pouch"—having reference to its bursiform sporangia.

# Pythium marsipium, sp. nov.

Mycelium hyalinum, ramosum, in hyphis 2-7.5  $\mu$  crassis consistens. Zoosporangiis modo sphaeroideis, papilla instructis, 20-70  $\mu$  diam., modo terminalibus, elongato-ovoideis vel utriformibus, saepius 25-70  $\mu$  longis et 20-45  $\mu$  latis, basi rotundatis, apice oblique rostratis, a latere transverse vel oblique ramo saepe 20-100  $\mu$  longo et 3-4  $\mu$  crasso applicatis; subinde per papillam vel per rostrum exinanientibus, sed saepius per tubulum usque 100  $\mu$  longum 2-8  $\mu$  crassum protoplasma eorum fundentibus. Zoosporis saepius 25-75 in vesicula evolutis, reniformibus, a latere biciliatis, quiescentibus globosis, 9-12  $\mu$ diam. Oogoniis terminalibus, subterminalibus, vel intercalaribus, globosis, 23-39  $\mu$  (saepe circa 30.9  $\mu$ ) diam., membrana .7-1.2  $\mu$  crassa tectis; antheridiis interdum singulis, plerumque binis ternisve, rarius quaternis, fere terminalibus, saepissime diclinis, cupulatis vel campaniformibus, 10-20  $\mu$  longis, 8-12  $\mu$  latis, apice ad oogonium late appositis; oosporis flavidis, sphaericis, 19-33  $\mu$  (saepe circa 26.2  $\mu$ ) diam., membrana 1.3-2.8  $\mu$ (saepe circa 1.95  $\mu$ ) crassa teetis, unam pilulam oleosam 11-22  $\mu$  (saepe circa 16.6  $\mu$ ) diam. et unum corpusculum nitidum globosum vel valde applanatum continentibus.

Habitat in foliis Nymphaeae tuberosae putrescentibus prope Butternut, Wisconsin.

Intramatrical mycelium in transparent agar media often slightly lustrous, of pronouncedly radiated appearance, capable of approximately 19  $\mu$  radial extension in 24 hours at 24° C, composed of hyphae 2 to 7.5  $\mu$  wide, the more delicate ramifications being present usually only in moderate quantity. Aerial mycelium often absent or rather sparingly developed; under aquatic conditions extramatrical growth rather scanty. Sporangia subspherical or often unsymmetrically utriform,—the subspherical speci-

Sporangia subspherical or often unsymmetrically utriform,—the subspherical specimens mostly 20 to 70  $\mu$  in diameter, papillate, produced mainly under moist rather than aquatic conditions, sometimes terminal but more often intercalary in wide filaments, the utriform specimens measuring 25 to 70  $\mu$  in length by 20 to 45  $\mu$  in width, broadly rounded at one end and skewly beaked at the other, formed under moist or under aquatic conditions for the most part terminally on branches often 20 to 100  $\mu$  long and 3 to 4  $\mu$ wide, their long axis transverse or oblique to the supporting filament; discharging sometimes by means of the beak or papilla without elongation of these parts, but more often by means of an evacuation tube, 2 to 8  $\mu$  wide and up to 100  $\mu$  long, which may prolong the beak or papilla, or may arise from a position nearby; proliferous in variable measure, usually by the production of a secondary sporangium within the empty envelope of a primary one. Zoospores bean-shaped, longitudinally grooved, laterally biciliate, produced within a moderately durable subspherical vesicle in numbers ranging from 2 to 125, usually from 25 to 75; after rounding up measuring mostly 9 to 12  $\mu$  in diameter.

Oogonia frequently terminal or subterminal on sturdy branches, but more often intercalary in the coarser axial hyphae, and then sometimes occurring adjacent to one another; subspherical, though often protruding noticeably toward an applied antheridium; measuring 23 to 39  $\mu$  (average 30.9  $\mu$ ) in diameter; provided individually with a sturdy wall .7 to 1.2  $\mu$  in thickness; and supplied with 1 to 4, usually 1 to 3, antheridia. Antheridia usually borne terminally on stout branches arising from filaments not closely connected with the oogonial hypha, though sometimes coming into a lateral position through prolongation of the supporting branch; irregularly expanded, prolate ellipsoidal, cupulate, or bell-shaped; measuring mostly 10 to 20  $\mu$  in length by 8 to 12  $\mu$ in width; at the distal end mostly rather broadly applied to the oogonium; intruding a fertilization tube often 2 to 5  $\mu$  long and 1.5 to 2.5  $\mu$  wide. Oospores usually yellowish, subspherical; measuring mostly 19 to 33  $\mu$  (average 26.2  $\mu$ ) in diameter; provided with a wall 1.3 to 2.8  $\mu$  (average 1.95  $\mu$ ) in thickness; containing a single spherical reserve globule 11 to 22  $\mu$  (average 16.6  $\mu$ ) in diameter; and having imbedded in the narrow parietal granular layer a single refringent body which measures about 5  $\mu$  in diameter when of subspherical shape, but which is often strongly flattened into an oblate ellipsoid and then measures 1.3 to 2.5  $\mu$  in thickness by 7 to 10  $\mu$  in width.

Occurring in discolored, watersoaked, or decaying leaves of Nymphaea tuberosa near Butternut, Wis.

## SUMMARY

Pythium oedochilum and P. palingenes resemble P. helicoides in the generally symmetrical conformation and proliferous development of their sporangia, which, however, owing to infrequence of distal branching in the supporting hyphae, are rarely borne in corymbose arrangement. Asexual reproduction in *P. palingenes* is often characterized by abundant iterative swarming; the repetitional development coming about both through direct evacuation of the cyst envelope, and through production of a miniature sporangium on a germ sporangiophore. The sexual reproduction of P. oedochilum and P. palingenes shows parallelism with that of P. helicoides not only in the longitudinal application of elongated antheridia to oogonia that often protrude along the regions of contact, but also in the distinctively multiplicate internal structure of the ripe oospore,—this spore containing plural reserve globules and plural refringent bodies instead of the single reserve globule and single refringent body found in the oospores of most species of *Pythium*. Helicoid involvement of the organial hypha by an antheridial branch, such as is associated with every unit of sexual apparatus in P. helicoides, occurs very rarely in P. oedochilum, and is concomitant with about one-third of the sexual units in *P. palingenes*.

A fungus isolated from affected portions of water-lily leaves is described as a new species under the name *Pythium marsipium*. In agar cultures its asexual reproduction is enormously increased by addition of various decaying plant materials. Apart from zoosporangia of commonplace subspherical shape, often intercalary in the longer mycelial filaments, it produces, for the most part terminally on fairly short branches, asymmetrical utriform zoosporangia of a type that in recent times has come to be considered more or less distinctive of the genus Pythiogeton. Its zoospores are fashioned within a spherical vesicle of at least ordinary durability, from a mass of protoplasm, which, previous to sporangial dehiscence, has usually undergone the preliminary changes that in most congeneric forms are accomplished during the first 5 minutes following dehiscence. Proliferous development of sporangia occurs under thoroughly aquatic conditions. As the fungus produces oospores of unitary internal structure—that is, oospores with a single reserve globule and single refringent body-it must be regarded as alien to the helicoides series.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,

U. S. HORTICULTURAL STATION,

BUREAU OF PLANT INDUSTRY, BELTSVILLE, MARYLAND. 1941]

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