PYTHIUM DEBARYANUM AND RELATED SPECIES IN SOUTH AUSTRALIA

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MATERIALS

During an investigation of pre-emergence rotting of peas at the Waite Institute it was evident that infections of seedlings by *Pythium* spp. were important. It was not known what species were concerned, and the writer undertook to investigate this problem during a brief sojourn in Adelaide.

Available for study were six isolations of *Pythium* from pea seedlings which had been grown in a red-brown earth at the Waite Institute, two from potatoes affected by "leak" disease and one from a tomato seedling. The isolations had been made by Mr. N. T. Flentje.

The various isolates were grown on potato-dextrose, oatmeal, cornmeal and water agars, respectively, as used by Middleton (1943), and on carrot agar as described by Johann (1928) and Schulz (1942). Since carrot agar, which has been autoclaved, was said to be unfavourable for oospore production (Schulz), it was sterilised by steaming it twice at 95° C. However, oospore reproduction was secured quite readily on the autoclaved media. The cultures studied were derived after a series of transfers from the edges of 24-26 hour old colonies, and each time the inoculum piece contained about half-a-dozen hyphal tips. Attempts were made to use single hyphal tips, but only in one case which is mentioned later was this successful. The use of single zoospores was precluded because their production was never observed. In all cases germination took place by means of a germ tube. The observation of oogonial and antheridial characters was facilitated by using cultures grown on a drop of potato agar in a small moisture chamber made from a glass ring and cover slip.

The diameters of the reproductive organs provide important criteria for differentiating species of *Pythium,* and it is desirable to measure large numbers of these structures to ascertain their range and mean value. It was found that diameters of oogonia and oospores were less variable than those of the sporangia, and that counts of 100-300 revealed that they followed a normal frequency distribution. It is appropriate then to describe the variability of means in terms of their standard errors calculated in the usual way. The sporangia, however, were much more variable in size and shape and only limits in size observed are described.

RESULTS

It was evident that the isolates could be referred to *Pythium debaryanum* Hesse or closely related species.

The genus *Pythium* has been treated systematically at least six times and has been the subject of several monographs. The isolates made locally have been referred to the species mentioned below and are reviewed in the light of recent descriptions and figures of Drechsler (1946), Matthews (1931), Sideris (1932), Van Luyk (1934, and especially, that of Middleton (1943).

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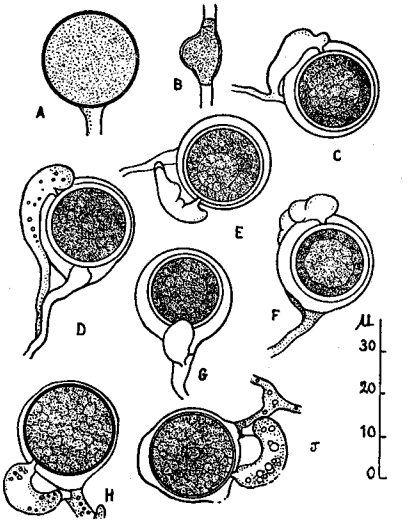


Fig. 1 *Pythium debaryanum* Hesse

A - D — Sporangia: E, Empty monoclinous antheridium; F, Diclinous antheridium; G, Oogonium penetrated by monoclinous and diclinous antheridia.

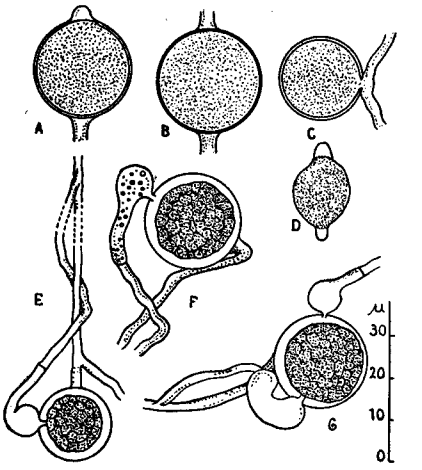


Fig. 2 *Pythium ultimum* Trow

A - B — Sporangia: C—J: Oogonia and oospores each with one monoclinous antheridium.

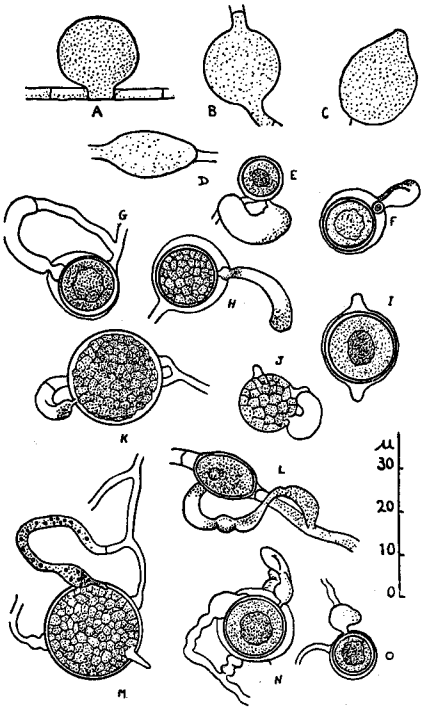


Fig. 3 *Pythium polymorphon* Sideris

A - D — Sporangia: F, G, H, Antheridium after fertilization; I, Intercalary oogonium; J, E, O, Small oogonia with curved swollen antheridia; K, L, M, Young oogonia with one antheridium. Antheridial stalk typically curved; N, Two antheridia to one oogonium.

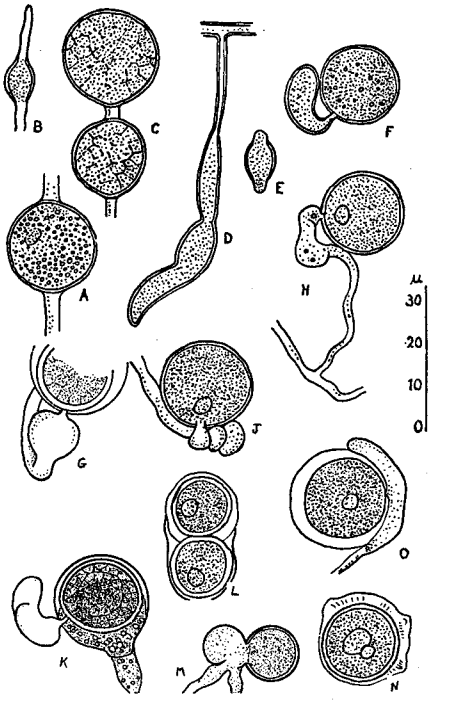


Fig. 4 *Pythium vexans* de Bary

A - C — Sporangia: D, Mycelial body; E, Germinating sporangium; F, Allantoid antheridium and young oogonium; G, H, J, O, Variations in antheridial shape; L, oogonium with two oospores; N, Aplerotic oogonium; K, Germinating oospore with empty antheridium; M, Germinating sporangium.

Pythium debaryanum Hesse

Hyphae 2·5-11 μ, usually 4-5 μ in diameter; sporangia spherical to oval terminal or intercalary, sometimes in chains ranging from 9-41 μ in diameter and germinating by germ tubes; oogonia smooth, spherical, terminal, 14-29 μ, means 20·7-23·1 μ in diameter. Antheridia one or more per oogonium generally diclinous but sometimes monoclinous when they arise some distance, 40-280 μ, below the oogonium. Antheridia stalked, crooked-necked with the terminal portion oblong, spherical or clavate and 5-11 μ in diameter, apex obtuse or narrow, tapering gradually to the oogonial wall to which it is more or less applied. Oospores smooth, aplerotic, 11-25 μ, averaging 17·4-19·9 μ in diameter, with a thick wall. Parasitic on *Pisum sativum* and *Solanum tuberosum* (see fig. 1).

The above description covers four of the isolates from peas and one from a potato affected by "leak." It agrees generally with those given by other authors, and at this point only the sizes of oogonia and oospores described above call for comment. If those quoted are compared with those of various authorities [Butler (1913), Braun (1925), Sideris (1932), Van Luyk (1934), Middleton (1943)], it is seen that the range of means we found is somewhat less than that described in the literature. Actually, statistical analysis reveals that the means for both oogonia and oospores in our different isolates may be significantly different. For example, the mean diameters of oogonia and oospores of isolate 5 from peas were 20·7 $\mu \pm 0{\cdot}09$ and 17·4 $\mu \pm 0{\cdot}18$ μ respectively, whereas corresponding measurements for isolate 2 (also referred to *Pythium debaryanum*), were 23·1 $\mu \pm 0{\cdot}19$ and 19· $\mu \pm 0{\cdot}22$ respectively. However, it is possible to arrange the isolates we classify as *Pythium debaryanum* in such a way that differences between successive pairs are not significant. The differences in means may indicate the existence of strains, but they offer little warranty for specific segregation.

Pythium ultimum Trow.

Hyphae 3-9 μ usually 4-5 μ in diameter with frequent irregular mycelial bodies; Sporangia terminal or intercalary, 7-25 μ diameter, germinating by germ tube; Oogonia smooth, terminal rarely intercalary, spherical 18-26 μ average 22·3 $\mu \pm 0{\cdot}09$ borne on laterals 15-90 μ long. Antheridia one per oogonium, monoclinous and arising from immediately below the oogonium (hypogynous) (fig. 2 C-J). Antheridium tube-like, swollen, curved with apex tapering towards oogonial wall, 5-8 μ x 11-23 μ. Ooospores aplerotic, single spherical 12-23 average 18·3 $\mu \pm 0{\cdot}09$ in diameter, thick walled. Isolated from *Solanum tuberosum.*

Middleton (1943), in concurring with Drechsler (1927) for the maintenance of *Pythium ultimum* as a distinct species, points out that its typically monoclinous swollen antheridia, which originate close to the oogonium, and curve sharply upwards to make a narrow apical contact with the basal portion of the oogonium, distinguish it from *Pythium debaryanum.* These features were characteristic of the isolate described and figured here. However, in another isolate, one from peas (Isolate I) at least two types of antheridial structure occurred in the one culture. One or more diclinous, long stalked, crook-necked antheridia typical of *Pythium debaryanum* occurred in the same culture as monoclinous, stalkless hypogynous antheridia characteristic of *Pythium ultimum.* There were also many gradations (fig. 2).

Middleton, while he refers to the infrequent occurrence of diclinous antheridia in *Pythium ultimum* says that "they are not distinctive enough to be utilized in the identification of the species." On the other hand, Van Luyk (1934), with others, doubts whether such differences between the antheridial characters as are mentioned above are sufficient to segregate the two species. He considers that *Pythium debaryanum,* among its different strains, exhibits differences of the type described; a view with which the writer concurs.

Pythium polymorphon Sideris

Hyphae 3-7 μ in diameter. Sporangia spherical, subspherical terminal or intercalary 7-33 μ in diameter and germinating by germ tube. Oogonia spherical, terminal or intercalary, smooth but sometimes with one or more papillae (fig. 3, J. M.) 9-25 μ av. 16·2 $\mu \pm 0{\cdot}22$ in diameter. Antheridia 1-2 per oogonium monoclinous, antheridial stalk typically falcate or sigmoid or with curved antheridial cell (fig. 3, K.L. M). Antheridial cell not greatly swollen and with a long fertilization tube, 8-20 μ long x 5-7 μ wide. After fertilization the apical portion is divided from the base by a narrow ring at junction with oogonial wall (fig. 3, F. G. H.). Swollen diclinous antheridia also occur but mostly in association with the smaller oogonia (fig. 3, J. E. O.). Oospores aplerotic, 7-23 μ, av. 13·4 $\pm$ 0·27 in diameter with somewhat thickened wall. Isolated from *Pisum sativum.*

The characteristic feature of this fungus is the shape of the antheridial branch, which may be curved like the letter C or double curved like the letter S. In our isolate the origin of the antheridial stalk was always monoclinous, arising some distance from the oogonium and generally curving in a wide bow to meet the oogonial wall in the way illustrated by Sideris.

The swollen diclinous antheridia applying to the smaller oogonia described above suggested the possible presence of more than one strain in our culture. However, it was possible to secure growth from a single hyphal tip of this isolate and the culture derived behaved in the way already described.

The isolate we describe is slightly different from *P. polymorphon* described by Middleton and appears to be the first record of the association of this fungus with *Pisum sativum.* Previous records associate *P. polymorphon* with Ananas *comosus* in Hawaii and *Nicotiana Tabacum* in the United States.

Pythium vexans De Bary

Hyphae 2-7 μ in diameter with irregular mycelial bodies associated (fig. 4, D). Sporangia terminal or intercalary, spherical to oval 10-26 μ in diameter and germinating by germ tubes (fig. 4, M). Oogonia spherical terminal, 15-26 μ av. 21·7 $\mu \pm 0{\cdot}10$ in diameter. Antheridia 1-2 per oogonium, diclinous. Antheridial cell very variable in shape (fig. 4, G. H. J. O.). In some cases, cylindrical and hardly curved or swollen, in others slightly swollen or so swollen that the shape is nearly spherical. The antheridial cell or its stalk may be so curved that application to the oogonial wall is narrow. In profile it may appear bell-shaped, and in another plane as an allantoid or disc-like structure covering the oogonium (fig. 4, G. O.). The cylindrical antheridia are 11-18 μ long by 7-11 μ wide, the spherical 7-9 μ in diameter. Oospores smooth, aplerotic, 13-24 μ av. 18·8 $\mu \pm 0{\cdot}11$ in diameter and with a thick brown wall. Isolated from *Lycopersicon esculentum.*

Middleton states that *"Pythium vexans* is readily distinguished from its congeners possessing aplerotic oospores by its typically monoclinous, stalked antheridium which arises in close proximity to the oogonium and by the clavate antheridial cell with the apex bell-shaped and broadly applied, sometimes fused with the oogonial cell."

In respect to the origin of the antheridia Middleton says that they are rarely diclinous, but Drechsler (1946 b), who cultivated *Pythium complectens* Braun, and like Middleton considers the name a synonym of *Pythium vexans,* remarks that "frequently the mycelial connection between the oogonial stalk and antheridial branch is too remote to be traced for certainty amid the confusion of ramifying hyphae." Thus the apparently diclinous character of antheridia in our isolate is not, in itself, sufficient to distinguish it from *Pythium vexans.* Antheridial shape in our isolate was very variable. It is true that in certain aspects, trumpet-shaped sstructures flaring out at the region of attachment, as described by Braund, bell-

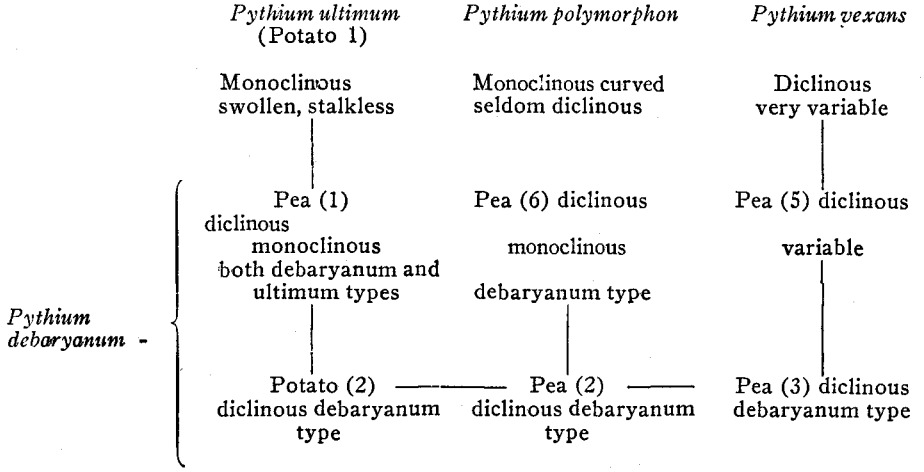
shaped apices as mentioned by Middleton, and the bilobate and biramous male cells, figured by Drechsler, were observed, but the antheridial cells were by no means uniformly of these shapes.

However, there is another feature in which our isolate resembled descriptions of *Pythium vexans.* According to Middleton the germination of oospores of *Pythium* is not common; it occurs more readily in *Pythium vexans,* a fact also noted by Butler (1907) and Drechsler (1946). The oospores of our isolate germinated fairly readily to produce sessile sporangia which in turn produced a germ tube (fig. 4, K). The sporangia, when they germinate first, produce a vesicle into which the contents of the sporangia passed. The vesicles then developed a germ tube.

General Discussion

Middleton considers that the origin and morphology of the antheridia afford valuable criteria for specific identification. Our isolates, because of close resemblance in other respects, have had to be looked at particularly in respect to antheridial characters, but we found these to be rather more variable than Middleton's descriptions might suggest. In one case (isolate 5) differently shaped antheridia were even found on one branch. (See also the discussion under *Pythium ultimum.*) Although we have distinguished our isolates in the terms discussed above, we should bear in mind the advice of Buisman (1927) not to define species too strictly: "It is not at all easy to determine if a special isolation belongs to the well-known *Pythium debaryanum* or not." It is clear, too, that in view of the methods of reproduction concerned and the obvious chances of hybridization, intermediate forms might be expected.

In view of the foregoing remarks, the following scheme of relationship of the isolates examined is suggested, remarks refer to salient antheridial features:—



Summary

The morphology of nine isolates of *Pythium* spp. grown in pure culture on various media have been studied. Four isolates from infected pea seedlings and one from a potato affected by "leak" disease were identified as *Pythium debaryanum* Hesse. A fifth isolate from peas was identified as *Pythium polymorphon* Sideris. A second isolate from potato with "leak" was identified as *Pythium ultimum* Trow and an isolate from a diseased tomato seedling as *Pythium vexans* De Bary.

All these species have a number of characters in common; spheroidal sporangia and smooth aplerotic oospores, and are considered to be closely related to one another, and this is discussed.

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