OLPIDIUM BRASSICAE (WOR.) DANG. AND ITS CONNECTION WITH *ASTEROCYSTIS RADICIS* DE WILDEMAN

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(With Plate V and 1 Text-figure)

Introduction

Olpidium Brassicae (Wor.) Dang. was first recorded in the roots of cabbage and figured by Woronin (1878). The resting spores are described as colourless or pale yellow with a thick membrane and more or less stellate. The contents are granular, with one large or several small oil drops (Pl. V, fig. 1). De Wildeman (1893) figured cysts of *O. Brassicae*, together with other cysts which he regarded as distinct and named *Asterocystis radicis*. These were stellate, with a large central refractive globule, and they could not be plasmolysed with glycerine, whereas those of *Olpidium Brassicae* were easily plasmolysed. His figures of the new species showed a star within a circle or ellipse (Pl. V, fig. 4). In the same paper De Wildeman figured oblong zoosporangia with one or more exit-tubes, and to these he gave the name *O. Borzii* n.sp., a name changed to *O. radicicolum* in a later paper (1896).

Marchal (1900), working with flax, found stellate cysts (Pl. V, fig. 5) identified as *Asterocystis radicis*, associated with zoosporangia, which appeared to discharge their zoospores through a small lateral opening, and not through a definite exit-tube.

Subsequently, stellate cysts and zoosporangia possessing exit-tubes of variable length have been found in a number of different plants. They have usually been recorded as *Asterocystis radicis* (Fron & Gaillat, 1925; Guyot, 1927; Vanterpool, 1930), more rarely as *Olpidium Brassicae* (Bensaude, 1923) or *O. radicicolum* (Bartlett, 1928), but most authors comment on the difficulty of distinguishing these species. This is hardly surprising, since the cysts of the first two species are described as stellate, and little additional information is given in the original papers. The distinction rests almost entirely on De Wildeman's drawings (1893), for he is one of the few workers who claims to have seen all three species. Němec (1912) also keeps them distinct. Both Guyot (1927) and Karling (1937) suggest that *Asterocystis radicis* and *Olpidium Brassicae* probably refer to the same organism, while Bartlett (1928) gives *Asterocystis radicis* and *Olpidium radicicolum* as synonyms.

In 1937, the opportunity arose of observing an *Olpidium* with stellate cysts in members of the Cruciferae and Gramineae, two of the families which most frequently serve as hosts for the above species. The *Olpidium* was first discovered in the roots of species of *Agrostis* growing in rather waterlogged soil. Seeds of cauliflower were sown in the same pots, and in two to three weeks their roots were abundantly parasitized by what appeared to be the same species, though not necessarily the same strain, of *Olpidium*. Observations were made on living roots and on material fixed and stained. For further comparison fresh material of *O. Brassicae* on cabbage was obtained by the kindness of Mr G. Samuel. To Mr Bartlett I am indebted for swedes bearing the so-called "hybridization nodules" and containing in their finer rootlets zoosporangia and cysts of an *Olpidium* which he identified as *O. radicicolum* De Wildeman.

The wide range in size and shape of the zoosporangia in the four collections of material examined, together with observations on the structure of the wall of the cyst, have convinced me that the parasite is the same on the four hosts. A careful comparison of this material with published records leads to the conclusion that *Olpidium Brassicae* (Wor.) Dang., *Asterocystis radicis* De Wildeman, and *Olpidium radicicolum* De Wildeman are synonyms, the first name having priority.

Examination of material from roots of *Agrostis*, cauliflower, cabbage and swede

(1) *Zoosporangia*

In all roots examined, the zoosporangia were extremely variable in size and shape, ranging from spherical units 12 μ in diameter, and opening by a single exit-tube, to elongated individuals 130 μ in length which may possess as many as four exit-tubes. Bartlett found sporangia as long as 220 μ. The diameter of each thallus is related to that of the host cell, which is not hypertrophied. Zoosporangia usually fill the short diameter of the cell, but sometimes exceptionally small zoosporangia are seen, and these may discharge zoospores into the cell itself. The same cell may contain small and large zoosporangia, and they are not uncommonly mixed with cysts (Text-figs. 1–18). In Woronin's figures of *Olpidium Brassicae*, spherical zoosporangia only are shown, and it was this which led De Wildeman to establish his species *O. radicicolum*. Possibly the fact that Woronin used transverse sections of the root may account for the absence in his drawings of long zoosporangia. Van der Meer (1926) and Bartlett (1928) also found spherical and elongated sporangia in the same root.

Confusion has arisen in regard to the length of exit-tubes, perhaps because the figure of *O. Brassicae* that found its way into most text-books showed only zoosporangia with long necks. In one of Woronin's

figures, however, both long and short exit-tubes are shown (Pl. V, fig. 1), and Woronin knew that the length of the tube depended upon the distance between the parasite and the outer wall of the root. This is quite clear in any material I have examined. It is not possible to observe an exit-tube on every mature thallus, possibly because of the position of the thallus in the cell, and this may, perhaps, explain the absence of exit-tubes noted by Marchal (1900) in his first record of the zoosporangia of *Asterocystis radicis*.

The gradation between the most extreme types of sporangia in all the material examined made it impossible to doubt that they belonged to one organism.

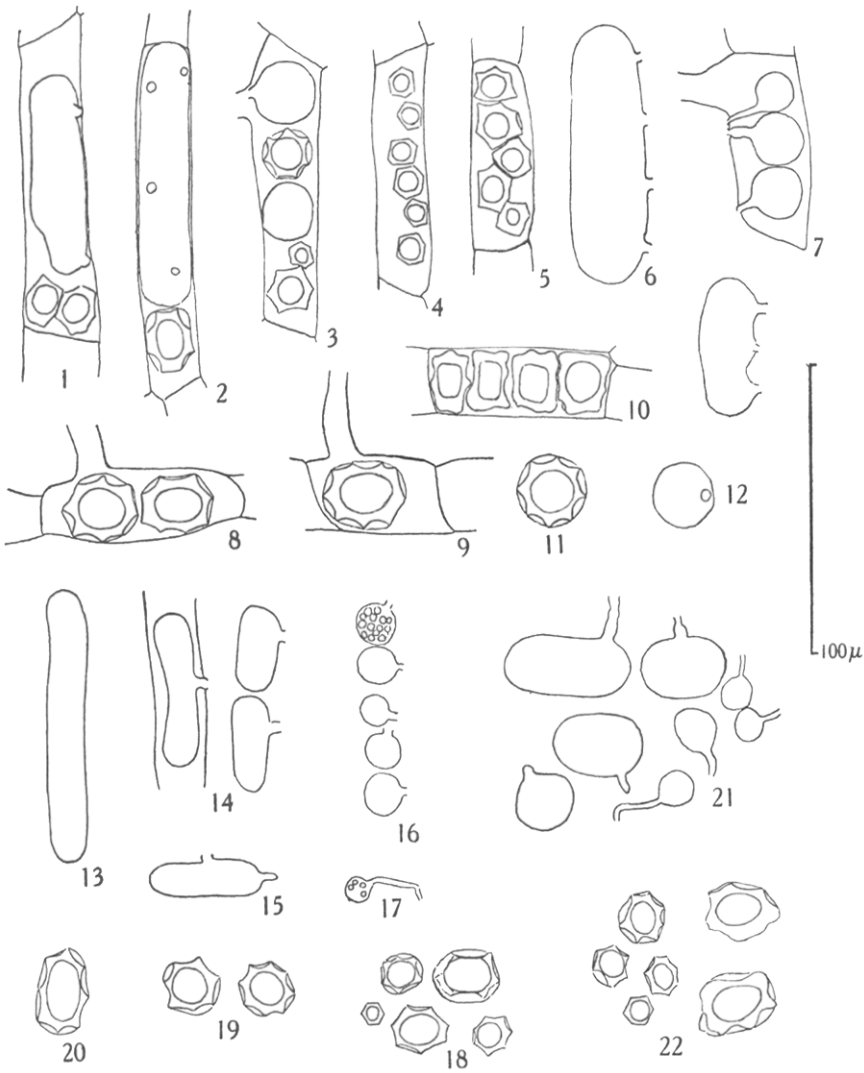
Zoospores escaped readily when suitable roots were mounted in fresh water. They are approximately 3 μ in diameter and possess a single long cilium which trails behind when swimming. The cilia are well shown in slides prepared with Noland's stain (Pl. V, fig. 8) (Noland, 1928).

(2) *Resting sporangia (cysts)*

The resting sporangia vary widely in size, ranging from 8 to 30 μ in diameter (Text-figs. 1–22). Fifty cysts taken at random from a single root of cabbage varied from 13 to 30 μ in diameter, fifty taken from a root of *Agrostis* gave a range of 8–26 μ, the modal length for both samples being 17 μ. De Wildeman gave 18–33 μ as the size of cysts of *Asterocystis radicis*, and Bartlett states that in his material they averaged 20–25 μ, but some reached 30 μ. Woronin did not provide measurements of *Olpidium Brassicae*, but it may be deduced from his figures that the cysts are 13–20 μ in diameter, and De Wildeman gave 7–10 μ as the size of cysts in this species. The cysts, like the zoosporangia, occur chiefly in the epidermal cells, only rarely in subepidermal tissue or in root hairs. They are solitary or in groups of two to six or more.

Diagnosis rests chiefly on the wall, the detailed structure of which has never been described. As in other species of *Olpidium* (Kusano, 1912, 1928–30; Sampson, 1932), the wall of the cyst is made up of three layers, a thin smooth hyaline endospore and a more strongly developed exospore with a space between. In the fungus now described the exospore is wrinkled in such a way as to form relatively high anastomosing ridges, which appear in optical section as the points of a star (Pl. V, figs. 7, 13, 15). It is usually impossible, as one realizes when trying to photograph the cysts, to get a sharp outline of several ridges in any one focal plane. When using a camera lucida a clear outline is obtained by slight changes of focus, and this gives the well-known stellate shape recognized at once as *Asterocystis radicis* (Text-figs. 4, 5, 8–11). The central circle or ellipse (= the refractive globule of De Wildeman?) represents the protoplast surrounded by the endospore, and the outer circle or ellipse in which the star appears to be enclosed is produced by the furrows.

Another way to sketch the cyst is to trace the ridges both on the surface and in profile in the hope (not always realized!) of giving the impression of a solid object. Cysts from *Agrostis* and cauliflower,



Text-figs. 1–22. Showing range in size and shape of zoosporangia and cysts of *Olpidium Brassicae* (Wor.) Dang. Figs. 1–12 from *Agrostis*; Figs. 13–18 from cauliflower; Figs. 19, 20 from cabbage; Figs. 21, 22 from swede bearing "hybridization nodules". × 370.

mounted in glycerine and drawn in this way (Pl. V, figs. 10, 11), are not altogether unlike some of De Wildeman's figures of *Olpidium Brassicae* (Pl. V, fig. 3).

Few authors have tried to show the sculpturing of the outer wall in surface view. Bensaude (1923) illustrates a cyst (tentatively identified as *O. Brassicae*) dissected from the root and drawn in perspective. A somewhat similar type of drawing is shown in Pl. V, fig. 14 of this paper.

Pl. V, figs. 12, 13, drawn under a 1/12th objective, using a camera lucida, show the ridges of the same cyst (*a*) in surface view, (*b*) in optical section. Both drawings are diagrammatic, in that images at different foci have been superimposed to give a continuous outline in one plane.

These familiar difficulties of portrayal have been mentioned only because I believe they have some bearing on the confusion which has surrounded the cysts of *O. Brassicae*. Few would doubt that the different cysts shown in Text-figs. 1–22 belong to the same organism, but they might be less ready to accept as one species figs. 7, 9–15 in Pl. V, yet their apparent differences depend only on the mounting medium, magnification and method of drawing.

In view of the scanty information supplied in Woronin's original description a fuller diagnosis is given.

Olpidium Brassicae (*Wor.*) *Dang.*

Synonyms: *Chytridium Brassicae* Wor.
Asterocystis radicis De Wildeman
Olpidium Borzii De Wildeman
O. radicicolum De Wildeman
Olpidiaster radicis (De Wildeman) Pascher.*

Zoosporangia solitary or aggregated in the host cell, thin-walled, spherical to elongate, the former 12–20 μ in diameter, the latter 20–45 × 25–220 μ, opening by one to four exit-tubes which vary in length according to the distance from the surface of the host.

Zoospores about 3 μ in diameter with a single cilium up to 17 μ in length.

Resting sporangia usually spherical, 8–25 μ in diameter (larger ones may be oval and up to 30 μ in length). Most of the cysts measure 17 μ. The exospore, which is coarsely and rather deeply wrinkled with ridges up to 3·5 μ in height, shows a stellate form in optical section, with six to nine points to the star. The endospore is thin and smooth, not united to the exospore. The cysts are probably binucleate (Němec, 1922). Their germination has not been observed.

Habitat. Roots of phanerogams, including the genera *Brassica*, *Capsella*, *Thlaspi*, *Linum*, *Plantago*, *Veronica*, *Limosella*, *Solanum*, *Avena*, *Triticum*, *Poa*, *Agrostis*, *Festuca*, *Lolium*. Associated with wet conditions.

* See Karling (1937) for a discussion of this binomial.

REFERENCES

BARTLETT, A. W. (1928). "*Olpidium radicicolum* De Wildeman and the 'Hybridization nodules' of swedes." *Trans. Brit. mycol. Soc.* XIII, 221–38.

BENSAUDE, M. (1923). "A species of *Olpidium* parasitic in the roots of tomato, tobacco and cabbage." *Phytopathology*, XIII, 451–54.

DANGEARD, P. A. (1886). "Recherches sur les organismes inférieurs." *Ann. Sci. nat. bot.*, VII, IV, 242–343.

FRON, M. & GAILLAT (Mlle) (1925). "Sur la maladie de la Brûlure des Racines de Graminées." *C.R. Acad. Agric. Fr.* XI, 119–22.

GUYOT, A. L. (1927). "Contribution à l'étude systematique et biologique de l'*Asterocystis radicis*." *Ann. Epiphyt.* XIII, 79–93.

KARLING, J. A. (1937). "Pascher and the genus *Asterocystis* of De Wildeman." *Mycologia*, XXIX, 291–94.

KUSANO, S. (1912). "On the life history and cytology of a new *Olpidium*, with special reference to the copulation of motile isogametes." *J. Coll. Agric. Tokyo*, IV, 141–99.

—— (1928–30). "Observations on *Olpidium Trifolii* Schroet." *J. Coll. Agric. Tokyo*, X, 83–99.

MARCHAL, E. (1900). "Recherches biologiques sur une Chytridinée parasite au Lin." *Bull. Agric. Belge*, XVI, 511–54.

MEER, VAN DER, J. H. H. (1926). "Rhizoctonia- en Olpidiumaantasting van bloemkoolplanten." *Tijdschr. PlZiekt.* XXXII, 209–42.

NĚMEC, B. (1912). "Zur Kenntnis der niederen Pilze IV. *Olpidium Brassicae* und zwei *Entophlyctis-Arten*." *Bull. Int. Acad. Sci. Bohême*, XVII, 16–24.

—— (1922). "Sexuality in *Olpidium Brassicae*," in *Zvláštní otisk ze Sborníku Klubu Přírodovědeckého* v. Praze, 1921.

NOLAND, L. E. (1928). "A combined fixative and stain for demonstrating flagella and cilia in temporary mounts." *Science*, LXVII, 535.

SAMPSON, K. (1932). "Observations on a new species of *Olpidium* occurring in the root hairs of *Agrostis*." *Trans. Brit. mycol. Soc.* XVII, 182–94.

VANTERPOOL, T. C. (1930). "*Asterocystis radicis* in the roots of cereals in Saskatchewan." *Phytopathology*, XX, 677–80.

WILDEMAN, E. DE (1893). "Notes mycologiques. I–III." *Ann. Soc. Belge Micro.* XVII, 5–32.

—— (1896). "Census Chytridinearum." *Mém. Soc. Roy. Bot. Belg.* XXXV, 7–69.

WORONIN, M. (1878). "*Plasmodiophora Brassicae*, Urheber der Kohlpflanzenhernie." *Jb. wiss. Bot.* XI, 548–74.

EXPLANATION OF PLATE V

The writer is indebted to Dr J. H. Western for the photographs.

Figs. 1, 2. *Olpidium Brassicae* (Wor.) Dang., zoosporangia and cysts after Woronin (1878).

Fig. 3. *Olpidium Brassicae* (Wor.) Dang., cysts (no. 22 was in glycerine) after De Wildeman (1893).

Fig. 4. Zoosporangia (*Olpidium Borzii*) and cysts (*Asterocystis radicis*) after De Wildeman (1893).

Fig. 5. Cysts from flax, identified as *Asterocystis radicis* (De Wildeman), after Marchal (1900).

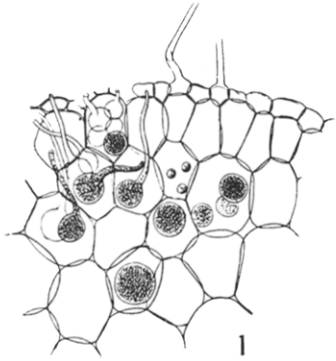
Fig. 6. Cysts and zoosporangia from swede, identified as *Olpidium radicicolum* (De Wildeman) after Bartlett (1928).

Figs. 7–15. *Olpidium Brassicae* (Wor.) Dang.

Fig. 7. Cyst in root of *Agrostis*, fixed Navashin's fluid and stained Haidenhain's Haematoxylin, photographed under 1/12th objective, oc. 12×.

Fig. 8. Zoospores stained Noland's fluid (Noland, 1928), photographed under 1/6th objective, oc. 12×.

Fig. 9. Relatively young cysts from cabbage, stained lacto-phenol cotton blue, drawn in optical secton under 1/6th objective, oc. 15×. ×590.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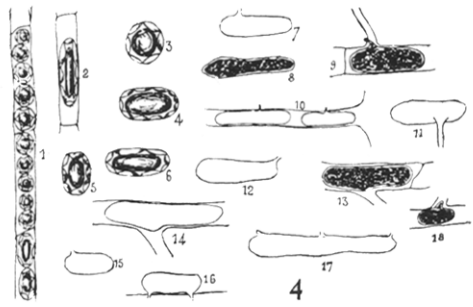
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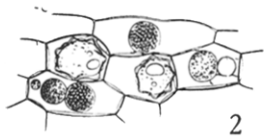
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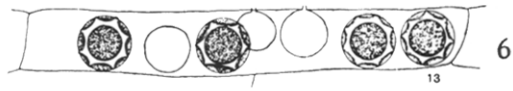
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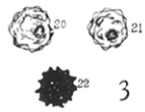
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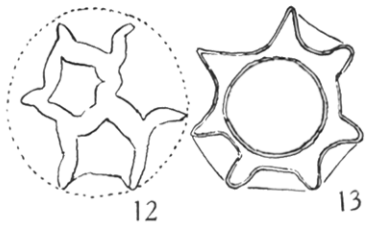
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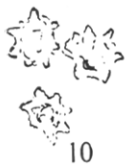
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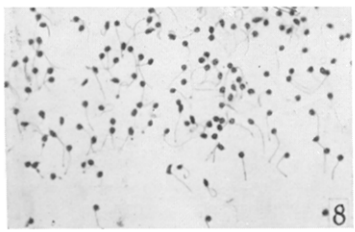
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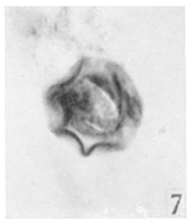
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Figs. 10 and 11. Cysts in pure glycerine, drawn as solid objects under 1/6th objective, oc. 15. ×590.
Fig. 10. From *Agrostis*.
Fig. 11. From cabbage.
Figs. 12–15. Cysts from *Agrostis*, fixed Navashin's fluid and stained Haidenhain's Haematoxylon, drawn under 1/12th objective, oc. 15. × 1720.
Fig. 12. Surface view.
Fig. 13. Optical section.
Fig. 14. Ridges in surface view.
Fig. 15. Ridges shown both in surface view and in profile.
The camera lucida has been used for all drawings with change of focus.

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