*Acta Bot. Neerl.* 21(6), December 1972, p. 633–639

# THE OCCURRENCE OF PYTHIUM IN THE NETHERLANDS

# III. PYTHIUM FLEVOENSE SP.N.

A. J. VAN DER PLAATS-NITERINK

Centraalbureau voor Schimmelcultures, Baarn

SUMMARY

A new species of *Pythium*, which in single culture produces only sporangia and zoospores, was isolated from soil and from Daphnia species. In dual cultures of compatible strains a sexual response was shown. The fungus is described as *Pythium flevoense*.

## 1. INTRODUCTION

In 1967 and 1968 CAMPBELL & HENDRIX described two heterothallic species of *Pythium: P. sylvaticum* and *P. heterothallicum*. Some other species which did not produce oogonia in single cultures or produced them only rarely were also shown to be heterothallic. These species were *P. intermedium* de Bary (VAN DER PLAATS-NITERINK 1968), *P. splendens* Braun (VAN DER PLAATS-NITERINK 1969), and *P. catenulatum* Matthews (HENDRIX & CAMPBELL 1969).

*P. sylvaticum*, *P. heterothallicum*, *P. intermedium*, and *P. splendens* do not commonly produce sporangia but form only spherical or slightly elongated hyphal swellings. In *P. catenulatum* there are, besides catenulate hyphal swellings, also sporangia consisting of irregularly swollen parts of the hyphae.

During the years from 1967 to 1971 many *Pythium* species were isolated by the author which produce non-swollen filamentous sporangia, but which do not show any sexual reproduction. These sporangia resemble vegetative hyphae or are slightly wider. The isolates do not form a homogeneous group, as there are differences in growth rate, colony pattern, and the shape of sporangia. Some of these isolates grow very slowly (3 mm in 24 hours at 25 °C), while others may have a growth rate of 7–10, 15, or even more than 20 mm in 24 hours at the same temperature. The colony pattern on agar media varies with different isolates and may be diffuse, radiate, or rosette-like.

As the possibility of heterothallism had to be considered, pairings were made between a number of these isolates.

## 2. MATERIALS AND METHOD

A great number of isolates producing only non-swollen filamentous sporangia was obtained from soils, water, and diseased plants or animals from different

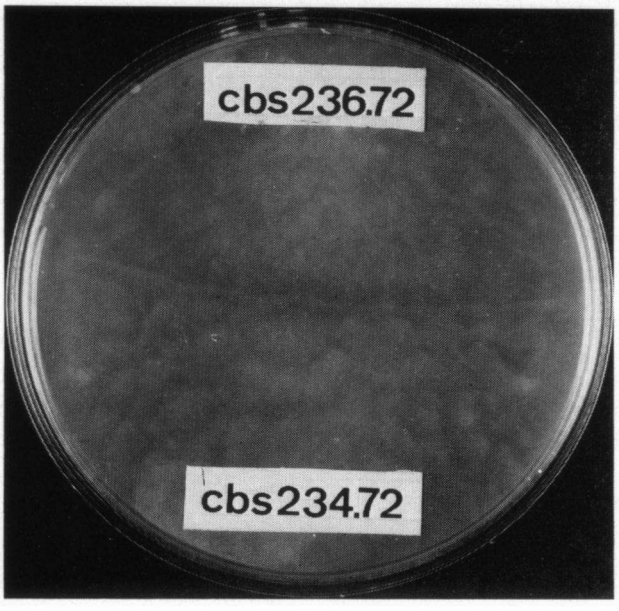


Fig. 1. Pythium flevoense CBS 234.72 × CBS 236.72, sexual response on potato carrot cholesterol agar after 8 days.

parts of the Netherlands. Among these, several isolates showed a daily growth rate of 7–10 mm at 25°C and on certain agar media a rosette pattern consisting of small triangles. These isolates and a number of others with different growth rates were used in the following mating experiments. Most isolates came from soil of the polder Zuidelijk Flevoland during the years 1968–1971. Two were from two diseased Daphnia species. The pairings were made by inoculating two isolates on opposite sides of a Petri dish containing a suitable agar medium. The media used were cornmeal agar, potato carrot agar, a mixture of cornmeal agar and potato carrot agar, hempseed agar, grass agar, and potato carrot agar to which was added 0.01 ppm chlolesterol. All pairings were kept at 20°C.

## 3. RESULTS

After about a week a small whitish line was observed in the zone of contact of both isolates in a number of the dual cultures. In this line many oogonia had been produced. The sexual response only occurred on potato carrot cholesterol agar. Neither in single cultures nor on the other media in the dual cultures were oogonia or antheridia formed. It was possible to trace the oogonial and the antheridial strain. In young cultures oogonia appeared on short lateral branches of feather-like branched hyphae near the approaching male mycelium. In ageing cultures the branching became more complicated and intercalary oogonia were also found. The oospore was in most cases aplerotic but it was difficult to discern in this stage the oogonial wall because of the amount of antheridial stalks wrapping themselves round the oogonium. The mycelium of the male strain usually had thinner hyphae and a tendency to form appressoria. In the

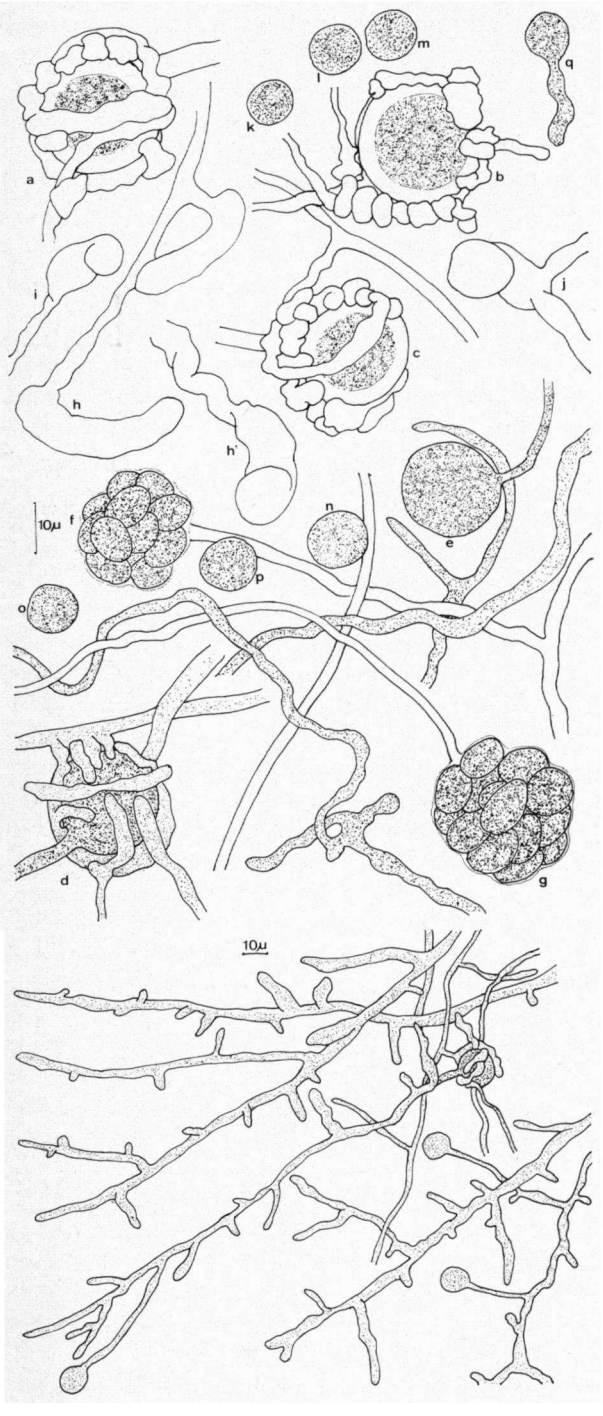


Fig. 2. Pythium flevoense. a–e, oogonia and antheridia (CBS 234.72 × CBS 236.72) f, g, vesicles just before the discharge of zoospores CBS 236.72, h, h', i and j, appressoria in CBS 236.72, k–p, encysted zoospores, q, germinating zoospore.

Fig. 3. Pythium flevoense, position of young oogonia (CBS 234.72 × CBS 236.72).

female strain this tendency was less strongly developed and, if appressoria were formed in the female strain, they were few and small. Some isolates showed an obvious rosette pattern, for example CBS 234.72. In others this pattern was only clearly visible when the culture was still young (CBS 236.72). Nevertheless it was always the same kind of rosette: small triangles becoming rounded towards the margin of the culture. Also the amount of aerial mycelium was variable: scarce and low in some isolates or none at all in others.

### 3.1. Pythium flevoense sp.n.

#### 3.1.1. Description

Hyphae vegetativae primariae ad 6μ latae; sporangia filamentosa a hyphis vegetativis haud differunt; vesiculas multas zoosporas parientes proferunt; oogonia post copulationem amborum sexuum formantur, plerumque ramulos laterales hypharum crassarum pinnate ramosarum terminantia, levia, tenuitunicata, 14–30 μ (plerumque 18–20 μ) diametro; oosporae apleroticae, nonnumquam fere pleroticae, leves 14–22 μ (plerumque 16–18 μ) diametro, pariete 2–4 μ crasso; antheridia diclina, singula vel numerosa, quorum pedicelli saepe juxta oogonium furcata vel alio modo ramosa oogonium complectuntur. In agaro et tuberibus solani et daucis mixto hyphae submersae, nonnumquam modicae et humiles hyphae aeriae, modo rosulari crescunt. Temperatura minima infra 5 °C, maxima supra 35 °C, incrementum radiale diurnum optimum (25 °C) 7–10 mm. Typus CBS 234.72, isolatus e terra, Zuidelijk Flevoland, Hollandia.

Type of growth on potato carrot agar: submerged, sometimes with a small amount of scarce low aerial mycelium and showing more or less clearly a rosette pattern. Main hyphae up to 6 μ in diameter; sporangia filamentous, not differing from the vegetative hyphae in appearance, when flooded with water producing spherical vesicles from which zoospores are discharged; oogonia only produced in dual cultures of compatible strains on potato carrot chlolesterol agar, mostly terminal on short side branches of feather-like branched hyphae, smooth, thin-walled, measuring 14–30 μ (mostly 18–20 μ) in diameter; oospores aplerotic, sometimes nearly plerotic, smooth, 14–20 μ (mostly 16–18 μ) in diameter, wall 2–3 μ thick; antheridia diclinous, one to many; antheridial stalks often bifurcate or otherwise branched near the oogonium and entwining it.

Cardinal temperatures: minimum under 5 °C, maximum over 35 °C. Optimal daily growth rate: 7–10 mm at 25 °C.

Isolated from soil from Zuidelijk Flevoland, the Netherlands, and from Daphnia species in the Netherlands.

#### 3.1.2. Cultures examined

CBS 234.72 (oogonial strain, Type culture), CBS 236.72 (antheridial strain), CBS 229.72 (oogonial strain), CBS 230.72 (oogonial strain), CBS 231.72 (oogonial strain), CBS 233.72 (oogonial strain), CBS 237.72 (oogonial strain), CBS 238.72 (oogonial strain), CBS 239.72 (oogonial strain), all isolated by the author from Zuidelijk Flevoland, the Netherlands, in 1968.

CBS 232.72 (oogonial strain) and CBS 235.72 (antheridial strain), both isolat-

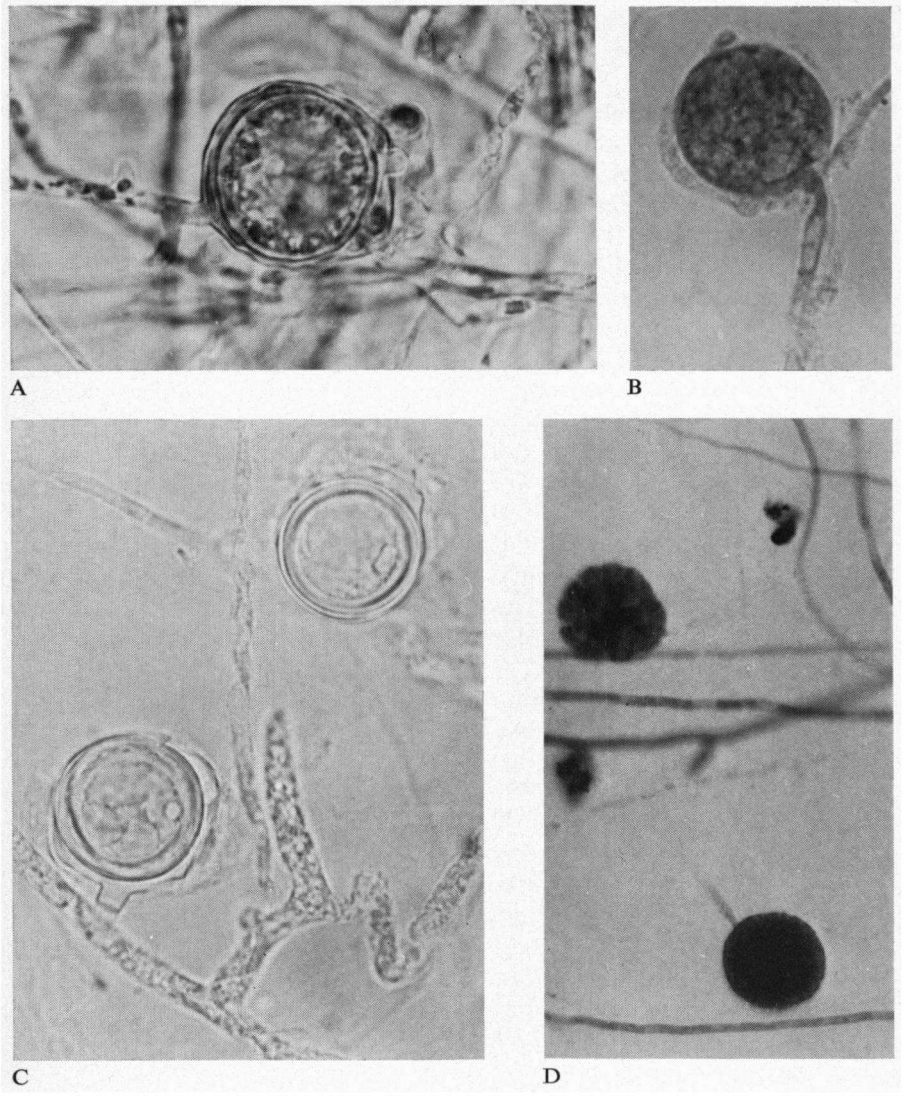


Fig. 4. Pythium flevoense. A, B, C, oogonia and antheridia in CBS 234.72 × CBS 236.72, D, sporangia and vesicles in CBS 236.72, stained with lactophenol-cotton blue.

ed by the author from two samples of Daphnia spec., the Netherlands. Strains CBS 237.72, CBS 238.72 and CBS 239.72 were isolated from the same soil sample.

## 4. DISCUSSION

Intercrossings of isolates of the group with a daily growth rate of 7–10 mm resulted in most cases in the production of oogonia and antheridia. Also a number of pairings were made with the slower or faster growing isolates. In these cases sex organs were never found.

Three *Pythium* species forming non-swollen filamentous sporangia but not producing any sex-organs have been described: *P. gracile* by SCHENK (1859), *P. reptans* by DE BARY (1860), and *P. afertile* by KANOUSE & HUMPHREY (1927). SCHENK (1859) described *P. gracile* from living fresh-water algae and considered it as a parasite. It is a doubtful species because its description is incomplete, no material is preserved, and several other species with filamentous non-swollen sporangia have been described later as parasites on green fresh-water algae, e.g. *P. dictyosporum* Raciborski, *P. tenue* Gobi, *P. apleroticum* Tokunaga, *P. angustatum* Sparrow and *P. adhaerens* Sparrow. BUTLER (1907) described a fungus from *Vaucheria* as *P. gracile* Schenk var. "a" which might be identical with Schenk's species as it showed the same kind of sporangia. This fungus, however, also formed sex-organs. Butler also mentioned a species with similar sporangia, but lacking sexual stages, which he often observed on *Spirogyra*. He was not certain about its identity, as both *P. tenue* and *P. dictyosporum* were also found on the same host. The fungi described under the name *P. gracile* by MATTHEWS (1931), and MIDDLETON (1943) and under the name *P. diclinum* Tokunaga by ITO & TOKUNAGA (1935) may be identical with Butler's *P. gracile*.

DE BARY described *P. reptans* in 1860, not being aware of *P. gracile* described by Schenk a short time before. In a footnote on page 182 of his publication he added that *P. reptans* was identical with *P. gracile* Schenk. *P. afertile*, described by KANOUSE & HUMPHREY in 1927, in addition to filamentous non-swollen sporangia, also produced "chlamydospores", spherical structures which in the author's opinion are similar to the hyphal swellings occurring in different *Pythium* species, so that the possibility that Kanouse and Humphrey dealt with a mixed culture cannot be excluded.

A later isolate identified as *P. afertile* (CBS 291.37) was studied by MIDDLETON (1943). This isolate differs from *P. flevoense* in growth rate and colony pattern. Also pairings of *P. flevoense* and *P. afertile* (CBS 291.37) did not result in a sexual response. *P. flevoense* has sporangia similar to those of *P. gracile* sensu Schenk, but differs in habitat. As many other species have the same kind of sporangia and nothing else is known about Schenk's isolate, there is no evidence of identity with this species.

Comparing *P. flevoense* with the other *Pythium* species forming filamentous non-swollen sporangia and thick-walled aplerotic oospores, the following differences are to be noticed. *P. flevoense* differs from *P. gracile* sensu Middleton in having several antheridia with often bifurcated stalks entwining the oogonium and from *P. aquatile* Höhner and *P. dissotocum* Drechsler in having diclinous antheridia. *P. adhaerens* has diclinous antheridia but these originate from a simple hypha, contrary to *P. flevoense*, in which the antheridia are formed on different hyphae. It may be better to consider *P. gracile* sensu Schenk and *P. afertile* as

doubtful species as the descriptions are incomplete and no type material is available.

ACKNOWLEDGEMENTS

The author would like to express her appreciation to Miss G. M. Waterhouse, Dr. J. A. von Arx and Dr. W. Gams for their interest and helpful criticism and to Miss J. B. Pannebakker for her technical help.

REFERENCES

BUTLER, E. J. (1907): An account of the genus Pythium and some Chytridiaceae. *Mem. Dept. Agr. India Bot.* **1**: 1–160.

CAMPBELL, W. A. & F. F. HENDRIX, JR. (1967): A new heterothallic Pythium from Southern United States. *Mycologia* **59**: 274–278.

DE BARY, A. (1860): Einige neue Saprolegnieen. *Jb. Wiss. Bot.* **2**: 169–192.

HENDRIX, F. F., JR. & W. A. CAMPBELL (1968): A new heterothallic Pythium from the United States and Canada. *Mycologia* **60**: 802–805.

— & — (1969): Heterothallism in Pythium catenulatum. *Mycologia* **61**: 639–641.

ITO, S. & Y. TOKUNAGA (1935): Notae Mycologicae Asiae Orientalis I. *Trans. Sapporo Nat. Hist. Soc.* **14**: 11–33.

KANOUSE, B. B. & T. HUMPHREY (1927): A new species of the genus Pythium in the subgenus Aphragmium. *Pap. Mich. Acad. Sci.* **8**: 129–138.

MATTHEWS, V. D. (1931): *Studies on the genus Pythium.* Univ. N. Carol. Press: 1–136.

MIDDLETON, J. T. (1943): The taxonomy, host range and geographic distribution of the genus Pythium. *Mem. Torrey Bot. Club* **20**: 1–171.

PLAATS-NITERINK, A. J. VAN DER (1968): The occurrence of Pythium in the Netherlands I. Heterothallic species. *Acta Bot. Neerl.* **17**: 320–329.

— (1969): The occurrence of Pythium in the Netherlands. II. Another heterothallic species: Pythium splendens Braun. *Acta Bot. Neerl.* **18**: 489–495.

SCHENK (1859): Algologische Mittheilungen. *Verh. phys.-med. Ges. Würzb.* **9**: 12–31.