

THE OCCURRENCE OF PYTHIUM IN THE NETHERLANDS. II ANOTHER HETEROTHALLIC SPECIES: PYTHIUM SPLENDENS BRAUN

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SUMMARY

Interpairings were made of nine strains of *Pythium splendens*. In seven strains a heterothallic response could be shown, while two strains also produced oogonia in single culture.

1. INTRODUCTION

In most isolates of *P. splendens* none or only very few oogonia are to be found. All isolates are characterized morphologically by large hyphal swellings. The rare occurrence of oogonia might be due to heterothallism. Intercrossings of the available strains were made to study this phenomenon.

2. MATERIALS AND METHODS

Only one of the available strains of *P. splendens* originated from the Netherlands. This strain CBS 252.25 was isolated in 1928 by Meurs from roots of *Nicotiana tabacum* L., which were cultivated in glasshouses. Other strains were isolated in Belgium (CBS 266.69, CBS 267.69), USA (CBS 462.48), Hawaii (CBS 338.29), the Congo Republic (CBS 268.69, CBS 269.69) and Costa-Rica (CBS 265.69). The origin of strain CBS 191.25 is not known. Crossings were made in the usual way by inoculating two isolates on opposite sides of a Petridish or of a slide covered with a thin layer of a suitable agar medium. The media used were potato carrot agar and a mixture of equal amounts of potato carrot agar and cornmeal agar.

3. RESULTS

On microscopical examination after 3 days, 5 strains showed sexual reactions with 2 other strains in the pairings on potato carrot agar. After 5 days the sexual response in these strains was also visible on the mixed medium of potato carrot agar and cornmeal agar. Two other strains formed many oogonia in single cultures.

3.1. *Pythium splendens* Braun 1925 in J. agr. Res. 30: 1061. *Pythium splendens* var. *hawaiianum* Sideris 1932 in Mycologia 24: 38-40.

3.1.1. Description

Main hyphae up to 9 μ . Sporangia and zoospores not produced. Hyphal swellings abundant in water and in agar media, spherical, smooth and thin-walled, mostly terminal, rarely intercalary, up to 49 μ mostly 25–43 μ in diameter, germinating with 1–6 germtubes, content often rather dark and densely granulated. Oogonia spherical, terminal and intercalary, 24–38 μ (mostly 27–32 μ) in diameter, wall smooth, thin. Oospores aplerotic, thick-walled, 20–33 μ (mostly 22–28 μ) in diameter. Oospore wall 1–3 (mostly about 2) μ thick. Antheridia 1–8, mostly 3–4, large, often 20 μ long and 15 μ broad, straight or crook-necked, declinous, rarely monoclinal, mostly terminal, sometimes intercalary. Antheridial stalk sometimes branched or bifurcate, often growing along the surface of the oogonium. Type of growth on potato carrot agar: submerged with some loose aerial mycelium, more or less radiate; on cornmeal agar: well developed cottony aerial mycelium. Optimal daily growth rate: > 35 mm at 25–30°C. No growth at 5°C or over 35°C.

3.1.2. Cultures examined

CBS 191.25 = ATCC 1457, sent to the CBS in 1925 by L. H. Leonian.

CBS 252.28 isolated by A. Meurs in 1928 from roots of *Nicotiana tabacum* in a greenhouse in Baarn, the Netherlands.

CBS 338.29 sent to the CBS in 1929 by C. P. Sideris as a new variety: *P. splendens* var. *hawaiianum*.

CBS 462.48 sent to the CBS in 1948 by J. T. Middleton, USA.

CBS 265.69 isolated by H. A. C. le Poole in 1966 from soil from Costa Rica.

CBS 266.69 isolated by A. Roos in 1964 from *Ericaceae* in a greenhouse in Ghent, Belgium.

CBS 267.69 isolated by A. Roos in 1964 from *Ericaceae* in a greenhouse in Ghent, Belgium.

CBS 268.69 sent to the CBS by G. Martin in 1968 from the Congo Republic.

CBS 269.69 sent to the CBS by G. Martin in 1968 from the Congo Republic.

4. DISCUSSION

Five strains produced oogonia in crossings with two other strains. On potato carrot agar the oogonia could be seen at the line of contact three days after inoculation. On the mixture of potato carrot agar after 5 days a band was formed on the line of contact. This band consisted of small whitish spots and was about 1 cm broad. In it numerous oogonia with antheridia were to be found. Most oogonia did not produce mature oospores, but degenerated, though on all oogonia always at least 1–5 antheridia were present. Only a few oospores became mature.

At the time that the antheridia are visible near the oogonium the oogonial branch has disappeared or is hardly visible. In the shape of the oogonia *P. splendens* shows some resemblance with *P. sylvaticum* Campbell et Hendrix and *P. intermedium* de Bary, but it differs from these species in the sizes of the oogo-

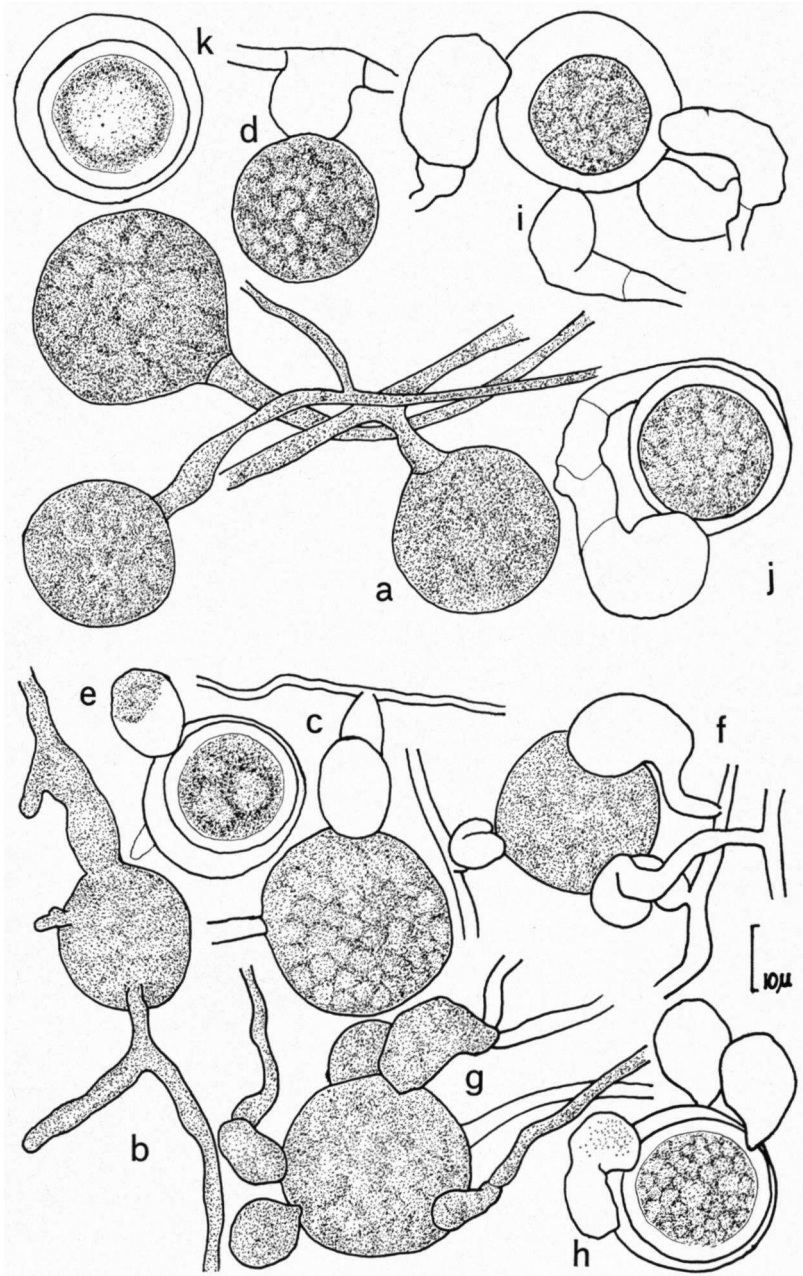


Fig. 1. *Pythium splendens* Braun
 a hyphal swellings strain CBS 462.48
 b hyphal swellings with 3 germ tubes strain CBS 252.28
 c, d, e, f, g, h, i, j, k oogonia and antheridia (strain CBS 462.48 × CBS 266.69)

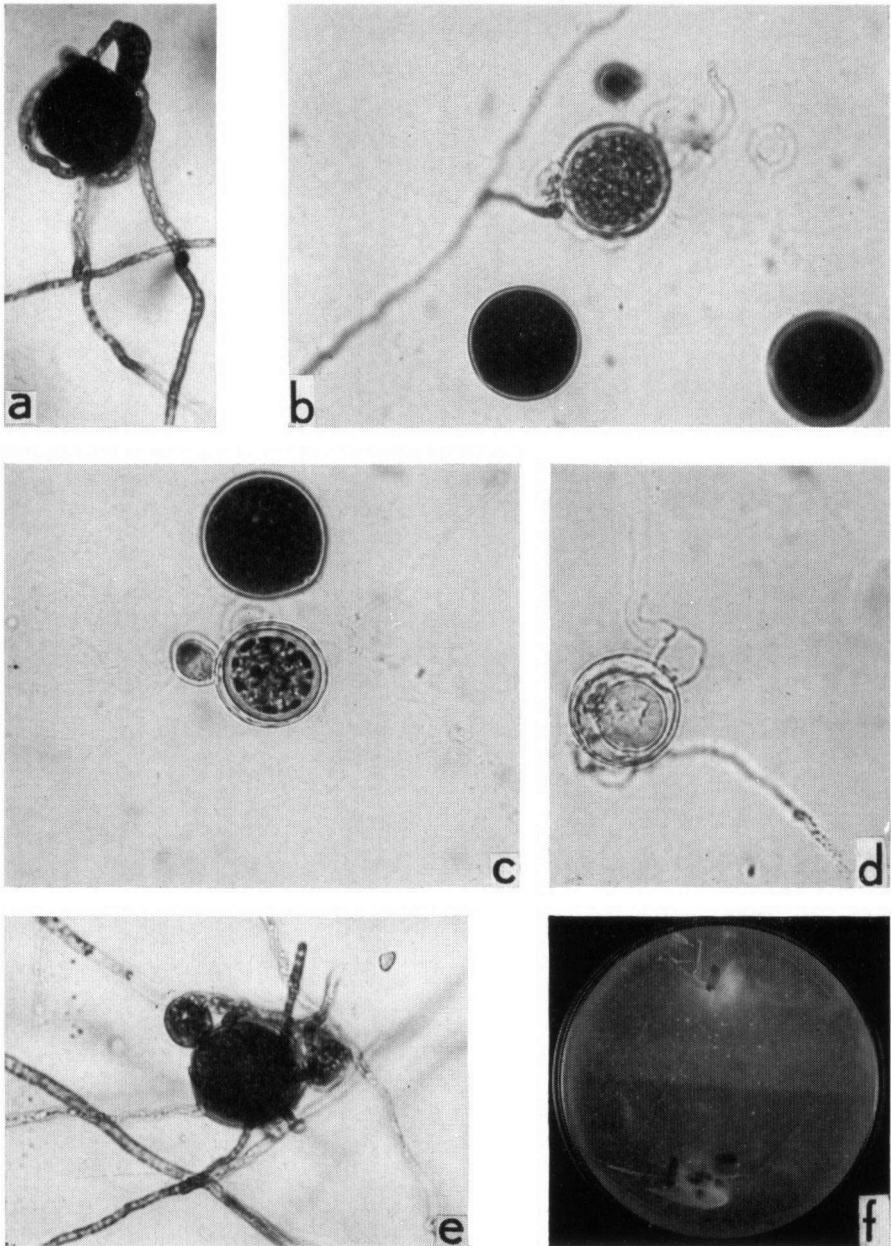


Fig. 2. *Pythium splendens* Braun strain CBS 462.48 \times CBS 266.69

a young oogonium with antheridia

b, c, oogonium, antheridia and hyphal swellings, d, e, oogonia and antheria.

f *Pythium splendens* Braun strain CBS 269.69 \times CBS 266.96, sexual response.

nia and hyphal swellings and in the thicker oospore wall. The hyphal swellings in *P. splendens* are much larger, mostly over 30 μ in diameter and often characterized by a rather dark and dense granular content and a slight thickening of the stalk under the hyphal swelling. Strain CBS 338.29 is the strain which was described by SIDERIS (1931) and considered as a new variety: "*P. splendens* var. *hawaiianum*". This strain is slightly different from the other strains in having larger hyphal swellings. In the experiments it shows a sexual reaction with strains CBS 266.69 and CBS 267.69 and according to Middleton (1943), it should be considered as insufficiently different from the species.

Among the nine strains investigated, CBS 252.28 and CBS 265.69 readily produced oogonia in single cultures and showed more or less homothallic behaviour. In one of these cultures (CBS 265.69) an increase of the number of oogonia could be seen on the places of contact in some matings. As the cultures never produced any zoospores it was not possible to make single-spore cultures. Therefore hyphal tip cultures were made and there may still be a possibility that these strains consist of a mixture of compatible strains. The shape and size of the oogonia, antheridia, hyphal swellings and the other characteristics are similar in most investigated strains.

In the Netherlands *P. splendens* has only once been recorded. MEURS (1928) isolated this species from roots of *Nicotiana tabacum*, BUISMAN (1927) and DIDDENS (1932) used the fungus for their experiments but they did not isolate it themselves. *Pythium splendens* was isolated from *Pelargonium* cuttings and described by BRAUN (1925). It has been recorded from different parts of the world: USA (MIDDLETON *c.s.* 1938; MIDDLETON 1942, 1947; BRAUN 1925), S. Africa (WAGER 1931, 1941), Malaya (SHARPLES 1930), N.S. Wales (anonymous 1956), Germany (SAUTHOFF & KRÖBER 1960), Hawaii (SIDERIS 1931; KLEMMER 1964) and many other countries (summarized in WATERHOUSE & WATERSTON 1966). It may be pathogenic to a variety of plants causing root-rot, seed-rot, pre-emergence blight or post-emergence damping off of seedlings of alfalfa and sweetclover (BUCHHOLTZ & MEREDITH 1938; HALPIN & HANSON 1953), aroids and easterlilies (TISDALE & RUEHLE 1949) *Pisum sativum* (MIDDLETON 1952; TEAKLE 1960), oil-palm (MOREAU & MOREAU 1958; ROBERTSON 1959), *Pinus elliotii* (TEAKLE 1960) and many other plants, mottle necrosis of *Ipomoea batatas* (HARTER & WHITNEY 1927) crown and stem rot of begonia and rhubarb (SWIFT 1932; MIDDLETON *c.s.* 1938, 1942; MIDDLETON 1947) and stem rot of *Pelargonium* cuttings (BRAUN 1925). Other records are from beans, cucumber, flax, lettuces, maize, pine-apple and a variety of other plants (summarized by WATERHOUSE & WATERSTON 1966).

Most records of *P. splendens* are from warm, moderate or subtropical area's. The German record originated from plants kept in greenhouses (SAUTHOFF & KRÖBER 1960). Among the material examined, the Dutch and Belgian strains were also isolated from heated greenhouses, while the other strains originated from a warm climate. In accordance with this are the facts that *P. splendens* shows hardly any growth under 10°C and requires a temperature of 25–30°C for optimal growth.

After completing this study the author received a letter from W. A. Campbell and F. F. Hendrix Jr, communicating similar results in *P. splendens*. In February 1969 they isolated *P. splendens* from several soil samples from horticultural nurseries and plantations in the Savannah, Georgia area. Among these isolates they also could distinguish homothallic and heterothallic strains.

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