

# THE OCCURRENCE OF PYTHIUM IN THE NETHERLANDS

## I. HETEROHALLIC SPECIES

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### SUMMARY

Among about 15 different species of *Pythium*, collected in the Netherlands, three species turned out to be heterothallic. By means of a great number of crossings they could be identified as: *Pythium sylvaticum* Campbell & Hendrix, *P. heterothallicum* Campbell & Hendrix and *P. intermedium* de Bary.

### 1. INTRODUCTION

During the years 1965–1968 several samples of soil, water and diseased plant material were collected from different regions of The Netherlands and examined for the occurrence of *Pythium* species. Also a number of isolates sent to the CBS by other investigators for identification is included in this study.

### 2. MATERIAL AND METHODS

The isolation of the fungi was made in various ways:

- A. Small particles of soil or diseased plant material were placed in Petri dishes containing a medium of 2% agar in water. Hyphal tips were taken from the rapidly growing Oomycetes and transferred to a cornmeal agar medium.
- B. Small particles of the material were placed in Petri dishes containing a small layer of water (pond-water/distilled water: 1/1) and two or three sterilized hempseeds. Swarming zoospores colonized the hempseeds, which were then transferred to cornmeal agar, to which had been added a certain amount of penicillin and streptomycin. In some cases other sterilized seeds or sterilized seedlings were used in the same way as hempseeds.
- C. Traps, consisting of different kinds of fruits (apple, pear, tomato) in a nylon net were placed under the water-level in ditches, canals or ponds. After some weeks they were collected and treated as diseased plant material.

When the isolates had been contaminated with bacteria a treatment with penicillin and streptomycin was applied or they were cultivated for a short time on 2% plain agar.

In many cases oogonia were produced on corn meal agar, but sometimes stimulation with wheat germ oil (500 mg/l) was necessary. As a rule sporangia are only formed in water cultures. Therefore small cubes taken from cultures on cornmeal agar were placed in dishes, containing a shallow layer of water and

a small piece of grass leaf. After a short time the sporangia are often to be found near the margin of the grass leaf. Transferring the grass leaves to fresh water after colonization or placing the dishes at a low temperature (5–10°C) for an hour or two may be favourable for zoospore-production. Slides were made from living cultures and mounted in water or in lactophenol-cotton-blue. All measurements are made from slides mounted in lactophenol-cotton-blue.

In case of heterothallic species crossings were made by transferring two or sometimes three isolates to different places in the same Petri-dish. When using compatible strains oogonia will be formed in the line where both mycelia meet. For tracing male and female branches transfers of compatible strains were made on opposite places of a slide covered with a thin layer of a favourable agar medium. To avoid contaminations and drying up these slides were kept on glass triangles in Petri-dishes, containing a small amount of water. After the mycelia had made contact an investigation could be made of the way in which the oogonia and antheridia had been formed.

### 3. RESULTS

Among the isolates several cultures failed to produce oogonia, sporangia or zoospores, but only formed globose intercalary or terminal hyphal swellings. Similar isolates had been sent to the CBS for identification, but could not be classified because of lack of sex organs and sporangia.

In 1967 CAMPBELL & HENDRIX gave a first account of a heterothallic *Pythium* which also produced globose hyphal swellings: *P. sylvaticum*. They could trace the oogonial and antheridial branches and made a differentiation between male and female strains. They will record a second one in 1968 which will be called *P. heterothallicum*. About 40 of the non-sporulating isolates were paired with male and female strains of *P. sylvaticum* and *P. heterothallicum*, kindly sent to the CBS by Dr. F. F. Hendrix Jr. Pairings were made on potato carrot agar, a transparent medium, on which oogonia could be seen easily by microscopic examination.

After some days 5 strains gave a positive sexual reaction with a male strain of *P. sylvaticum*, 5 other strains showed a positive reaction with a female strain of *P. sylvaticum* and two isolates produced oogonia in pairings with the male strain of *P. heterothallicum*. Intensive interpairings of the remaining strains in some cases demonstrated the production of oogonia on the places of mycelial contact. In these strains catenulate hyphal swellings were also produced, resembling those of *P. intermedium* de Bary. Pairings with strains of *P. intermedium* from the CBS collection also resulted in the production of oogonia. As the production of sex-organs was rather poor, the potato carrot agar was mixed with an equal amount of cornmeal agar. On this medium far more oogonia and oospores were formed.

All isolates were made from hyphal tip cultures, as none of the strains produced any zoospores.

3.1. *Pythium sylvaticum* Campbell & Hendrix, 1967 in *Mycologia* 59: 274–278.  
Fig. 1, 4a, 5e.

3.1.1 Description

Main hyphae up to  $11\mu$  in diameter. Sporangia and zoospores not produced. Hyphal swellings globose or lemon-shaped, intercalary or terminal, resembling young oogonia, scarce to abundant, up to  $32\mu$  in diameter. Appressoria single or complex, clavate or sickle-shaped. Oogonia produced sometimes scarcely in old single cultures, but abundantly in pairings with an opposite strain, spherical, intercalary or terminal,  $15\text{--}29\mu$  in diameter, wall smooth, thin. Oospores aplerotic,  $13\text{--}28\mu$  in diameter. Oospore wall smooth,  $1\text{--}2\mu$  thick. Antheridia 2–4 per oogonium. Antheridial stalks mostly bifurcate, dividing at a short distance under the oogonium, declinuous, soon vanishing after fertilisation. Antheridial cells inflated, soon losing their content. Growth type on potato carrot agar: radiate and submerged, on corn meal agar: cottony. Optimal daily growth rate: 30 mm or more at  $25\text{--}30^\circ\text{C}$ . Growth at  $0^\circ\text{C}$ : 3–5 mm, at  $35^\circ\text{C}$ : 6–15 mm.

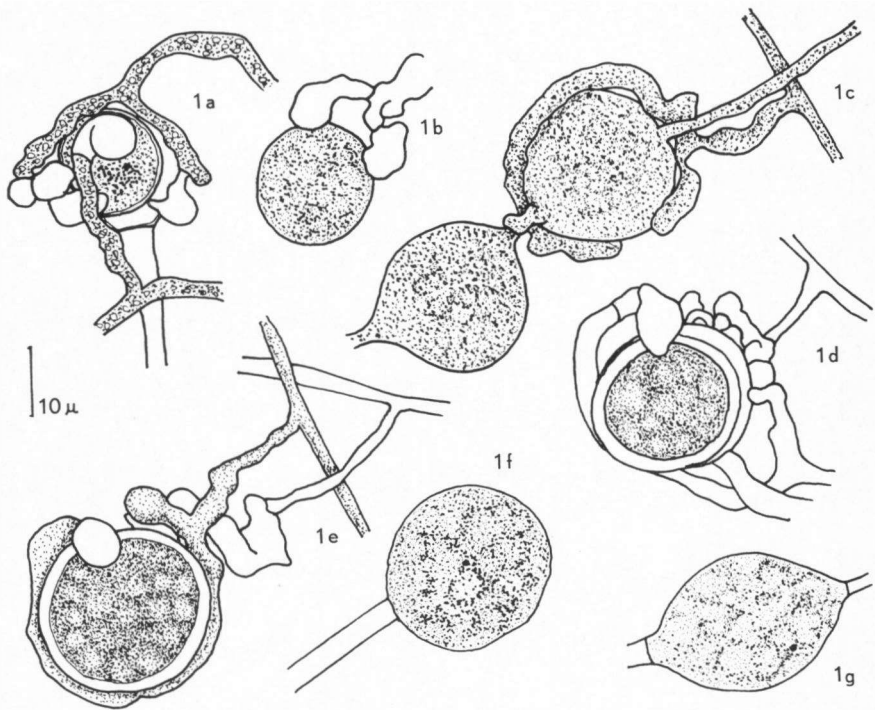


Fig. 1. *Pythium sylvaticum* Campbell & Hendrix.  
a, b, c, oogonia (strain CBS 231.68 ♀ × CBS 234.68 ♂).  
d, e, oogonia (Strain CBS 230.68 ♀ × CBS 234.68 ♂).  
f, g, hyphal swellings (strain CBS 230.68 ♀).

### 3.1.2. Cultures examined

- CBS 452.67 male strain, isolated in 1967 by F. F. Hendrix and W. A. Campbell from soil in Georgia.
- CBS 226.68 male strain, isolated by G. J. Saaltink in 1965 from *Crocus* sp. in The Netherlands.
- CBS 227.68 male strain, isolated by Ir. J. van der Spek in 1962 from *Pisum* sp. in The Netherlands.
- CBS 228.68 male strain, isolated by Ir. J. van der Spek in 1962 from *Pisum* sp. in The Netherlands.
- CBS 234.68 male strain, isolated by Ir. J. H. van Emden in 1967 from soil in The Netherlands.
- CBS 235.68 male strain, isolated by Ir. J. H. van Emden in 1967 from soil near Wageningen in The Netherlands.
- CBS 453.67 female strain, isolated by F. F. Hendrix Jr. and W. A. Campbell in 1967 from soil in Georgia.
- CBS 232.68 female strain, isolated by the author in 1965 from soil from Oostelijk Flevoland in The Netherlands.
- CBS 230.68 female strain, isolated by the author in 1967 from *Ipomoea* sp. in The Netherlands.
- CBS 229.68 female strain, isolated by the author in 1966 from soil near Rhenen in The Netherlands.
- CBS 231.68 female strain, isolated by the author in 1965 from soil from Oostelijk Flevoland in The Netherlands.
- CBS 233.68 female strain, isolated by Ir. J. van der Spek in 1962 from *Pisum* sp. in The Netherlands.

### 3.1.3. Discussion

Every female strain could be paired with every male strain. When the mycelia of compatible strains make contact a broad band of whitish mycelium is developed in which numerous oogonia and oospores are to be found. Toward the male strain this band is sharp-lined, but toward the female strain a diffuse boundary is formed. Although the nature of the reaction-line was the same in all the pairings, slight differences in the various strains could be noticed. In single cultures most female strains also produced some oogonia near the transferred pieces of the culture. This has only been found in one male strain. In old cultures of some female strains on cornmeal agar patches with oogonia are sometimes formed. In two female strains the average diameter of the oogonia was about  $21\mu$ , in two other strains about  $25\mu$  and in one strain  $22.5\mu$ . The mating male strain had no influence on the diameter of the oogonia.

*Pythium sylvaticum* is a rather common species in The Netherlands. It was collected from different plant material and from soil from different regions. Nothing is published about pathogenicity of this species, but experiments, recently taken in Wageningen, proved that it causes a rot in apple-seedlings (MULDER 1969).

3.2 *Pythium heterothallicum* Campbell & Hendrix, 1968 in *Mycologia* 60, in press. Fig. 2, 4b, 5d.

3.2.1. Description

Main hyphae up to  $7\mu$  diameter. Sporangia and zoospores not seen. Hyphal swellings globose or lemon-shaped, intercalary or terminal, abundant, mostly  $15\text{--}21\mu$ , but sometimes up to  $25\mu$  in diameter. Oogonia spherical, intercalary and terminal  $20\text{--}29\mu$  in diameter, wall smooth, thin. Oospores aplerotic,  $18\text{--}29\mu$  in diameter, oospore wall smooth,  $1.5\text{--}2\mu$  thick. Antheridia up to 8 per oogonium, antheridial cells slightly inflated, antheridial stalk branched, declinuous, sometimes forming a hyphal knot around the oogonium.

Growth type on potato carrot agar: submerged, slightly rosette, on cornmeal agar: some aerial mycelium developed, slightly zonate and rosette.

Optimal hyphal daily growth rate: 20 mm at  $25^{\circ}\text{C}$ . No growth at  $0^{\circ}\text{C}$ , growth at  $35^{\circ}\text{C}$  0–3 mm.

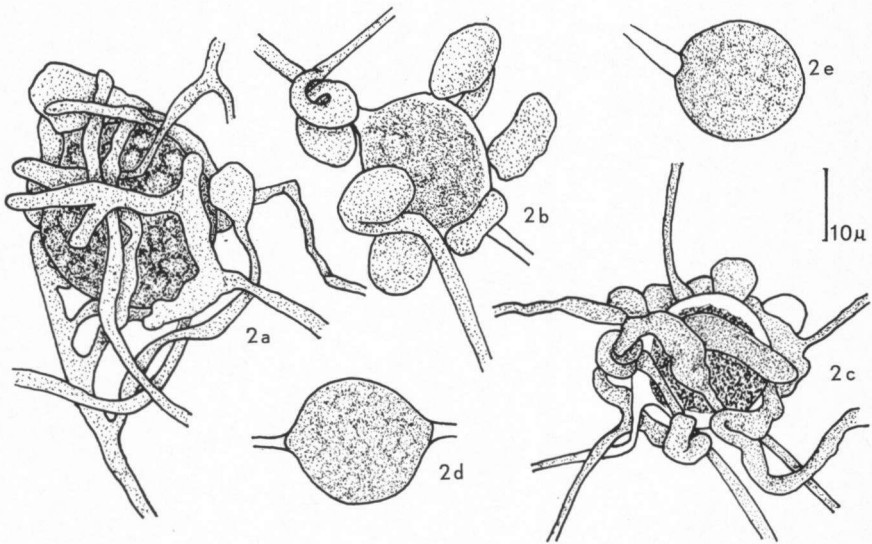


Fig. 2. *Pythium heterothallicum* Campbell & Hendrix.  
a, b, c, oogonia (strain CBS 207.68 ♀ × CBS 450.67 ♂).  
d, e, hyphal swellings (strain CBS 450.67 ♂).

3.2.2. Cultures examined

CBS 450.67 male strain isolated by Dr. A. W. Henry in 1967 from soil in Canada.

CBS 451.67 female strain, isolated by Dr. A. W. Henry in 1967 from soil in Canada.

CBS 207.68 female strain, isolated in 1966 by the author from soil in Rhenen in The Netherlands.

CBS 208.68 female strain, isolated by Le Poole from soil in Costa Rica.

3.2.3. Discussion

A strain which gave a positive sexual reaction with the Canadian male culture was isolated from only one of the Dutch soil samples. Also a strain from Costa Rica turned out to be *P. heterothallicum*. All three female strains could be paired readily with the male strain. At the place of contact of the mycelia, first a sharp white line was formed, later on becoming slightly broader and somewhat diffuse toward the female strain. Oogonia were only to be found in the places where male and female strains met. They appear about five days after inoculation. All efforts to induce the production of sporangia and zoospores failed. No investigations have been made about pathogenicity of this species.

3.3. *Pythium intermedium* de Bary, 1881. *Bot. Zt.* 39: 553–558, fig. 3, 4c, 5a, b, c. *Artotrogus intermedius* Atkinson, 1895 in *Cornell Agr. Exp. Sta. Bull.* 94: 233–272.

3.3.1. Description

Main hyphae up to  $7\mu$  in diameter. Sporangia and zoospores rarely produced. Hyphal swellings abundant in agar and water cultures, mostly formed terminally at nearly all tips of the hyphae, often closely catenulate, but readily deciduous, spherical or sometimes pear-shaped, mostly  $18\text{--}20\mu$  in diameter, sometimes up to  $25\mu$  in diameter. Appressoria sickle-shaped, simple or complex. Oogonia only formed in dual cultures, spherical, intercalary or terminal,  $18\text{--}27\mu$  in dia-

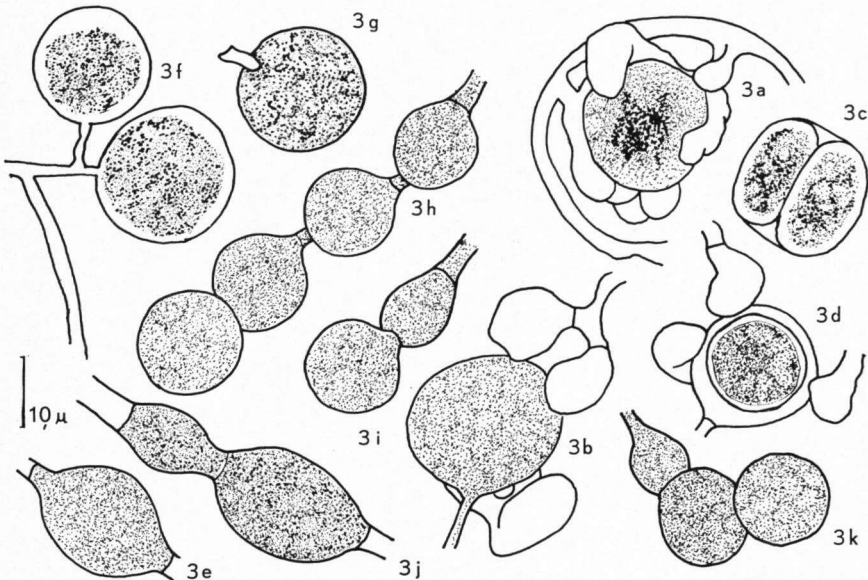


Fig. 3. *Pythium intermedium* de Bary.  
 a, b, oogonia (strain CBS 267.38 - × CBS 221.68 +).  
 c, d, oogonia (strain CBS 234.68 - × CBS 221.68 +).  
 e, f, g, h, i, j, k, hyphal swellings (strain CBS 267.38).

meter, wall smooth, thin. Oospores aplerotic, 13–22 $\mu$  in diameter, oospore wall smooth, 1–2 $\mu$  thick. Mostly one, but often two oospores per oogonium. Antheridia declinuous, antheridial stalk rather long, branched, sometimes bifurcate near the oogonium, antheridial cells inflated, crook-necked, 1–7 per oogonium.

Growth type on potato carrot agar and on potato agar: submerged, radiate; on cornmeal agar: mostly submerged; on oatmeal agar: some aerial mycelium developed.

Optimal daily growth rate: about 30 mm at 20–25°C. Minimum at about 0–5°C, maximum at about 30°C. No growth at 35°C.

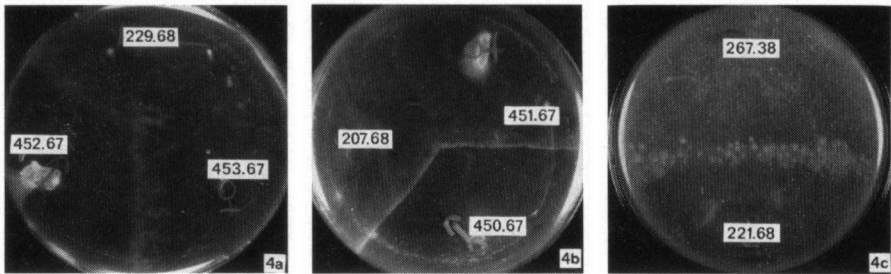


Fig. 4. Sexual response in a, *P. sylvaticum*, b, *P. heterothallicum*, c, *P. intermedium*.

### 3.3.2. Cultures examined

- CBS 266.38 — strain isolated in 1938 by A. van Luyk from *Agrostis stolonifera* L., The Hague, The Netherlands.
- CBS 267.38 — strain isolated in 1938 by J. G. ten Houten from *Pinus silvestris* L., in The Netherlands.
- CBS 268.38 — strain isolated in 1938 by J. G. ten Houten from *Pinus nigra* Arn. var. *austriaca* Ant., The Netherlands.
- CBS 380.34 — strain isolated in 1934 by A. Meurs from *Ricinus communis* L. from a greenhouse in Baarn, The Netherlands.
- CBS 221.68 + strain isolated in 1966 by the author from soil near Rhenen, The Netherlands.
- CBS 222.68 — strain isolated in 1965 by G. J. Saaltink from *Hyacinthus spec.*, from Lisse, The Netherlands.
- CBS 223.68 + strain isolated by the author from diseased *Scilla spec.* near Egmond, The Netherlands.
- CBS 224.68 — strain isolated in 1967 by the author from diseased *Scilla spec.* near Egmond, The Netherlands.

### 3.3.3. Discussion

Among the non-sporulating isolates some showed catenulate hyphal swellings. No oospores were produced in crossings of these strains with strains of *P. sylvaticum* or *P. heterothallicum*. In some cases interpairings of these strains resulted after six days in the production of oogonia and oospores. Also in dual

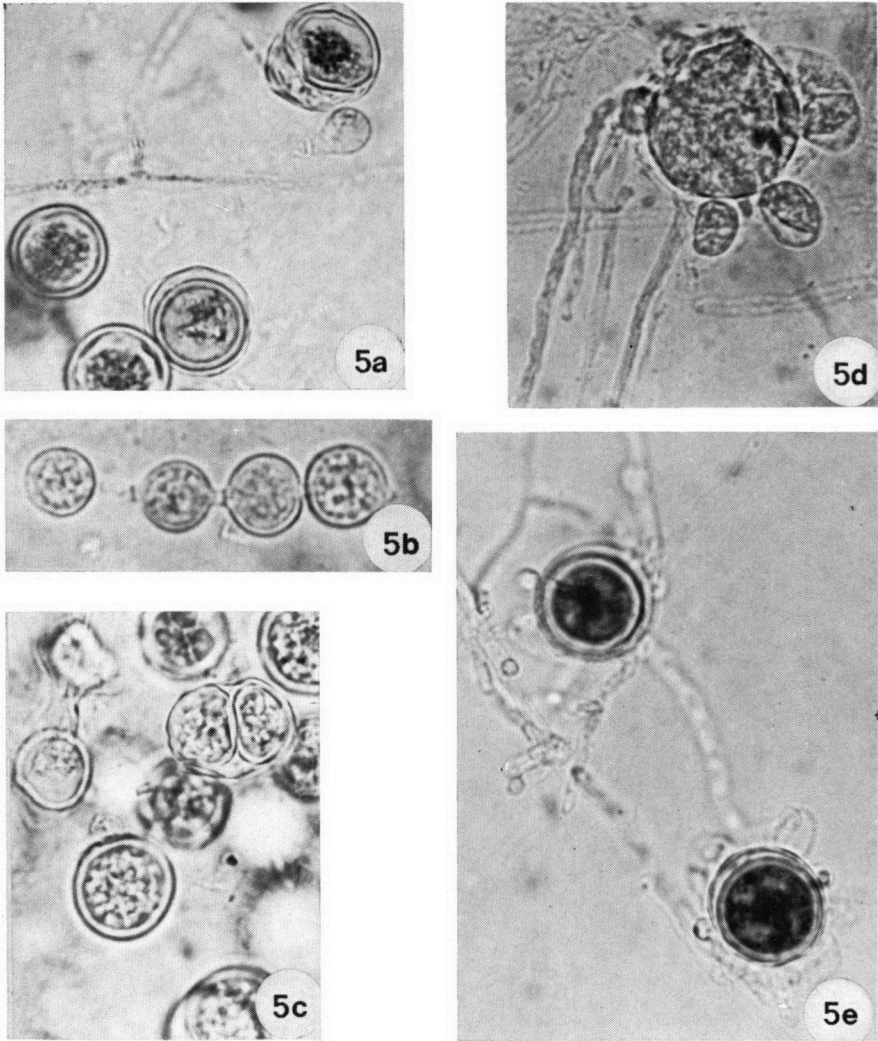


Fig. 5. a, b, c, *P. intermedium*, d, *P. heterothallicum*, e, *P. sylvaticum*.

cultures with strains of *P. intermedium* from the CBS culture collection a sexual reaction was to be seen on the places of contact. On potato carrot agar only a few oogonia were produced, but on a mixture of potato carrot agar and cornmeal agar a conspicuous broad line, consisting of patches with oogonia was formed at the meeting place of both mycelia. As this was formed only after at least six days much submerged mycelium was produced too. For this reason it was not possible to trace antheridial or oogonial hyphae. Therefore strain CBS 267.38 was preliminary indicated as a - strain and its compatible strains as + strains.



*P. intermedium* shows much resemblance with *P. sylvaticum* especially in the shape and size of the oogonia and the antheridia. It differs from *P. sylvaticum* in the number of antheridia and in having catenulate hyphal swellings, which are mostly smaller than the hyphal swellings in *P. sylvaticum*. *P. intermedium* sometimes has two oospores in one oogonium and produces less aerial mycelium on cornmeal agar than *P. sylvaticum*. Dual cultures of compatible strains of *P. sylvaticum* make abundant oogonia within three days on potato carrot agar, while in *P. intermedium* only few oogonia are produced after at least 6 days on this medium. Also the contact lines of the mycelia in dual cultures are different in these two *Pythium* species. The optimal growth is in *P. sylvaticum* at 25–30°C, in *P. intermedium* at 20–25°C. At 30°C growth in *P. intermedium* is already much decreased, while in *P. sylvaticum* growth still occurs at 35°C. *Pythium intermedium* has been reported from several hosts and from several countries: Germany (DE BARY 1881; SCHULZ 1939, 1950), England (HULL 1949; GATES & HULL 1954; MONTGOMERY 1954), Belgium (MARCHAL & MARCHAL 1921), U.S.A. (MIDDLETON 1938, 1942; NANCE 1939), Argentina (FREZZI 1956), Hawai (SIDERIS & PAXTON 1931). In the Netherlands *Pythium intermedium* is a common fungus and has often been isolated (BUISMAN 1927; MEURS 1928; WESTERDIJK & BUISMAN 1929; WESTERDIJK *c.s.* 1931; VAN LUIJK 1938; VAN EEK 1938). From soil from France, England and Germany this species was isolated by BUTLER 1907.

*P. intermedium* is strongly pathogenic to tomato and *Pinus* seedlings (MONTGOMERY 1954; TEN HOUTEN 1939) and to *Pelargonium* and *Chrysanthemum* cuttings (BUISMAN 1927). It causes a rootrot of pansies (VAN EEK 1938), a stemrot of tuberous begonias (MIDDLETON 1938) and may be a cause of black leg disease of sugarbeets (GATES & HULL 1954). Moreover this fungus may be capable of attacking *Lupinus* (SCHULZ 1950), flax (DIDDENS 1931) and prothallii of ferns and *Equisetum* sp. (ATKINSON 1895; DE BARY 1881). According to an information from Dr. D. Mulder the strains CBS 267.38 and CBS 221.68 are strongly pathogenic to apple-seedlings (MULDER 1969).

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