Species of Pythium in the Netherlands

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Abstract

A review is given on observations of *Pythium* spp. so far published. In personal investigations about 4000 isolates of *Pythium* species were obtained from soil, water, Daphnias and plants during 1966–1972.

Special attention was paid to the progressive colonization of the newly reclaimed polder Zuidelijk Flevoland: the numbers of species per sample increased from 0 in 1966 to 13 in 1972. The percentage of samples which did not contain any *Pythium* decreased from 54 in 1968 to 0 in 1972. From Zuidelijk Flevoland altogether 21 species were isolated, from Oostelijk Flevoland 22, of which 19 occurred in both the polders. The soil depth down to 10 cm had little influence on the occurrence of *Pythium* species.

From soils near Rhenen and Zutphen 12 and 19 species respectively were isolated, from flax and flaxfields 11, from forests 8, from roots of different plants 17 and from water 11. Besides species already previously recorded, 15 new records of *Pythium* species for the Netherlands are documented.

Species with filamentous non-swollen sporangia preferentially occurred in water and wet soils while species with spherical sporangia or hyphal swellings dominated in cultivated soils, forests and in the roots of phanerogams. In 1968 species with filamentous non-swollen sporangia comprised 89% of the isolations from Zuidelijk Flevoland; this percentage decreased to 9 in 1972. The percentage of isolates with spherical sporangia or hyphal swellings from cultivated soils, forest soils or plant material was mostly over 90. *P. rostratum* Butler, though found in all kinds of soils, was often the only species which could be isolated from dry sandy soil samples. *P. sylvaticum* Campbell & Hendrix was the most common species in cultivated soils in the Netherlands (37% of the isolates in 1968–1972), fo llowed by *P. oligandrum* Drechsler (22%) and *P. paroecandrum* Drechsler (11.5%).

Introduction

In 1907 Butler described 18 species of the genus *Pythium* Pringsheim. Matthews (1931) monographed 41 species. In 1943 Middleton dealt with 66 species in his comprehensive work about the taxonomy, host range and geographic distribution of *Pythium*. The most recent useful contributions on the genus *Pythium* are a key and a compilation of diagnoses of *Pythium* by Waterhouse (1967, 1968). Since Middleton's monograph 38 new species have been described of which 27 are now recognized as valid species.

Species of *Pythium* can be found all over the world. Many species, e.g. *P. hydnosporum* (Mont.) Schroet., *P. intermedium* de Bary, *P. irregulare* Buisman, *P. mamillatum* Meurs, *P. middletonii* Sparrow, *P. monospermum* Pringsheim, *P. rostratum* Butler and *P. ultimum* Trow have a large area of distribution. Also *P. debaryanum* Hesse and *P. gracile* Schenk have been often recorded, but probably these records do not always refer to these species. A number of the records of *P. debaryanum* certainly

are identical with heterothallic species such as *P. sylvaticum* Campbell & Hendrix. A part of the records of *P. gracile* may concern *P. gracile* as described by Middleton (1943), but others probably belong to species which produce only filamentous non-swollen sporangia and which are possibly heterothallic. Other species are mentioned only from one or a few places, possibly because of insufficient investigation or because the species is very rare indeed.

Species of the genus *Pythium* are known from plants, soil, water and aquatic animals. They can be common fungi and occur in great numbers, but they also may cause severe plants diseases, often appearing as damping-off of seedlings and root rots. Studies of the genus *Pythium* were mostly carried out by or in cooperation with plant pathologists, and the strains studied usually originated from diseased plant material (Table 1). Because little is known about the distribution in the Netherlands, the occurrence of *Pythium* in the soil in this country was studied from a taxonomic point of view; but since most of the species encountered are potential plant pathogens, it might have also a phytopathological bearing.

Materials and methods

From 1966 to 1972 more than 250 samples of soil, water and diseased plants and animals originating from different parts of the Netherlands were examined. The soil samples were usually taken from the same localities at different times of the year; in the other cases the diseased plants or animals or pure cultures were sent to the CBS for examination and identification.

The isolations were made in various ways:

- 1. Small amounts of soil or small parts of plants or animals were placed on 2% water agar.
- 2. Similarly particles were placed on cornmeal agar to which after sterilization were added 5000 units of penicillin and 10.000 units of streptomycin per Petri dish.
- 3. Small parts of the material were put in Petri dishes containing sterile water (1/3 pond water + 2/3 aq. dest.) and 3 sterilized hempseeds. Sometimes other seeds were used too. After colonization the seeds were placed on an agar medium.
- 4. A soil sample was diluted with about 15 times its volume of sterile sand. About half this volume of water and three sterilized seeds (corn, hemp, garden cress) were added. After 1-24 hours the seeds were removed and laid out on agar.
- 5. For isolating *Pythium* and other Oomycetes from water, nylon nets containing fruits (apple, pear, tomato, etc.) were placed under the water surface of ditches, rivers, lakes, etc. After 1-3 weeks small parts of the fruits were placed on agar.
- 6. More recently a low concentration of benomyl (< 50 ppm) was added to the agar to suppress the growth of other fungi.

Hyphal tips of the growing colonies were transferred as soon as possible to a cornmeal-agar plate. When the isolates were still contaminated with bacteria, a passage over penicillin and streptomycin-containing agar was applied.

For identification it was mostly necessary to cultivate the isolates in water: small agar blocks (about 0.5 cm diam.) were placed in a Petri dish containing water (1/3) pond water (1/3) aq. dest.) together with a piece of a grass leaf of approximately 2.5 cm length which was sterilized by boiling for 10 minutes. In many cases sporangia were soon formed, often especially along the margin of the grass leaf. The temperature

Table 1. Published records of Pythium in the Netherlands.

Species	Substrate and symptoms	Author
P. afertile Kanouse & Humphrey	pansies (root rot)	Van Eek (1937, 1938)
P. aphanidermatum (Edson) Fitzpatrick	pansies (root rot)	Van Eek (1937, 1938)
P. artotrogus (Mont.) de Bary (= P. hydnosporum (Mont.) Schroet.)	Pinus seedlings (damping-off) Spinacia, Arabis (root rot), soils from flax fields	Ten Houten (1939) Meurs (1928)
P. debaryanum Hesse	aster, reseda (damping-off)	Ritzema Bos (1896)
	potatoes (rot), Beta (root rot)	Staes (1896)
	Lepidium cotyledons	Oudemans (1897)
	Beta seedlings (root rot) Spergula (rot)	Ritzema Bos (1902)
	Pinus (damping-off)	Ritzema Bos (1903) De Koning (1927)
	Beta, Spinacia (root rot),	Meurs (1928)
	Phaseolus, Lens (root rot),	Wicuis (1926)
	Sinningia (bulb and stem rot)	
	grasses (from brown patches in golf links in Hilversum)	Van Luijk (1934)
	pansies (root rot)	Van Eek (1937, 1938)
	Pinus seedlings (damping-off)	Ten Houten (1939)
P. debaryanum Hesse var.	Spinacia, Pisum, Phaseolus, Lens,	Meurs (1928)
pelargonii Braun	Aquilegia, Eschscholtzia,	
(= P. sylvaticum Campbell & Hendrix)	Arabis, Viola, Lupinus, Godetia, (root rot), soil from flax fields,	
Campbell & Heliditx)	Sinningia (bulb and stem rot)	
	Cedrus seedlings	Ten Houten (1939)
P. echinulatum Matthews	pansies (root rot)	Van Eek (1937, 1938)
P. flevoense Van der Plaats- Niterink	Daphnia sp., soil from Zuidelijk Flevoland	Van der Plaats-Niterink (1972)
P. heterothallicum Campbell & Hendrix	soil near Rhenen, Netherlands	Van der Plaats-Niterink (1968)
P. intermedium de Bary	Chrysanthemum cuttings (root rot)	Buisman (1927)
	Pisum, Phaseolus, Fragaria (root rot), cucumber seeds, Aquilegia, Arabis, Hesperis, Viola, Godetia (root rot), soil under Beta	Meurs (1928)
	Agrostis (brown patches in golflinks near Hilversum)	Van Luijk (1934)
	pansies (root rot)	Van Eek (1937, 1938)
	Pinus seedlings (damping-off)	Ten Houten (1939)
	Scilla (root rot), soil near Rhenen	Van der Plaats-Niterink (1968)
n. / n:	hyacinth (root rot)	Saaltink (1969)
P. irregulare Buisman	Pisum (root rot), Lupinus, cucumber seeds	Buisman (1927)
	Pisum, Phaseolus, Lens, Sinapis (root rot), Viola, Antirrhinum, Calceolaria, Nemesia (root rot)	Meurs (1928)
	Agrostis (brown patches in golf links near Hilversum)	Van Luijk (1934)
	hyacinth (root rot)	Saaltink (1969)

Species	Substrate and symptoms	Author
P. mamillatum Meurs	Beta (root rot)	Meurs (1928)
	Beta (root rot)	Van Poeteren (1930)
	flax ('vlasbrand')	Diddens (1931, 1932)
P. megalacanthum de Bary	Pelargonium cuttings, Chrysanthemum cuttings (rot)	Van Poeteren (1938)
P. megalacanthum de Bary	flax ('vlasbrand')	Buisman (1927)
ss. Buisman	flax ('vlasbrand')	Van der Meer (1928)
	flax ('vlasbrand')	Diddens (1931, 1932)
	flax ('vlasbrand')	Wiersema (1955)
P. monospermum Pringsheim	Richardia, Spinacia (root rot)	Meurs (1928)
P. paroecandrum Drechsler	hyacinth (root rot)	Saaltink (1969)
P. perniciosum Serbinov	pansies (root rot)	Van Eek (1937, 1938)
P. splendens Braun	Nicotiana (damping-off)	Meurs (1928)
P. sylvaticum Campbell & Hendrix	soil from Zuidelijk Flevoland, soil near Rhenen and near Wageningen, Crocus, Pisum (roots), Ipomoea (seeds)	Van der Plaats-Niterink (1968)
	apple (root rot)	Mulder (1968)
	hyacinth (root rot)	Saaltink (1969)
P. torulosum Coker & Patterson	grasses (brown patches in golf links in Hilversum)	Van Luijk (1934)
	Abies and Pinus seedlings (damping-off)	Ten Houten (1939)
P. ultimum Trow	tulips (root rot)	Moore & Budding (1937)
	hyacinth (root rot)	Saaltink (1969)
P. undulatum Petersen	water in pond in Baarn	Van Beverwijk (1948)
P. vexans de Bary	Hydrangea	C. Dorsman, listed in Centraalbureau voor Schimmelcultures, List of Cultures (1962)
P. violae Chesters & Hickman P. volutum Vanterpool & Truscott	hyacinth (root rot) grasses (brown patches in golf links in Hilversum)	Saaltink (1969) Van Luijk (1934)

Tabel 1. Gepubliceerde vondsten van Pythium in Nederland.

may be of importance: many species produce sporangia and also oogonia at a temperature of 20°C, other species require a temperature of 17°C or even 5°C. Some species occurring in warmer countries and in glass-houses, however, often prefer 25–30°C.

Identification was sometimes difficult because many isolates produced only sporangia but no oogonia. A number of heterothallic species may still be among them. The imperfect strains were subdivided according to sporangial characteristics into four groups:

Group F: species with filamentous non-swollen sporangia
Group T: species with filamentous swollen sporangia
Group P: species with spherical proliferating sporangia

Group HS: species with hyphal swellings only.

Group F is heterogeneous. At least 4 sub-groups were distinguished by their growth characteristics. Group T consists possibly of two or more species and group P of one or more species. In group HS it was not possible to identify species properly before 1967. By means of mating these isolates with known heterothallic species most of them could be identified. Yet in a number of cases no sexual response was shown.

These isolates are collected in group HS. Species which only produced a few oogonia, but not enough for identification are indicated as *Pythium* spec.

Results

1. Zuidelijk Flevoland (Table 2)

Colonization of the freshly reclaimed polder soil by *Pythium* was followed from the beginning in 1966 (Table 2). Then this polder had just fallen dry and a sample of the soil was studied, but no *Pythium* species could be isolated from it. During the years 1968–1972 ten times a series of 8–15 samples was examined. In 1968 some parts of the new land were not yet covered with plants. In other parts most of the area was covered by a vegetation of *Senecio congestus* and *Phragmites australis*. As all this land was not yet firm enough to walk on because of the presence of quicksands or marshland, most of the samples had to be taken just inside the dike which surrounds the polder. Always the upper 10 cm layer of the soil was sampled. At each sam pling the

Table 2. Survey of the *Pythium* species isolated from soil of Zuidelijk Flevoland during 1 968–1972. The figures indicate the numbers of samples from which a species was isolated.

	1968			1969			1970			1972	Total	
	May	Aug.	Oct.	Мау	Aug.	Oct.	May	July	Oct.	May	number of samples	
Series (and											_	
number of samples)	1(10)	2(10)	3(15)	4(12)	5(10)	6(9)	7(10)	8(8)	9(9)	10(10))	
P. adhaerens	_	_	_	1		1	1	_	-	_	3	
P. angustatum	_	-		~	_	_	_	1	_	1	2	
P. dissotocum	~	_	-	-		1	_	_	~	_	1	
P. flevoense	4	-	1	~	_	_	1	_	-	1	7	
P. gracile	-	_	2	_	-	2	_	-	2	_	6	
P. heterothallicum	-	_	-	-	_	1	-	-	-	-	1	
P. intermedium	_	-	_	_	-	1	-	_	-	_	1	
P. irregulare	-	_	-	-	-	_	-	_	1	-	1	
P. middletonii	-	_	_	-		_	_	1	-	-	1	
P. monospermum	-	-	-	1	-	_	1		-	2	4	
P. oligandrum	-	-	-	-	-	_	-	1	-	2	3	
P. paroecandrum	-		-	-	-	_	1	1	1	3	6	
P. rostratum	_		-	-	_	_	1	1	1	7	10	
P. sylvaticum	_	_	_	1	- .	_	1	2	_	2	6	
P. torulosum	_	1	-	-	_	-	1	_	4	1	7	
P. ultimum	_	_	-	-	_	-	_	1	-	1	2	
P. spec.	_	_	_	-	_	_	2	_	1	1	4	
P. group F	4	1	10	6	2	6	6	4	5	4	48	
P. group HS	-	_	-	-	_	_	-	_	3	3	6	
P. group P	1		-	1	3	1	-	1	3		10	
P. group T	-	-	1	-	2	2	5	6	5	5	26	
Number of species Number of samples		2	4	5	3	8	10	10	10	13	21	
without <i>Pythium</i> spp		9	5	6	6	2	3	0	2	0	38	

Tabel 2. Overzicht van de Pythium isolaties uit de grond van Zuidelijk Flevoland gedurende 1968-1972.

same route was followed so that in each series samples were taken from the same area. Altogether 103 samples were involved in these investigations (Table 2). The number of species increased from 3 in May 1968 to 10 in May 1970 and 13 in May 1972. An unusually low number of species in August 1969 (series 5) may be due to a warm and dry period just before the samples were collected. In 1968 54% of the samples did not contain any species of *Pythium*, in 1969 this percentage was 45 and in 1970 19. Each sample of series 10 contained *Pythium* spp.

Influence of soil depth on the distribution of Pythium spp. Not much is known about the occurrence of Pythium in different levels of the soil. Most species have been isolated from the upper layers, but a few records are given of finds at a greater depth. Harder and Uebelmesser (1957) found Pythium spp. at depths of 70, 75 and 355 cm in samples from a trench in Göttingen, but not in layers between 120 and 200 cm. Apinis (1964) isolated Pythium spp. from layers at depths of 60–100 cm in England. Vaartaja and Bumbieris (1964) obtained 10 species at a depth of 7–10 cm.

Soil samples (109), mostly originating from Zuidelijk Flevoland consisted of two parts: one from the upper 5 cm layer, the second from a layer at a depth of 5-10 cm below the surface. In 21 cases no *Pythium* spp. were found in both layers. In 16 cases only the upper contained *Pythium* and in 6 cases only the lower level. 28 Samples contained the same number of species in both levels. In 41 cases more species were isolated from the upper layer than from the lower layer and in 19 cases more from the lower layer. The number of the different species in the samples varied from 0-7. In both levels the same species occurred with an exception of *P. dissotocum* Drechsler (observed only twice in the lower level), *P. flevoense* (only twice in the upper layer) and *P. heterothallicum* (only once in the upper layer). Common species such as *P. sylvaticum*, *P. paroecandrum*, *P. intermedium*, *P. oligandrum* Drechsler and *P. rostratum* were present as well in the samples from the upper as from the lower level. On the whole there was little influence of soil depth down to 10 cm.

2. Oostelijk Flevoland (Table 3)

In 1957 this polder had fallen dry and now it consists for the greatest part of cultivated land. From October 1969 to July 1971 six series of soil samples were collected from different parts of Oostelijk Flevoland: from dry sandy soil near bird's nests or rabbit burrows, from fertile arable fields, from pastures and verges (Table 3). Most samples were taken from the south-western part of this polder between Lelystad and Harderwijk.

In the dry soils mostly only *P. rostratum* was found or no *Pythium* at all, in rare cases 1 or 2 other species were present in such a sample: *P. sylvaticum*, *P. intermedium*, *P. oligandrum* and a few isolates belonging to group F.

Pythium spp. were often abundant in the samples from other places ranging in number from 4 to 10 per sample.

Comparing the occurrence of *Pythium* spp. in Zuidelijk Flevoland with observations of Oostelijk Flevoland (Table 4) it is obvious that most species present in Oostelijk Flevoland also have appeared in Zuidelijk Flevoland after some years. Of the 22 species isolated in Oostelijk Flevoland only 3 have not yet been found in Zuidelijk Flevoland: *P. acanthicum* Drechsler, *P. periplocum* Drechsler and *P. dissimile* Vaartaja.

Table 3. Survey of *Pythium* species from soil of Oostelijk Flevoland during 1969–1971.

	Date (and number of samples)												
	October 1969(3)	May 1970(4)	July 1970(9)	October 1970(7)	May 1971(10)	July 1971(13)							
P. acanthicum	_	+	_		_	_							
P. adhaerens	_	_	_	_	_	+							
P. angustatum	_	_	-	_	_	+-							
P. dissimile	-	_		+	_								
P. dissotocum	_	_	_	+	_	+-							
P. gracile	+	+	+	+	_	_							
P. intermedium	+		_	+	+	+							
P. irregulare	+	_		_	_								
P. middletonii	+	+	+	_	_								
P. monospermum	+	_	_	_		_							
P. oligandrum	+	+	+	+	+	+							
P. paroecandrum	+	+	+	+	+	+-							
P. periplocum	_	_	+	_	_	+							
P. rostratum	+	+	+	+	+	+-							
P. sylvaticum	+	+	+	+	+	+-							
P. torulosum	+	_	+	+	+	+							
P. ultimum	_	+	+	+	+	+							
P. spec.	+			+	+	+							
P. group F	+	+	+	+	+	+							
P. group HS	_	+	-	+	+	+							
P. group P	+		+	_	_	+							
P. group T	+	+	+	+	+	+							
Number of species	14	11	12	14	11	16							

Tabel 3. Overzicht van de Pythium isolaties uit grond van Oostelijk Flevoland gedurende 1969-1971.

These three species were only found in a limited number of samples. Two species found in Zuidelijk Flevoland were not isolated from soil from Oostelijk Flevoland: *P. heterothallicum*, which occurs in other parts of the Netherlands, but is certainly not a common species here, and *P. flevoense*, so far only isolated from soil from Zuidelijk Flevoland and from diseased Daphnias. This species may be more common, but its identification is not easy as it is a heterothallic species and its oogonia are produced only on special media.

3. Rhenen

A soil sample, originating from a place near Rhenen, where a garden bordered on the outer marches of the river Rhine, was investigated every month from April till September in 1967. In all 12 species of *Pythium* were found and though there was some variation in the species, the number of species in the samples varied between 6 and 8 in the different times of the year, with the exception of August when 12 species were isolated. *P. acanthicum*, *P. ultimum* and also species of the group HS were present in each sample; *P. irregulare*, *P. oligandrum* and *P. torulosum* could be isolated from 5 of the 6 samples. The frequency of the group HS is rather high because of the impossibility of identifying the different heterothallic species in 1967. A few cultures of this

Table 4. Comparison between species distribution in Zuidelijk (Z) and Oostelijk (O) Flevoland.

	Date, place (and number of samples)												
	1968	1969		1970		1971	1972						
	Z(35)	Z(31)	O(3)	Z(27)	O(20)	O(23)	Z(10)						
P. acanthicum			-		+								
P. adhaerens		+		+		+							
P. angustatum				+	_	+	+						
P. dissimile		_		_	+								
P. dissotocum		+	*****	-	+	+							
P. flevoense	+	_	_	+		_	+						
P. gracile	+	+	+	+	+		-						
P. heterothallicum	_	+			_								
P. intermedium		+	+		+	+							
P. irregulare			+	+									
P. middletonii	_		+	+	+								
P. monospermum		+	+	+			+						
P. oligandrum			+	+	+	+	+						
P. paroecandrum			+	+	+	+	+						
P. periplocum	****				+	+	-						
P. rostratum	_		+	+	+	+	+						
P. sylvaticum		+	+	+	+	+	+						
P. torulosum	+		+	+	+	+	+						
P. ultimum				+	+	+	+						
p. spec.	_	_	+	+	+	+	+						
e. group F	+	+	+	+	+	÷	+						
P. group HS			_	+	+	+	+						
P. group P	+	+	+	+	+	+							
P. group T	+	+	+	+	+	+	+						
Number of species	6	10	14	18	18	16	13						

Tabel 4. Vergelijking tussen de verspreiding van soorten in Zuidelijk (Z) en Oostelijk Flevoland (O).

group of isolates were maintained and later turned out to be *P. sylvaticum* and *P. heterothallicum*. Other species isolated were *P. dissotocum* (from 3 samples), *P. mamillatum* (4), *P. paroecandrum* (1) and *P. rostratum* (2).

4. Zutphen

In 1970 4 soil samples originating from the river forelands of the river Yssel near Zutphen were studied. The samples were collected from about the same place under grass vegetation in March, April, May and October. 19 different species were isolated. The number of species per sample varied from 8 to 11. The following species were isolated: P. angustatum Sparrow (from 1 sample), P. dissimile (3), P. dissotocum (2), P. heterothallicum (1), P. irregulare (2), P. middletonii Sparrow (2), P. monospermum (3), P. oligandrum (1), P. paroecandrum (4), P. periplocum (1), P. rostratum (4), P. sylvaticum (4), P. torulosum (1), P. ultimum (2), P. spec. (1), P. group F (2), P. group HS (2), P. group P (2), P. group T (1).

Table 5. Survey of the species distribution in flax fields in Friesland and Noord-Holland. Experimental fields (Plantenziektenkundige Dienst, Wageningen) situated: Si near Oude Bildtzijl, J near St. Annaparochie, S, K and V near Slootdorp. (d = diseased, h = healthy).

	Loc	Localities, state (and number of samples)														
	Isol	ated	fror	n soi	l			Isolated from roots of plan ts								
	Frie	slan	d	No	ord-I	Holla	ınd		Frie	eslan	d	Noc	ord-F	Iollai	nd	
	Si	J		S		K	V		Si	J		S		K	V	
	h(1)	d(1) h(1) d(2) h(1)	-) d(1	d(1)	h(1)	h(1)	d(1)) h(1)	d(3)	h(1)	d(1)	d(1)) h(1)
P. heterothallicum	+	+	-			_	_			_	+		_	_		
P. intermedium	+	+		+	+	+	+	+		+	+	+		+	+	+
P. irregulare	_				-1		+	+			_	+	_	+		+
P. oligandrum	÷	+	+	-	+	+	+	+				+		_	+	
P. paroecandrum	_	_		- -							+	+	+			+
P. rostratum	_		-	*****	-		+-		-		_	+		_		
P. sylvaticum	÷	+	+	+	+	+	+	+		+	+	+		+		+
P. ultimum	_			+							_	+		_	+	
P. spec.	_			-	_						_	+		_	+	_
P. group HS	+		+								+	+		_	_	
P. group P	-					_	+	-			_	_		_	_	
Number of species	5	4	3	6	4	3	6	4	1	2	5	9	1	3	4	4

Tabel 5. Overzicht van de verspreiding van soorten in vlasvelden in Friesland en Noord-Holland. Proefvelden (Plantenziektenkundige Dienst, Wageningen) gelegen: Si bij Oude Bildtzijl, J bij St. Annaparochie, S, K en V bij Slootdorp. (d = ziek, h = gezond).

5. Flax and flax fields (Table 5)

In flax a disease called 'scorch', 'fire' or 'vlasbrand' can cause large fallow patches as a result of stunting and wilting of the young plants. This disease is caused by *P. megalacanthum* de Bary sensu Buisman (Buisman, 1927; Diddens, 1931). Elucidation of the identity of this fungus was the reason for collecting a number of soil and plant samples from flax fields. The oogonia of the fungus could be seen in the roots of plants with symptoms of 'vlasbrand' but the isolation of the fungus did not succeed. 5 soil samples were collected from diseased fields and 4 from places where no 'vlasbrand' occurred; 6 root samples originated from diseased plants, 4 from healthy ones (Table 5).

The *Pythium* populations of the two fields in Friesland were similar but differed from those in Noord-Holland which showed a greater variation in *Pythium* spp. and were more different among themselves. Altogether 11 common soil-inhabitant species were isolated, more species were obtained from the diseased than from the healthy material. *P. heterothallicum* which is not common in the Netherlands occurred only in the Friesland samples, while *P. rostratum* and *P. ultimum* were isolated only from the Noord-Holland material. No correlation in species distribution was found with occurrence of flax scorch.

6. Forest soils

Amongst 11 samples of different forest soils in the central part of the Netherlands five did not contain any *Pythium* species. From the remaining six only a few isolates were

obtained, altogether 8 species (2-4 per sample). In 2 samples *P. dissotocum*, a species with filamentous non-swollen sporangia, was present. One of these samples originated from soil near a brook, the other from a marshy part of the forest under sphagnum near Nijmegen. Isolates with spherical sporangia or hyphal swellings comprised 91% of the isolates. The other species isolated from forest soil were *P. intermedium* (from 3 samples), *P. irregulare* (1), *P. oligandrum* (1), *P. paroecandrum* (2), *P. sylvaticum* (6), *P.* spec. (1), *P.* group HS (1).

7. Diseased plants (Table 6)

From 30 samples of 14 diseased species of phanerogams 104 strains of *Pythium* spp. were isolated. Many other isolates from diseased plant material were sent to the CBS for identification.

In 1961 Rangaswami reviewed the literature on plant diseases caused by *Pythium* and *Phytophthora*. Recently Hendrix and Campbell (1973) published a review about *Pythium* as plant pathogens. Only publications after 1961 on the phytopathological importance will be cited below.

P. sylvaticum was the most frequent isolate. This species occurred on 12 of the 14 species investigated, though in none of these cases it seemed to be the cause of a disease. Nevertheless it may become pathogenic to phanerogams according to Hendrix

Table 6. Survey of the Pythium species isolated from different phanerogams.

	Host plants (and number of samples)													
	flax (8)	hyacinth (12)	iris (10)	tulip (1)	Scilla (2)	garden-cress (2)	marigold (1)	cucumber (1)	snapdragon (3)	Saintpaulia (1)	Zinnia (2)	Ipomoea (1)	Billbergia (1)	strawberry (4)
P. dissotocum	_	+	+											+
P. heterothallicum			+			-								
P. intermedium	+	+	+	_	+	+			+	+			_	
P. irregulare	+	+	+		+	_			+	_		+	+	+
P. mamillatum					_						+	_	+	+
P. monospermum								+						
P. oligandrum	+	_						_						-
P. paroecandrum	+	+	+		+				+		+			+
P. rostratum	+													
P. spinosum				_			_		_		+		_	
P. sylvaticum	+	+	+	+			+	+	+	+	+	+	+	+
P. ultimum	+	+	+	+					+	+		+		+
P. violae		+		*****	+				_	_				_
P. spec.	+					_				+	_	+		+
P. group F			_		_				_		_		+	+
P. group HS	+	+	+		+				+			+	_	+
P. group T	_							_						+
Number of species	9	8	8	2	5	1	1	2	6	4	4	5	4	10

Tabel 6. Overzicht van de van verschillende phanerogamen geïsoleerde Pythium soorten.

and Campbell (1968), Gams and Domsch (1969), Mulder (1969) and Blok (1970). P. ultimum, already known as a pathogen of tulips and many other plants, was isolated from 8 different species and occurred frequently on tulips and irises. P. irregulare was isolated from 8 plant species, P. intermedium and P. paroecandrum from 7. Though these species are common soil inhabitants they may become pathogenic to higher plants (Vaartaja, 1967; Hendrix and Campbell, 1968).

P. violae, known as a pathogen of pansies and hyacinths (Saaltink, 1969), and P. spinosum Sawada, a parasite on several ornamentals (Hendrix and Campbell, 1966), which are certainly no common soil organisms in the Netherlands, were also isolated from diseased material: P. violae from hyacinth and Scilla, P. spinosum from Zinnia and also from a compost heap in the botanical garden 'Cantons Park' in Batarn. The latter fungus has not previously been recorded in the Netherlands.

P. mamillatum, which may be pathogenic to a number of phanerogams (Takahashi and Kawase, 1965; Vaartaja, 1967) was isolated from Zinnia, Billbergia and strawberry. P. oligandrum was only isolated from flax, from diseased plants as well as from healthy ones. The species occurs mostly in cultivated soils and has been recorded from several plants all over the world. Several investigators considered it as a pathogen. According to Schmitthenner (1964) it is not pathogenic to alfalfa. Till now there is no indication that this species is responsible for any serious plant disease in the Netherlands.

8. Isolations from water and aquatic animals

During the years 1967-1973 13 water samples were studied. From six samples of water from ditches near Bergen, Egmond aan Zee, Utrecht, Baarn and Eemnes nire species of Pythium were obtained: P. acanthicum (1), P. adhaerens Sparrow (1), P. dissotocum (1), P. paroecandrum (1), P. sylvaticum (4), P. spec. (1), P. group F (5), P. grou p HS (1), P. group T (3); of three samples from water in canals one did not contain any Pythium in one only P. rostratum was present and in the third only P. group F. From lake and pond water (4 samples) were isolated: P. angustatum (1), P. sylvaticum (1), P. group F (3) and P. group HS (1). The number of species per water sample was 0-5. In all 11 species of Pythium were isolated.

Two samples of diseased *Daphnia* spp. sent to the CBS were investigated for the presence of Oomycetes. *P. flevoense* and species belonging to *Pythium* group F were obtained form both samples.

Species of group F comprised 82% of the 222 isolates from water and Daphnias. The remaining isolates consisted for about 7% of other species with filamentous non-swollen sporangia (P. adhaerens, P. dissotocum, P. flevoense and P. angusta tum), for 7% of species of P. group T and for the rest of a small number of strains of P. acanthicum, P. paroecandrum, P. rostratum, P. sylvaticum, P. spec. and P. group HS. In 3 samples Pythium group T was frequent or dominating (44, 66 and 100% of the isolates, respectively).

Discussion

Of the 26 Pythium species and 4 groups from circa 4000 isolates 11 species were already recorded before for the Netherlands. Other species recorded before were not

found: P. afertile, P. aphanidermatum, P. debaryanum, P. echinulatum, P. hydnosporum, P. megalacanthum de Bary, P. perniciosum, P. splendens, P. undulatum and P. volutum. P. debaryanum, though often recorded, was not isolated again. The possibility that these records are due to wrong identifications cannot be excluded. Some of these strains could be traced and were identified as P. sylvaticum and P. ultimum, both common species in the Netherlands. P. afertile is a doubtful species, as only its filamentous non-swollen sporangia are known. This species is included here in Pythium group F. P. aphanidermatum and P. splendens were only recorded from glass-houses. Both species are known from warmer countries. P. hydnosporum, P. volutum, P. echinulatum, P. perniciosum and P. vexans were recorded only once, the last three were possibly present under glass-house conditions. It is not clear if van Poeteren (1938) dealt with P. megalacanthum de Bary or with P. megalacanthum ss. Buisman.

A number of species not previously recorded in the Netherlands were isolated from soil samples from the polders Zuidelijk and Oostelijk Flevoland and from soils near Rhenen and Zutphen: P. acanthicum Drechsler (CBS 434.68), P. adhaerens Sparrow (CBS 520.74), P. angustatum Sparrow (CBS 522.74), P. dissimile Vaartaja (CBS 523.74), P. dissotocum Drechsler (CBS 524.72), P. gracile Schenk (CBS 526.74), P. middletonii Sparrow (CBS 528.74), P. monospermum Pringsh. (CBS 529.74), P. oligandrum Drechsler (CBS 530.74), P. periplocum Drechsler (CBS 532.74), P. rostratum Butler (CBS 533.74), P. spinosum Sawada (CBS 276.67) and P. group T. Some of these species were also present in water (P. adhaerens (CBS 521.74), P. rostratum (CBS 534.74) and P. group T) or in plants (P. dissotocum (CBS 525.74), P. oligandrum (CBS 531.74), P. spinosum Sawada (CBS 275.67) and P. group T). The following species were isolated from plants or soil: P. helicandrum Drechsler (CBS 527.74), isolated from azalea by H. Rattink, P. oedochilum Drechsler (CBS 252.70), isolated from soil by Miss I. Blok, P. polymastum Drechsler (CBS 810.70), isolated from lettuce by J. H. van Emden and P. tracheiphilum Matta (CBS 870.72), isolated from lettuce and sent by the Plantenziektenkundige Dienst, Wageningen.

Species with filamentous non-swollen sporangia were generally frequent in water and wet soils (Table 7). 89% of the isolates from water and Daphnias belonged to this group of Pythium species. In Zuidelijk Flevoland in 1968 also 89 % of the isolates had filamentous non-swollen sporangia. In the course of time this percentage decreased to 9 in 1972, which is only little higher than the percentage occurring in the polder Oostelijk Flevoland. Other wet soils near rivers also showed a high percentage of species of the group with filamentous non-swollen sporangia. As many as 94% of the isolates from the banks of the river Vecht in the province of Overijssel belonged to this group. A sample from the outer marches of the river IJssel at a distance of about 100 m from the river yielded 26% isolates with filamentous non-swollen sporangia. In cultivated soils these proportions were quite different. 18 out of 28 samples yielded no Pythium species with filamentous non-swollen sporangia at all, while all samples showed a dominance of *Pythium* spp. with spherical sporangia or hyphal swellings, especially of P. sylvaticum which was present in all samples from arable fields, meadows, gardens and also from river forelands. However, this fungus was isolated only a few times from dry sandy places and not at all from marshes. Besides P. sylvaticum also P. oligandrum and P. intermedium were frequent in arable fields. P. paroecandrum was present in soil samples of most arable fields (oats, wheat, rape) especially in the polder Oostelijk Flevoland (90%), but only in 14% of the samples originating from

Table 7. Comparison between the distribution of *Pythium* species with filamentous non-swollen sporangia and species with spherical sporangia or hyphal swellings.

			isolates with filamentous wollen sporangia	non-p	% of isolates with spherical, non-proliferating spora ngia or hyphal swellings				
Soil	Z. Flevoland 1968Z. Flevoland 1969Z. Flevoland 1970Z. Flevoland 1972	89 76 43 9		0 2.: 12 36	5				
Soil	O. Flevoland 1969 O. Flevoland 1970 O. Flevoland 1971	6.5 4 6		50 85 85					
Soil	arable fields, garden soil, meadows 1966–1972	3	(2 samples 25-33%; 8 samples 2-11%; 18 samples 0%)	90	(24 samples 80– 1 00%; 4 samples 63–77 %)				
Soil	forests 1968-1972	7	(1 sample 30%; 1 sample 10%; 4 samples 0%; 5 samples without <i>Pythiut</i>	91 n)	(4 samples 95–100%; 2 samples 69–75 %; 5 samples without Pythium				
Soil	Zutphen, banks of the river IJssel 1970	26		54					
Soil	Marienberg, Overijssel, banks of the river Vecht, 1970	94		6					
Aquatic	ponds, lakes, ditches, canals, Daphnia, 1968–1973	89	(9 samples 80–100%; 1 sample 50%; 1 sample 17%; 2 samples 0%; 2 samples without <i>Pythium</i>)	5	(1 sample 100%; 1 sample 45%; 3 samples 8–17%; 8 samples 0%; 2 samples without <i>Pythium</i>)				
Plants	14 species of Phanerogams 1968–1972	1.5	(4 samples 30–50%; 1 sample 6%; 43 samples 0%; 1 sample without <i>Pythium</i>)	94	(44 samples 90–1 00%; 2 samples 68–70%; 2 samples 50–60%; 1 sample without <i>Pythium</i>)				

Tabel 7. Vergelijking tussen de verspreiding van Pythium soorten met niet verdikte, hyphenvormige sporangiën en soorten met ronde sporangiën of hyphenzwellingen.

flax fields in Noord-Holland and Friesland. Of about 1000 isolates from cultivated soils during the years 1968–1972 37% could be identified as *P. sylvaticum*, 22% as *P. oligandrum* and 11.5% as *P. paroecandrum*. Dry sandy places, forests and marshes were poor in *Pythium* species. Samples from dry sandy places, often originating from the neighbourhood of rabbit burrows or bird's nests showed only a few species of *Pythium* among which *P. rostratum* was nearly always present. Nevertheless this species was also found in samples originating from much wetter soils. It may be a very common species which is not so often isolated because it is a slow grower (about 7 mm in 24 hours at 25°C) and soon overgrown by quicker growing species such as *P. sylvaticum* or *P. oligandrum*.

Sometimes one species may suddenly dominate. In 1967 the soil samples from a

garden in Rhenen showed a number of different species among which species with ornamented oogonia were rather frequent. A sample from the same place in 1966 showed only one species with smooth oogonia which was parasitized by an unknown Chytrid. The species could not be identified because of the lack of sporangia and was not isolated in 1967.

Samenvatting

Pythiumsoorten in Nederland

Gedurende de jaren 1966–1972 werden ongeveer 4000 isolaties van 26 Pythium soorten verkregen uit grond, water, Daphnia's en planten. Vooral werd er aandacht geschonken aan de voortschrijdende kolonisatie in de nieuwe polder Zuidelijk Flevoland; het aantal soorten in de bodem van deze polder nam toe van 0 in 1966 tot 13 in 1972 (Tabel 2). Het percentage grondmonsters waarin geen Pythium spp. voorkwamen nam daarentegen af van 54 in 1968 tot 45 in 1969, 19 in 1970 en 0 in 1972. De diepte van de laag waaruit de Pythium spp. geïsoleerd werden had tot 10 cm weinig invloed. Uit de bovenste laag van 5 cm dikte werden vrijwel dezelfde soorten geïsoleerd als uit de laag van 5 cm dikte er onder. Uit grond van Oostelijk Flevoland werden 22 soorten geisoleerd (Tabel 3). In 1972 waren 19 van deze soorten eveneens uit de bodem van Zuidelijk Flevoland verkregen (Tabel 4).

Uit grond uit de omgeving van Rhenen (op de grens van een tuin en de uiterwaarden) werden in 1967 12 soorten geïsoleerd, uit grond van de uiterwaarden van de IJssel bij Zutphen zelfs 19. Invloed van het jaargetijde was niet duidelijk aanwezig. Bosgrond bevatte meestal slechts 0-4 soorten per monster. In totaal werden uit bosgrond 8 soorten geïsoleerd. In grond van vlasvelden in Noord-Holland en Friesland en in gezonde en vlasbrandzieke vlasplanten bleken 11 soorten aanwezig. De Friese velden kwamen in hun *Pythium* populatie onderling sterk overeen, maar toonden verschillen met de Noordhollandse akkers, die overigens onderling ook variabeler waren dan de Friese (Tabel 5).

Van zieke planten werden in totaal 17 Pythium soorten geïsoleerd (Tabel 6), zieke Daphnia's leverden 2 soorten op en in water werden 11 soorten gevonden. Aan de vroeger reeds gevonden soorten konden 15 voor Nederland nieuwe soorten worden toegevoegd. De soorten die niet gedetermineerd konden worden, werden naar de bouw van de sporangiën in 4 groepen (F, HS, P en T) ondergebracht. Soorten met niet verdikte hyphevormige sporangiën bleken een voorkeur te hebben voor water en natte gronden (Tabel 7). Zij werden vooral geïsoleerd uit water (89% van de isolaties), Zuidelijk Flevoland in 1968 (89%), de oever van de Overijsselse Vecht (94%) en de uiterwaarden van de IJssel bij Zutphen (26%), hoewel in dit laatste geval de monsters op vrij grote afstand van de rivier (meer dan 100 m) genomen werden. In cultuurgrond, bosgrond en ook op planten werd een veel lager percentage van deze soorten gevonden. Hier overheersten soorten met ronde, niet prolifererende sporangiën of hyphenzwellingen. In droge zandgrond bleek dikwijls P. rostratum de enig aanwezige Pythium soort te zijn, hoewel deze soort ook op andere minder droge plaatsen te vinden was. P. sylvaticum was de meest voorkomende soort in cultuurgronden (37% van alle isolaties in 1968-1972), gevolgd door P. oligandrum (22%) en P. paroecandrum (11,5%).

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References

Apinis, A. E., 1964. Concerning occurrence of Phycomycetes in alluvial soils of certain pastures, marshes and swamps. Nova Hedwigia 8: 103-126.

Beverwijk, A. L. van, 1948. Observations on submerged watermoulds in the Netherlands. Antonie van Leeuwenhoek 14: 223-250.

Blok, I., 1970. Pathogenicity of Pythium sylvaticum. Neth. J. Pl. Path. 76: 296-298.

Buisman, C. J., 1927. Root rots caused by Phycomycetes. Diss. Univ. Utrecht.

Butler, E. J., 1907. An account of the genus *Pythium* and some Chytridiaceae. Mem. Dep. Agric. India, bot. Ser. Vol. 1 (5): 1–160.

Centraalbureau voor Schimmelcultures, Baarn, 1962. List of Cultures 1961, Suppl. 1, 1962.

Diddens, H. A., 1931. Onderzoekingen over den vlasbrand, veroorzaakt door *Pythium megcalacanthum* de Bary. Diss. Univ. Amsterdam.

Diddens, H. A., 1932. Untersuchungen über den Flachsbrand. Phytopath. Z. 4: 291-313.

Eek, Th. van, 1937. Wortelrot van Viola tricolor L. Diss. Univ. Amsterdam.

Eek, Th. van, 1938. Root rot of Viola tricolor maxima Hort. Phytopath. Z. 11: 217-282.

Gams, W. & Domsch, K. H., 1969. Bemerkungen zu einigen schwer bestimmbaren Bodenpi Izen. Nova Hedwigia 18: 1-29.

Harder, R. & Uebelmesser E., 1957. Notiz zur Frage des Vorkommens von Chytridineen und anderen Pilzen in tiefen Bodenschichten. Arch. Mikrobiol. 26: 353-357.

Hendrix, F. F. Jr & Campbell, W. A., 1966. Root rot organisms isolated from ornamental plants in Georgia. Plant Dis. Reptr 50: 393-395.

Hendrix, F. F. Jr & Campbell, W. A., 1968. Pythiaceous fungi isolated from forest nursery soils and their pathogenicity to pine seedlings. Forest Sci. 14: 292–297.

Hendrix, F. F. Jr & Campbell, W. A., 1973. Pythiums as plant pathogens. A. Rev. Phytopath. 11: 77-98.

Houten, A. Thzn, J. G. ten, 1939. Kiemplantenziekten van coniferen. Diss. Univ. Utrecht.

Koning, M. de, 1927. Ziekten van kiemplanten. Tijdschr. PlZiekt. 33: 44-47.

Luijk, A. van, 1934. Untersuchungen über Krankheiten der Gräser. Meded. phytopath. Lab. Willie Commelin Scholten 13: 1-28.

Matthews, V. D., 1931. Studies on the genus Pythium. Chapel Hill, Univ. N. Carolina Press.

Meer, J. van der, 1928. Vlasbrand. Tijdschr. PlZiekt. 34: 126-146.

Meurs, A., 1928. Wortelrot veroorzaakt door schimmels uit de geslachten Pythium en Aphanomyces de Bary. Diss. Univ. Utrecht.

Middleton, J. T., 1943. The taxonomy, host range and geographic distribution of the genus Pythium. Mem. Torrey bot. Club 20: 1-171.

Moore, W. C. & Buddin, W., 1937. A new disease of tulip caused by species of *Pythium*. Ann. appl. Biol. 24: 752–761.

Mulder, D., 1969. The pathogenicity of several *Pythium* species to rootlets of apple seedlings. Neth. J. Pl. Path. 75: 178-181.

Oudemans, C. A. J. A., 1897. Revision des champignons tant supérieurs qu'inférieurs trou vés jusqu'à ce jour dans les Pays Bas, II. Verh. K. ned. Akad. Wet. Sect. 2, Afd. Natuurk., 2: 4-5.

Plaats-Niterink, A. J. van der, 1968. The occurrence of *Pythium* in the Netherlands. I. Heterothallic species. Acta bot. neerl. 17: 320–329.

Plaats-Niterink, A. J. van der, 1972. The occurrence of *Pythium* in the Netherlands. III. *Pythium flevoense* sp. n. Acta bot. neerl. 21: 633-639.

Poeteren, N. van, 1930. Verslag over de werkzaamheden van den Plantenziektekundige Dienst in het jaar 1929. Versl. Meded. plziektek. Dienst. Wageningen 62: 1-42.

Poeteren, N. van, 1938. Verslag over de werkzaamheden van den Plantenziektekundige Dienst in het jaar 1937. Versl. Meded. plziektek. Dienst, Wageningen 87: 1-84.

- Rangaswami, G., 1961. Pythiaceous fungi, a review. Indian Counc. Agric. Res., New Delhi.
- Ritzema Bos, J., 1896. Het omvallen van kiemplanten door de werking van *Pythium debaryanum* Hesse. Tijdschr. PlZiekt. 2: 1-4.
- Ritzema Bos, J., 1902. Phytopathologisch Laboratorium WCS. Verslag over de onderzoekingen gedaan in en inlichtingen gegeven vanwege bovengenoemd laboratorium in het jaar 1901. Tijdschr. PlZiekt. 8: 1-34.
- Ritzema Bos, J., 1903. Phytopathologisch Laboratorium WCS. Verslag over de onderzoekingen gedaan in en over de inlichtingen gegeven vanwege bovengenoemd laboratorium in het jaar 1902. Tijdschr. PlZiekt. 9: 1-61.
- Saaltink, G. J., 1969. Root rot of Hyacinths caused by species of *Pythium*. Neth. J. Pl. Path. 75: 343-354.
- Schmitthenner, A. F., 1964. Prevalence and virulence of *Phytophthora*, *Pythium* and *Aphanomyces*, *Rhizoctonia* and *Fusarium* isolated from diseased alfalfa seedlings. Phytopathology 54: 1012–1018. Staes, G., 1896. Paardestaarten en aardappelziekte. Tijdschr. PlZiekt. 2: 170–172.
- Takahashi, M. & Kawase, Y., 1965. Ecologic and taxonomic studies on *Pythium*. *Pythium* as pathogenic soil fungi. V. Several species of *Pythium* causing root rot of strawberry. Ann. phytopath. Soc. Japan 30: 186–191.
- Vaartaja, O., 1967. Damping-off pathogens in S. Australian nurseries. Phytopathology 57: 675-770.
 Vaartaja, O. & Bumbieris, M., 1964. Abundance of *Pythium* species in nursery soils in South Australia. Aust. J. biol. Sci. 17: 436-445.
- Waterhouse, G. M., 1967. Key to Pythium Pringsheim. Mycol. Pap. 109. pp. 1-15.
- Waterhouse, G. M., 1968. The genus Pythium Pringsheim. Mycol. Pap. 110: 1-71.
- Wiersema, H. T., 1955. Flax scorch. Euphytica 4: 197-205.

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