CHAPTER 2

PYTHIUM SPECIES ASSOCIATED WITH WILT AND ROOT ROT OF HYDROPONICALLY GROWN CROPS IN SOUTH AFRICA

Abstract

Eight *Pythium* species, *P. acanthicum*, *P. aphanidermatum*, *P. coloratum*, *P. diclinum*, *P. irregulare*, *P. myriotylum*, *P. perplexum*, *P. spinosum*, and representatives of five heterothallic *Pythium* groups, F, G, HS, P and T, were isolated from roots and crowns of crops and from nutrient solutions, substrates, water sources and run-off water in 11 hydroponica in South Africa. *P.* group F was isolated most frequently and from the greatest variety of crops, followed by *P. irregulare*, *P. spinosum*, *P. aphanidermatum* and *P.* group HS. Various new *Pythium*/host associations for South Africa were recorded, e.g. *P. acanthicum* on strawberry, *P. aphanidermatum* on parsley, *P. coloratum* on lettuce, *P. irregulare* on Chinese cabbage and lettuce, *P. perplexum* on tomato, *P.* group G on lettuce, and *P.* group HS on cucumber and lettuce. In artificial inoculation studies, *P.* group F proved to be pathogenic to various lettuce cultivars and to the herbs endive, fennel and sorrel. *P. spinosum* was highly virulent on cucumber.

INTRODUCTION

Root infection by *Pythium* species poses a major constraint to the cultivation of crops in hydroponic systems (Stanghellini & Rasmussen, 1994). Of the various *Pythium* species associated with hydroponically-grown crops, *Pythium aphanidermatum* (Edson) Fitzp. has been reported the most frequently and from the greatest variety of crops (Bates & Stanghellini, 1984; Stanghellini, 1984; Zhou & Paulitz, 1993; Chérif *et al.*, 1994; Moulin *et al.*, 1994; Menzies & Belanger, 1996; Stanghellini *et al.*, 1996; Paulitz, 1997). Another important species is *Pythium ultimum* Trow. It has been recorded as a pathogen on hydroponically grown cucumber (*Cucumis sativus* L.), geranium (*Pelargonium hortorum* Baily), lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum* Mill.) (Zinnen, 1988; Hausbeck *et al.*, 1989). *Pythium dissotocum* Drechsler and *Pythium tracheiphilum* Matta are known to attack hydroponically grown lettuce, the latter particularly under

cool conditions (Tortolero & Sequeira, 1978; Stanghellini & Kronland, 1986; Moller & Hockenhull, 1997). *P. dissotocum* is mostly associated with subclinical infections, though infection can result in significant yield losses (Stanghellini & Kronland, 1986; Favrin *et al*, 1988). *Pythium myriotylum* Drechsler commonly occurs in irrigation water and causes root rot of cucumber, lettuce and tomato (Gill, 1970; Jenkins & Averre, 1983; Schuerger & Pategas, 1985). Other *Pythium* species and groups that have been associated with hydroponically grown crops include *Pythium spinosum* Sawada, *Pythium irregulare* Buisman, *Pythium coloratum* Vaartaja and *Pythium* groups F and G (Favrin *et al*, 1988; Chen *et al*., 1992; McCullagh *et al*., 1996; Chérif *et al*., 1997).

It is estimated that commercial hydroponica in South Africa cover an area of approximately 800 ha. Although local production manuals, e.g. Lewies (1998) and Thompson & Labuschagne (2001), refer to the presence of *Pythium* species such as *P. aphanidermatum*, *Pythium aristosporum* Vanterpool, *P. dissotocum*, *P. myriotylum* and *P.* groups F and G on crops in closed hydroponic systems, no survey of *Pythium* species occurring in hydroponica in the country has yet been conducted. To propose control measures based on accurate experiments it is necessary to determine which *Pythium* species are responsible for yield reductions in a particular hydroponicum (Moulin *et al*., 1994). The purpose of this study therefore was to identify the *Pythium* species and groups associated with root disease of crops in selected hydroponica in South Africa.

MATERIALS AND METHODS

Survey of *Pythium* species in hydroponic systems

Celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers.), Chinese cabbage (*Brassica rapa* L. subsp. *Pekinensis* Laur.), cucumber, lettuce, parsley (*Petroselinum crispum* (Mill.) A.W. Hill), strawberry (*Fragaria* sp.) and tomato plants exhibiting stunted growth, wilt and root rot, as well as plants without visible disease symptoms, were collected between 1998 and 2001 from eight hydroponica in South Africa (Table 1). Samples were also taken of other potential sources of *Pythium* infestation, e.g. water sources, growth media and substrates (Goldberg & Stanghellini, 1990; Stanghellini & Rasmussen, 1994), in five of the above and three additional hydroponica.

Plants were collected at various stages of maturity and transported in plastic bags to the laboratory in Pretoria. Four to five water samples were taken at 5 to 10 minute intervals at four hydroponica by submerging a sterilised 1 l Schott bottle 2-3 cm deep into the water source until the bottle was filled with water. Run-off water was collected from three to five randomly selected hydroponic beds in four hydroponica. The contents of the bottles from each source at each hydroponicum were pooled and taken to the laboratory. Growth medium and substrate samples were randomly collected in plastic bags at five hydroponica.

In the laboratory, roots and crowns were rinsed in sterile distilled water (SDW) till clean. In accordance with Stanghellini & Kronland (1986), no further surface-disinfestation was applied. Five root tip segments, *ca.* 10 mm in length, and five internal crown tissue sections (*ca.* 2 mm^3) per plant were excised and plated on the selective medium of Masago *et al.* (1977), modified by Botha & Coetzer (1996): 1% water agar + 50 mg benomyl, 25 mg nystatin, 50 mg tolclofos-methyl, 20 mg rifampicin and 50 mg ampicillin l^{-1}. Plates were incubated at 25 °C.

Isolation of *Pythium* from water and substrate samples was accomplished by baiting with citrus leaf discs (Grimm & Alexander, 1973). About 200 ml of each water sample was transferred to a 250 ml plastic cup and five 5-mm-diameter leaf discs, sprayed with 70% ethanol and rinsed in SDW, were floated on the surface for 3-4 days. The discs were then plated on the above selective medium and incubated at 25 °C. With the growth media and substrates, approximately 80 ml of each sample was placed in a plastic cup and SDW added to a volume of 200 ml. Growth media containing perlite was covered with a mesh before adding water to prevent the perlite from levitating to the surface. Leaf discs were floated, plated and incubated as described previously.

After 3-6 days incubation, hyphal tips from *Pythium* colonies were transferred to 1% water agar supplemented with 30 µg l^{-1} ß-sitosterol to enhance development of sexual structures (Botha & Coetzer, 1996). Morphological observations were made directly on the culture plates after one week's incubation at 25 °C in the dark. Sporangia were produced by incubating 5-mm-diameter plugs from water agar cultures for 24 hours at 25 °C in non-sterile soil extract under near-UV light. Zoospores were released by exposing the cultures for 4 hours to 4 °C and then returning them to room temperature (*ca.* 25 °C). Sexual and asexual structures were observed under a

compound microscope at 400x and 1000x magnification. The key compiled by Dick (1990) based on oogonial criteria was used in conjunction with Van der Plaats-Niterink (1981) to identify the isolates, and the identifications were verified by W. J. Botha of the Agricultural Research Council - Plant Protection Research Institute (PPRI), Pretoria. Isolates were maintained on cornmeal or V8-juice agar plugs in SDW in McCarty bottles stored at 25 °C. Voucher cultures of representative isolates were deposited in the National Collection of Fungi, PPRI, Pretoria.

Pythium host range in gravel culture and ebb-and-flow systems

In a separate study, various crops in gravel culture hydroponicum #1 (Table 1) were screened for root infection by *Pythium* species. The crops included the lettuce cultivars Butter *Lutetia*, Lolla Rossa *Sesam*, Lolla Bionda *Bergamo*, Cos *Bambi*, Cos *Junior*, Cos *Wallop*, Cos *Pinnocio*, Batavia Red *Ascona*, Red Oak Leaf *Red Salad Bowl* and Green Oak Leaf *Krizet*, the herbs basil (*Ocimum basilicum* L.), chive (*Allium schoenoprasum* L.), fennel (*Foeniculum vulgare* L), mint (*Mentha* spp.), rocket (*Delphinium* spp.), sorrel (*Rumex* spp.) and watercress (*Nasturtium officinale* L.), the Oriental vegetables Chinese cabbage, pak choi (*Brassica rapa* L. subsp. *chinensis* L.) and tah tsai (*Brassica chinensis* L. var. *rosularis* L.), and other crops such as celery, endive (*Cichorium endiva* L.) cv. Oxalie, radicchio (*Cichorium intybus* L.) cv. Firebird, and viola (*Viola* spp.). A similar survey was conducted on cucumbers grown in the ebb-and-flow hydroponicum (#11 in Table 1). Diseased and symptomless plants collected at the two hydroponica were processed as described in the previous section.

Subcultures of selected *Pythium* isolates from each host were maintained on V8-juice agar at 25 °C for inoculation purposes. Pathogenicity of the isolates was determined by artificially inoculating 4-week-old cucumber, endive 'Oxalie', fennel, sorrel, and lettuce cultivar Batavia Red *Ascona*, Butter *Lutetia* and Lolla Bionde *Bergamo* seedlings in a hydro culture system in the greenhouse. The system consisted of 5 l plastic vessels, 23 cm in diameter, with four 3-cm-diameter holes spaced 9 cm apart in the lid of each vessel. Each vessel was filled with a nutrient solution consisting of 0.9 g Agrasol® 'O 3:2:8 (Fleuron, P.O. Box 31245, Braamfontein, 2017), 0.6 g calcium nitrate monohydrate and 0.3 g Micromix® (Fleuron) l^{-1} tap water. Aeration was provided by a compressor supplying air through a 5-mm-diameter tube inserted through a hole in the lid of each vessel (Bates & Stanghellini, 1984). The 4-week-old seedlings were

transplanted from steam-pasteurised Canadian peat moss growth medium into the holes in the lids of the vessels, one seeling per hole, their roots submerged in the nutrient solution and their shoots supported by strips of foam rubber. Three vessels were used for each crop / isolate.

Cucumber seedlings were inoculated with a zoospore suspension of an isolate of *P. spinosum*, whereas the other crops were each inoculated with a *P.* group F isolate from the respective crop. The isolates were cultured for 7-10 days on V8-juice agar. Zoospores were released as described above, immobilised by vortexing, and the zoospore cysts enumerated with the aid of a haemacytometer (Moulin *et al.*, 1994; Wulff *et al.*, 1998). Each vessel was inoculated by adding 15 ml of a 10^5 ml^{-1} zoospore cyst suspension to the nutrient solution one week after transplanting.

Symptoms (wilting and root rot) were recorded weekly. Plants were harvested 4 weeks after transplanting. Three root segments, *ca.* 10 mm long, were excised from each plant in each container, rinsed in SDW, and plated on the above selective medium. After incubation for 3-6 days at 25 °C, colonies were transferred to water agar supplemented with 30 µg l^{-1} ß-sitosterol for identification.

RESULTS

Survey of *Pythium* species in hydroponic systems

A total of 143 isolates, representing eight *Pythium* species and five heterothallic groups, were collected fom the 280 plant, 32 water source, 49 substrate and 30 run-off water samples (Table 1). *P.* group F was the most prevalent, representing 41% of all the isolates. Twenty per cent of the isolates were identified as *P. irregulare*, 6% as *P. spinosum*, and 5% as *P. aphanidermatum* and *Pythium* group HS, respectively. *Pythium acanthicum* Drechsler, *Pythium diclinum* Tokunaga, *P. myriotylum*, *Pythium perplexum* Kouyeas & Theohari, and *Pythium* groups G, P and T each contributed 2% or less to the total number of isolates.

Besides being encountered the most frequently, *P.* group F was also isolated from the greatest number of crop species (5/7) and hydroponica (5/11). It did, however, occur preferentially in recirculating gravel systems and, despite its prevalence in roots, borehole water and substrates,

could not be isolated from any of the run-off water samples. *P. irregulare* was isolated from three of the seven crop species in two recirculating gravel and two open dripper systems. It also frequently occurred in run-off water. *P. aphanidermatum* and *P.* groups HS were each isolated from two crop species, and the first two also from substrates and/or borehole water in three hydroponica. *P. acanthicum*, *P. coloratum*, *P. perplexum* and *P.* groups P and T were each isolated from one crop species only, whereas *P. diclinum* and *P. myriotylum* were not associated with a specific host. *P. coloratum*, although apparently infecting only lettuce, was isolated from water and/or substrate sources in three of the five recirculating gravel systems.

As almost 70% of the plant samples comprised lettuce, albeit from only two hydroponica, it was not surprising that this crop yielded the greatest diversity of *Pythium* species/groups (*P. coloratum*, *P. irregulare*, *P.* groups F, G, HS and T). Cucumber, which represented 15% of the plant samples, also yielded a variety of species/groups (*P. aphanidermatum*, *P. irregulare*, *P. spinosum*, *P.* groups F and HS). Of the crop species which contributed less than 10% to the total number of plant samples, celery, parsley and tomato each produced only one *Pythium* species/group, and Chinese cabbage two. A notable exception was strawberry, of which the three plants (1.1% of the total) from one hydroponicum yielded *P. acanthicum* and *P.* groups F and P. Strawberry was also the only crop from which a species/group (*P.* group F) could be isolated from crown tissue.

As indicated above, *P. coloratum* occurred only, and *P.* group F mostly, in recirculating gravel systems. *P. aphanidermatum* and *P. spinosum*, on the other hand, were isolated only from ebb-and-flow and open dripper systems. Due to the greater number of gravel system samples that were processed, a somewhat greater diversity of *Pythium* species/groups was retrieved from these systems, but the diversity was not consistently related to the number or variety of samples processed per hydroponicum.

Pythium host range in gravel culture and ebb-and-flow systems

Crops that were sampled in gravel system #1 showed stunting of the aboveground parts and slight to moderate browning of the root tips. Cucumber plants from which isolations were made in the ebb-and-flow system exhibited severe wilting of the foliage and were weakly anchored due to underdeveloped root systems. Symptomless infection without typical wilting and root rot

associated with *Pythium* disease also occurred in some crops, e.g. basil, celery, chive, mint, pak choi and tah tsai.

P. group F was isolated from all the crops evaluated in the gravel culture system, and *P. irregulare* from the lettuce cultivars Lolla Rossa *Sesam*, Cos green *Junior* and Batavia Red *Ascona* (Table 3). The incidence of *Pythium* in roots of the various lettuce cultivars ranged from 70 to 100% (mean 85%). Compared to the 7-83% (mean 38%) of the other crops, this was relatively high. Cucumber plants in the ebb-and flow system yielded only *P. spinosum.*

The different *Pythium* isolates varied in the time required for disease symptoms to appear after artificial inoculation, but all inoculated plants eventually developed some root rot and wilting or stunting of aboveground parts. Cucumber plants inoculated with *P. spinosum* showed symptoms of disease shortly after inoculation. The symptoms characteristically comprised wilting and stunting of aboveground parts which eventually resulted in the death of more than half of the plants. Watersoaked lesions appearing on the stems and root systems were severely necrotic. The herbs and lettuce cultivars inoculated with *P.* group F mainly showed stunting of aboveground parts and light to moderate root rot, but severe wilting was not observed. Roots of inoculated plants consistently yielded the same *Pythium* species/group the particular crop or cultivar was inoculated with. Uninoculated plants remained healthy. Symptoms observed on the crops are depicted in Figs 1-5.

DISCUSSION

The 11 hydroponica surveyed in this study comprised only a portion of the total number of hydroponic units in South Africa, and the *Pythium* species and groups that were isolated therefore do not represent the entire hydroponicum industry. Nevertheless, various new host / pathogen associations have been established for South Africa, e.g. *P. acanthicum* on strawberry, *P. aphanidermatum* on parsley, *P. coloratum* on lettuce, *P. irregulare* on Chinese cabbage and lettuce, *P. perplexum* on tomato, *P.* group G on lettuce, *P.* group HS on cucumber and lettuce, *P.* group P on strawberry, *P.* group T on lettuce and strawberry, and *P.* group F on all the crops it was isolated from. The study is also the first to report the isolation of *P. coloratum*, *P. diclinum*, *P. perplexum*, and *P.* groups HS and P from any crop or substrate in South Africa.

In accordance with the observation by Domsch *et al.* (1980) that nematosporangiate *Pythium* species preferentially occur in aquatic or semi-aquatic habitats, more than twice as many of the species/groups in this study (*P. coloratum*, *P. diclinum*, *P. myriotylum*, *P.* group F, *P.* group T) produce filamentous sporangia as those that produce strictly globose or subglobose sporangia (*P. acanthicum*, *P. irregulare*). Of all the species/groups, *P.* group F was isolated the most frequently and from the greatest variety of crops. Indeed, the only crops it could not be isolated from were parsley and tomato. This is in conflict with Chérif *et al.* (1997), who described *P.* group F as a common, albeit not very virulent, pathogen of hydroponically-grown tomatoes. It should, however, be kept in mind that tomato was sampled in only one hydroponicum, where only *P. perplexum* was the only *Pythium* sp. present at the time of sampling. *P.* group F is important in hydroponica (Rafin *et al.*, 1995) and is known to occur in hydroponica in South Africa (Lewies, 1998; Thompson & Labuschagne, 2001), and has also been associated with root rot of cabbage, wheat and pine cultivated conventionally (Linde *et al.*, 1994; Botha & Coetzer, 1996; Meyer & Wehner, 2000). Pathogenicity of *P.* group F to endive, fennel, sorrel and lettuce, as found in the present study, has not previously been reported anywhere in the world.

The second-most prevalent species, *P. irregulare*, is one of the most widespread and pathogenic *Pythium* species in temperate zones (Domsch *et al.*, 1980) and is commonly associated with hydroponically-grown crops elsewhere (Schuerger & Pategas, 1985; Favrin *et al.*, 1988), but not referred to by Lewies (1998) and Thompson & Labuschagne (2001) as a pathogen in hydroponica in South Africa. It has, however, been reported from 14 field-grown plant species in 10 families in the country (Crous *et al.*, 2000), including cucumber (Botha & Coetzer, 1996). *P. irregulare* produces a growth factor that stimulates the growth of pathogens such as *Rhizoctonia solani* J. G. Kühn, *Aphanomyces euteiches* Drechsler and several other *Pythium* species (Yang, 1969), and could therefore be particularly significant in disease complexes. *P. coloratum*, the third most prevalent species in this survey, is also not listed by Lewies (1998) and Thompson & Labuschagne (2001) as present in hydroponica in South Africa. Although it was isolated only from lettuce in gravel culture, it occurred commonly in substrates and run-off water in gravel systems, which is in accordance with Favrin *et al.* (1988) who could isolate the species only from potting mixes in British Colombian greenhouses. *P. coloratum* is a relatively rare species in soil (Van der Plaats-Niterink, 1981), but has been reported to cause root rot of cucumber and onion (*Allium cepa* L.) (Favrin *et al.*, 1988; Shishkoff, 1989).

The remaining species/groups contributed between <1 and 6% to the total number of isolates. Of these, *P. aphanidermatum*, *P. myriotylum* and *P.* group G have been described by Lewies (1998) and Thompson & Labuschagne (2001) as present in South African hydroponica. *P. myriotylum*, *P.* group G, and particularly *P. aphanidermatum* are also regarded as important in hydroponica elsewhere (Gill, 1970; Jenkins & Averre, 1983; Favrin *et al.*, 1988; McCullagh *et al.*, 1996; Stanghellini *et al.*, 1996). Indeed, *P. aphanidermatum* is considered to be the dominant *Pythium* in many types of hydroponic systems all over the world (Favrin *et al.*, 1988; Cherif *et al.*, 1994; Moulin *et al.*, 1994; Sanchez *et al.*, 1999). Being a thermotolerant species (Van der Plaats-Niterink, 1981) causing root damage mainly at higher temperatures (Hendrix & Campbell, 1973), it understandably is also very common in South African soils and has locally been reported as a root pathogen of 19 plant species in nine families (Crous *et al.*, 2000). The reason for its relative paucity in the present study is unclear, but could not have been due to temperatures in local hydroponica being too low as these temperatures are essentially the same as those in the environment. Particularly conspicuous in its absence was *P. ultimum*, another common *Pythium* species in hydroponica elsewhere (Mc Cullagh *et al.*, 1996; Cherif *et al.*, 1997) and the commonest *Pythium* species in South Africa, with 32 host species in 20 families recorded in Crous *et al.* (2000). The absence of *P. ultimum* in local hydroponica remains unresolved, but is nevertheless in accordance with Lewies (1998) and Thompson & Labuschagne (2001).

In conclusion, this study has shown that *Pythium* is omnipresent in South African hydroponica. The presence or absence of some, but not all, previously reported "hydroponic" species/groups was confirmed and new species and groups have been added to the list. Most of the species/groups have not previously been reported in South Africa from their present hosts in conventional production systems, the exceptions being *P. aphanidermatum*, *P. irregulare* and *P. spinosum* which are associated with root rot of greenhouse-cultivated cucumber (Botha & Coetzer, 1996). It is furthermore evident from the results that the *Pythium* species/group composition of hydroponica is not consistent. For instance, the first survey of gravel system #1 yielded *P. coloratum*, *P. irregulare* and *P.* groups F, G and HS from three crop species, whereas only *P.* group F and, to some extent, *P. irregulare* could be isolated from 16 crop species and 10 lettuce cultivars in the second survey. A similar shift occurred at the ebb-and-flow system with the apparent 'disappearance' of *P. aphanidermatum*. It is thus clear that hydroponica should be

monitored regularly to remain abreast of changes in *Pythium* populations.

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TABLE 1 Hydroponica in South Africa surveyed for *Pythium* species

Hydroponicum	System	Locality	Number of samples collected [a]
1	Recirculating gravel[b]	Pretoria	Le (121), Ce (15), GM (6), GC (23) BW (11), WW (19)
2	Recirculating gravel[b]	Vereeniging	Le (70), Ce (6), Ch (12), GC (6), BW (6), WW (5)
3	Recirculating gravel[b]	Port Elizabeth	BW (3)
4	Recirculating gravel[b]	Muldersdrift	WW (3)
5	Recirculating gravel[b]	Skeerpoort	St (3)
6	Open dripper[c]	Centurion	RS (3)
7	Open dripper[c]	Marikana	Cu (3), RW (2), DW (2), WW (3)
8	Open dripper[c]	Kemptonpark	Pa (5)
9	Open dripper[d]	Benoni	Cu (21), PE (7)
10	Open dripper[d]	Badplaas	To (6)
11	Ebb-and-flow[e]	Benoni	BW (8), Cu (18), QS (4)

[a]Ce = celery, Ch = Chinese cabbage, Cu = cucumber, Le = lettuce, Pa = parsley, St = strawberry, To = tomato, BW = borehole water, DW = dam water, RW = river water, WW = run-off water, GC = granite chips, GM = growth medium (Canadian peat moss), PE = perlite, QS = quarts sand, RS = river sand.

[b]Recirculating systems consisting of gullies with granite chips as substrate.

[c]Open dripper systems consisting of plastic bags with perlite as substrate.

[d]Open dripper systems consisting of plastic bags with pine wood shavings as substrate.

[e]Ebb-and-flow system consisting of gullies filled with coarse quartz sand.

TABLE 2 ***Pythium*** **species and groups isolated from commercial hydroponica in South Africa**

Pythium sp / group[a]	% Incidence[b]	Host	Number of isolates[c]		Hydroponic system[d]
P. acanthicum	1.5	Strawberry	2	Roots	5
P. aphanidermatum	5	Cucumber	1	Roots	11
		Parsley	1	Roots	8
			1	Sand	6
			4	Borehole water	11
P. coloratum	11	Lettuce	2	Roots	1, 2
			3	Growth media[e]	1
			6	Run-off water	1, 4
			5	Gravel substrate	2
P. diclinum	1.5		2	Run-off water	2
P. group F	41	Celery	5	Roots	2
		Chinese cabbage	4	Roots	2
		Cucumber	2	Roots	9
		Lettuce	39	Roots	1, 2
		Strawberry	1	Roots	5
			1	Gravel substrate	1, 2
			1	Growth media[e]	1
			5	Borehole water	1, 2, 3
P. group G	1.5	Lettuce	2	Roots	1
P. group HS	5.5	Cucumber	2	Roots	9
		Lettuce	4	Roots	1
			1	Gravel substrate	2
			1	Borehole water	1
P. group P	2	Strawberry	3	Stem	5
P. group T	2	Lettuce	3	Roots	2

TABLE 2 (continued)

P. irregulare	20	Cucumber	4	Roots	7, 9
		Lettuce	16	Roots	1, 2
		Chinese Cabbage	4	Roots	2
			2	Gravel substrate	2
			3	Run-off water	1, 2, 7
P. myriotylum	2		3	Gravel substrate	2
P. perplexum	1.5	Tomato	2	Roots	10
P. spinosum	5.5	Cucumber	8	Roots	9, 11

[a] Identified according to the keys compiled by Dick (1990) based on oogonial criteria and the revised key of Van der Plaats-Niterink (1981).

[b] Percentage frequency of species/group out of a total of 143 isolates.

[c] Number of isolates recovered from different hosts and other sources.

[d] Hydroponic systems according to Table 1.

[e] Canadian peat moss growth media for seedlings.

TABLE 3 ***Pythium*** **species and groups isolated from crops in gravel culture hydroponicum #1 and ebb-and-flow hydroponicum #11** [a]

Crop	% *Pythium* incidence in roots[b]	*Pythium* sp./group isolated[c]
GRAVEL CULTURE		
Lettuce cultivars		
Batavia Red *Ascona*	83	*P.* Group F, *P. irregulare*
Butter *Lutetia*	75	*P.* Group F
Cos green *Bambi*	93	*P.* Group F
Cos green *Junior*	97	*P.* Group F, *P. irregulare*
Cos green *Wallop*	73	*P.* Group F
Cos green *Pinnocio*	83	*P.* Group F
Lolla Rossa *Sesam*	70	*P.* Group F, *P. irregulare*
Lolla Bionda *Bergamo*	100	*P.* Group F
Green Oak Leaf *Krizet*	100	*P.* Group F
Red Oak Leaf *Red Salad Bowl*	80	*P.* Group F
Herbs		
Basil	80	*P.* Group F
Chives	17	*P.* Group F
Fennel	60	*P.* Group F
Mint	27	*P.* Group F
Rocket	43	*P.* Group F
Sorrel	7	*P.* Group F
Watercress	7	*P.* Group F
Oriental vegetables		
Chinese cabbage	20	*P.* Group F
Pak choi	30	*P.* Group F
Tah tsai	53	*P.* Group F

TABLE 3 (continued)

Other crops		
Celery *Victoria*	27	*P.* Group F
Endive *Oxalie*	40	*P.* Group F
Raddiccio *Firebird*	33	*P.* Group F
Violas	83	*P.* Group F
EBB-AND-FLOW CULTURE		
Cucumber	70	*P. spinosum*

[a] See Table 1.

[b] Percentage root segments out of 30 yielding the particular species/group.

[c] Identified according to the keys compiled by Dick (1990) based on oogonial criteria and the revised key of Van der Plaats-Niterink (1981).

FIGURE 1 a **Butterhead Lettuce uninfected (left) and naturally infected (right) with *P.* group F in a gravel culture system**

FIGURE 1 b **Butterhead Lettuce uninfected (left) and artificially infected (right) with *P.* group F in a water culture system**

FIGURE 2 a **Endive uninfected (left) and naturally infected (right) with *P.* group F in a gravel culture system**

FIGURE 2 b **Endive uninfected (left) and artificially infected (right) with *P.* group F in a water culture system**

FIGURE 3 a **Sorrel uninfected (left) and naturally infected (right) with *P.* group F in a gravel culture system**

FIGURE 3 b **Sorrel uninfected (left) and artificially infected (right) with *P.* group F in a water culture system**

FIGURE 4 a **Fennel uninfected (left) and naturally infected (right) with *P.* group F in a gravel culture system**

FIGURE 4 b **Fennel uninfected (left) and artificially infected (right) with *P.* group F in a water culture system**

FIGURE 5 a **Cucumber uninfected (left) and naturally infected (right) with *P. spinosum* in a ebb-and-flow system**

FIGURE 5 b **Cucumber uninfected (left) and artificially infected (right) with *P. spinosum* in a water culture system**