European Journal of Plant Pathology **104:** 69–76, 1998.

Inoculation of cucumber roots with zoospores of mycoparasitic and plant pathogenic *Pythium* species: Differential zoospore accumulation, colonization ability and plant growth response

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Accepted 21 October 1997

Key words: biocontrol, hydroponics, *Pythium aphanidermatum, Pythium* 'group F', *Pythium oligandrum, Pythium periplocum*

Abstract

Hydroponically grown cucumber (*Cucumis sativus*) seedlings were inoculated with zoospores of 1 mycoparasitic (*Pythium oligandrum*) and 2 pathogenic (*Pythium aphanidermatum* and *Pythium* 'group F') *Pythium* spp. During the first 2 days after inoculation, all the *Pythium* spp. caused reduction in the root length. However, roots treated with *Pythium oligandrum* quickly reached the length of the control and on the 8th day, and for the rest of the experimental period, stimulation of root elongation was noted. *Pythium oligandrum* was not pathogenic on cucumber and no differences in the fresh weights of control and *Pythium oligandrum* inoculated plants were observed in the course of the experiment. *Pythium* 'group F' and *Pythium aphanidermatum* were pathogenic on cucumber seedlings, but their pathogenicities differed. Thus, while *Pythium* 'group F' had a constant, negative influence on root length and plant growth, measured as fresh weight, *Pythium aphanidermatum* caused generalized necroses of the root system, inhibiting consistently root elongation and plant growth and finally causing plant death. Moreover, the zoospores of 2 mycoparasitic species, *Pythium oligandrum* and *Pythium periplocum*, were not attracted to roots of cucumber and accumulated on the roots in very low numbers compared to those of the pathogenic species, *Pythium aphanidermatum*, which were strongly attracted and accumulated in large numbers. Finally, it was also found that *Pythium oligandrum* colonized the roots very poorly, while *Pythium* 'group F' and *Pythium aphanidermatum* were significantly better root colonizers. The significance of these findings is discussed in relation to the ecology of *Pythium* species and biocontrol.

Introduction

In greenhouse production systems, including hydroponics, *Pythium* spp. are common root pathogens of many plant species (Jenkins and Averre, 1983; Stanghellini and Rasmussen, 1994). On cucumber (*Cucumis sativus* L.), root rot may be caused by several pathogenic *Pythium* species (Jenkins and Averre, 1983; Favrin et al., 1988). Among these, *Pythium aphanidermatum* (Edson) Fitzp. is one of the most serious pathogens capable of causing catastrophic yield losses (Favrin et al., 1988). Other *Pythium* species may occur in the rhizosphere as 'minor pathogens' [*sensu* Salt (1979)], since infected plants, under normal conditions, present no visible symptoms except for reduced growth (Funck-Jensen and Hockenhull, 1983). Isolates belonging to *Pythium* 'group F' [*sensu* Plaats-Niterink (1981)], which have been isolated from hydroponic systems from apparently healthy cucumber (Funck-Jensen and Hockenhull, 1983) and tomato roots (Rafin and Tirilly, 1995) seem to be thus a kind of a minor pathogen.

The interaction between plant roots and mycoparasitic *Pythium* is still incompletely understood,

and while there is evidence that *Pythium oligandrum* Drechsler (McQuilken et al., 1990; Kratka et al., 1994) and *Pythium periplocum* Drechsler (Hockenhull et al., 1992) have no pathogenic effects on crop plants, other evidence suggests that *P. oligandrum* may be capable of damaging healthy plant roots, but the effect is transient and reversible (Vesely, 1987; Kratka et al., 1994). Promotion of plant growth (Vesely, 1987; Cother and Gilbert, 1993; Kratka et al., 1994) and increased phosphorus uptake by the plant (Kratka et al., 1994) have also been attributed to *P. oligandrum*.

The mycoparasitic abilities of *P. oligandrum* and *P. periplocum* are well documented, and especially *P. oligandrum* has been utilized for biological control of several plant pathogens (Lutchmeah and Cooke, 1985; Martin and Hancock, 1987; McQuilken et al., 1990). Most of the biocontrol experiments performed with *P. oligandrum* have utilized mycelia and oospores to give seed protection (Lutchmeah and Cooke, 1985; Martin and Hancock, 1987; McQuilken et al., 1992). Unfortunately, as mentioned by Madsen et al., (1995), inoculant formulations containing mycelia are difficult to store successfully, and oospore formulation of *P. oligandrum* germinated poorly and slowly (McQuilken et al., 1992). Treatment of seedlings growing in hydroponics with zoospores of *P. oligandrum* may be a good alternative for protecting plants in this and other growing systems. Mycoparasitic activity has been observed both with cysts and motile zoospores of *P. oligandrum* (Madsen et al., 1995), and if zoospores of mycoparasites such as *P. oligandrum* are to be used as inoculum sources for biocontrol, it is important to know to what extent *P. oligandrum* can establish, multiply and spread on plant roots. While the attraction of zoospores of *P. aphanidermatum* and several other plant pathogenic species of *Pythium* to plant roots is well documented (Jones et al., 1991; Wester et al., 1991), no work on the behaviour of zoospores of mycoparasitic *Pythium* spp. towards roots appears to have been published. Thus, better knowledge of zoospore behaviour and root colonization patterns of mycoparasitic *Pythium* species would be useful.

The investigations reported here were carried out on cucumber seedlings growing in a simple hydroponic system using zoospores as the inoculum source. The aim was to compare mycoparasitic and plant pathogenic *Pythium* spp. with regard to their: i) effect on seedling growth, ii) ability of zoospores (cysts) to accumulate on cucumber and other crop plant roots and iii) root colonization abilities.

Materials and methods

Fungal isolates

The isolates of *Pythium oligandrum* and *Pythium* 'group F' were isolated in Denmark from roots of pea (*Pisum sativum* L.) by Nini Leroul and hydroponically grown pepper (*Capsicum annuum* L.) by Connie N. Rosendahl, respectively. The isolates of *Pythium periplocum* and *Pythium aphanidermatum* were isolated from Indonesian soil (Hockenhull et al., 1992).

Plant material and growth condition

The experiments conducted to examine the effect on plant growth and root colonization ability were carried out on cucumber (*Cucumis sativus* L. cv. Corona) seedlings inoculated with 1 mycoparasitic (*P. oligandrum*, isolate number 1010) and 2 pathogenic [*P. aphanidermatum* (170) and *Pythium* 'group F' (11)] *Pythium* species growing in a simple hydroponic system. To investigate the ability of zoospores of *Pythium* spp. to accumulate on plant roots, experiments were conducted using seedlings of cucumber, French bean (*Phaseolus vulgaris* L.), lettuce (*Lactuca sativa* L.), radish (*Raphanus sativus* L.), tomato (*Lycopersicon esculentum* L.) and dill (*Anethum graveoleus* L.), inoculated with 2 mycoparasitic [*P. oligandrum* (1004) and (1010, cucumber only) and *P. periplocum* (1048)] and 1 plant pathogenic [*P. aphanidermatum* (170)] *Pythium* species. Seedlings were produced as described below.

Seeds were surface sterilized by immersion in 70% ethanol for 1 min, followed by immersion in a solution of 2% sodium hypochlorite for 5 min. Subsequently, the seeds were washed twice in sterile distilled water (SDW) and left to soak overnight. The seeds were then germinated aseptically in Petri dishes containing sterile filter paper moistened with SDW. The Petri dishes were covered with aluminum foil and incubated for 72 h at room temperature. When the cotyledons had emerged, the foil was removed. Three to 5 days after sowing, seedlings with identical root systems measuring 3 cm in length were selected. The chosen seedlings were mounted in plastic strips (10 cm $\times$ 2 cm), containing 4 holes (0.5 cm in diameter), one plant per hole. The plastic strips with the seedlings were placed over plastic tanks (8.0 cm $\times$ 5.5 cm $\times$ 4.5 cm) filled with 150 ml of nutrient solution, prepared with 1000 ml autoclaved tap water and 1 ml liquid fertilizer (Substral ^{R}, Henkel, Taastrup, Denmark). No special precautions were taken to grow the seedlings aseptically. The tanks were

kept in a greenhouse at 22/25 °C with 16 h of coolwhite fluorescent light (145FEm^{-2} s^{-1}) and topped up with the nutrient solution every second day.

Zoospore production

Production of zoospores of the mycoparasitic *Pythium* spp. and *P. aphanidermatum* was based on the method of Rahimian and Banihashemi (1979). Briefly, 90 mm Petri dishes containing V8-agar [V-8 juice (Campbell, Soup Company, USA), 200 ml; Difco Agar (Difco Laboratories, Detroit, USA), 20 g; distilled water, 1000 ml) were inoculated with the isolates and grown at 35 °C under continuous light provided by two 18 watt fluorescent tubes, placed 35-40 cm above the Petri dishes. When growth had reached the perimeter of the plates, the culture was cut into 5 mm strips and half of the strips were moved to an empty plate. Twenty ml of sterile-filtered pond water (PW) was added to each plate. After 30 min the PW was decanted and fresh added after which the plates were incubated under continuous light at 35 °C in order to induce sporangium formation (Rahimian and Banihashemi, 1979). Formation of sporangia required induction periods of 24 h for *P. aphanidermatum* and 48 h for *P. oligandrum* and *P. periplocum*. To induce vesicle formation and zoospore release, the PW was changed again and the strips incubated at 20 °C. Approximately 10^4 zoospores per ml were harvested 4 h later.

Zoosporogenesis of *Pythium* 'group F' was performed according to Chérif et al. (1991) with minor modifications. Ten mm diameter plugs were cut from the margin of a 2-day-old culture of *Pythium* 'group F' (growing on V-8 juice agar at 27 °C), transferred to a sterile Petri dish, flooded with SDW and incubated at room temperature. The SDW was changed 24 h later, and the Petri dishes incubated in the dark at 20 °C. Approximately 10^5 zoospores per ml were harvested 3 h later.

Zoospore inoculation

Zoospores for counting were immobilized by vigorous shaking on a vortex mixer and the number of cysts was determined in a haemocytometer. Seedlings were inoculated with 3000–5000 zoospores per ml by adding the zoospores to the nutrient solution. In the tanks containing the control plants, 1 ml of a V-8 agar solution, prepared by liquidizing 15 ml V8-agar in 100 ml SDW, was added instead of the zoospore suspension.

Pathogenic and growth stimulation effects on cucumber following inoculation with P. oligandrum, Pythium *'group F' and* P. aphanidermatum

The effects of the 3 *Pythium* spp. on cucumber seedlings were assessed by measuring the total length of the root system and by determining the total fresh weight of the plants on the 3rd, 4th, 6th, 8th, 10th and 12th day after inoculation. After measuring and weighing, the plants were returned to their respective tanks. The experiments consisted of 3 completely randomized blocks each with 4 treatments and 3 plants per treatment.

Accumulation of zoospores of P. oligandrum, P. periplocum *and* P. aphanidermatum *on roots of cucumber and other plant species*

In order to simplify observation of zoospore attachment and accumulation, roots were coated with a double layer of calcium alginate by the method of Jones et al. (1991). Uncoated roots and glass coated capillary tubes served as controls. Immediately after treatment, and prior to inoculation with zoospores, the coated and uncoated roots and coated capillary tubes were rinsed in SDW.

The roots and capillary tubes were exposed to the zoospores for 30 min, after which they were mounted in water in open-ended chambers (Jones et al., 1991) and viewed using dark-field transmitted light microscopy (Nikon Optiphot). The numbers of cysts attached to the transparent layers of calcium alginate along 2 sides of the root or capillary tube were recorded per mm. Similarly, roots of 3–4 day-old seedlings of French bean, lettuce, radish, tomato and dill, together with capillary tube controls, were inoculated with zoospores of *P. oligandrum*, *P. periplocum* and *P. aphanidermatum*. Experiments were repeated 3 times and the number of encysted zoospores was calculated with the average of the three repetitions.

Colonization of cucumber roots by P. oligandrum, Pythium *'group F' and* P. aphanidermatum

Root colonization was assessed using CMA-P_{10}ARP selective medium (Jeffers and Martin, 1986). Samples of inoculated and non-inoculated (control) roots were selected at random and analysed on the 2nd, 4th, 6th and 8th day after inoculation. Roots were segmented (10 mm), plated on the selective medium, and incubated at 27 ° C. Based on the assumption that each species

Table 1. Length (cm) of the root system of cucumber seedlings following inoculation with mycoparasitic and pathogenic *Pythium* spp.

Treatment	Days following inoculation [1]					
	2	4	6	8	10	12
Control	4.54^a	6.40^a	7.31^a	8.38^a	10.03^a	11.35^a
P. oligandrum	3.84^b	5.92^a	7.24^a	9.58^b	11.26^b	13.12^b
Pythium 'group F'	3.19^c	3.39^b	4.19^b	5.18^c	6.98^c	8.72^c
P. aphanidermatum	3.13^c	3.22^b	3.19^c	3.28^d	ND[2)]	ND[2)]

In each column, values with the same letter are not significantly different (P=5%).
[1)] Length of root system at inoculation time was 3.00 cm.
[2)] ND: Not determined, since half of the plants were dead.

produces similar amounts of growth on CMA-P_{10}ARP per colonization unit, assessment was performed by counting the number of *Pythium* hyphae that grew out from the root segments. The time for assessment was chosen according to the fungal growth rate. Thus, root colonization assessment took place approximately 3 h, 6 h and 7 h after plating on CMA-P_{10}ARP for *P. aphanidermatum*, *Pythium* 'group F' and *P. oligandrum* respectively.

Results were expressed using the following colonization index: index 0 = no hyphae detected; index 1 = 1–20 hyphae per segment; index 2 = 21–40 hyphae per segment; index 3 = 41–60 hyphae per segment; index 4 = 61–80 hyphae per segment; index 5 = 81–100 hyphae per segment; index 6 = more than 100 hyphae per segment. The root colonization index was calculated using the index from 5 plants, 5 root segments per plant.

Statistical analysis

Analysis of variance was conducted using the General Linear Models (GLM) of the Statistical Analysis Systems (SAS) package (Statistical Analysis Systems Institute Inc., Cary, NC, USA). The mean of each treatment, in the different experiments, was compared using the Student-Newman-Keuls test (SAS Institute Inc.). In the case of the experiment on the accumulation of *Pythium* zoospores on the roots, the untransformed data (number of encysted zoospores per isolate) were compared using the T-test according to Rudemo (1979).

Results

Pathogenic and growth stimulation effects on cucumber following inoculation with P. oligandrum, Pythium *'group F' and* P. aphanidematum

On day 2, all plants inoculated with *Pythium* showed a similar response with significantly inferior root growth compared to the control plants (Table 1). However, roots inoculated with *P. oligandrum* were less negatively affected than roots inoculated with *Pythium* 'group F' and *P. aphanidermatum*. On the 4th and 6thday, there were no significant differences between roots of control and *P. oligandrum* inoculated plants. A significant, positive effect on the root elongation was observed in plants treated with *P. oligandrum* on the 8th day after inoculation and for the rest of the experimental period (Table 1). Roots treated with *Pythium* 'group F' and *P. aphanidermatum* were, in contrast, significantly shorter than control roots and roots treated with *P. oligandrum*. Whereas inhibition of the root elongation was observed during the whole experimental period, on the 6th and 8th day after inoculation, *Pythium* 'group F' inhibited the root elongation less than *P. aphanidermatum.* On the 10th and 12th day after inoculation, while roots were still severely inhibited by *Pythium* 'group F', half of the plants inoculated with *P. aphanidermatum* were dead by this time.

Although there was a tendency towards an increase in fresh weight due to inoculation of roots with *P. oligandrum* (Table 2), no significant effect on the fresh weight of the plants was found during the course of the experiment. Seedlings inoculated with *Pythium* 'group F' initially showed a strong reduction in the fresh weight, but on the 6th and subsequent days, the plants appeared to be less affected than plants treated

Table 2. Fresh weight (g/plant) of cucumber seedlings inoculated with mycoparasitic and pathogenic *Pythium* spp.

Treatment	Days following inoculation					
	3	4	6	8	10	12
Control	0.63^a	0.85^a	1.17^a	1.69^a	2.62^a	3.63^a
P. oligandrum	0.61^a	0.84^a	1.23^a	1.75^a	2.73^a	3.89^a
Pythium 'group F'	0.44^b	0.54^b	0.84^b	1.30^b	1.96^b	2.59^b
P. aphanidermatum	0.36^c	0.44^b	0.54^c	0.55^c	ND[1)]	ND[1)]

In each column, values with the same letter are not significantly different (P=5%).
[1)] ND: Not determined, since half of the plants were dead.

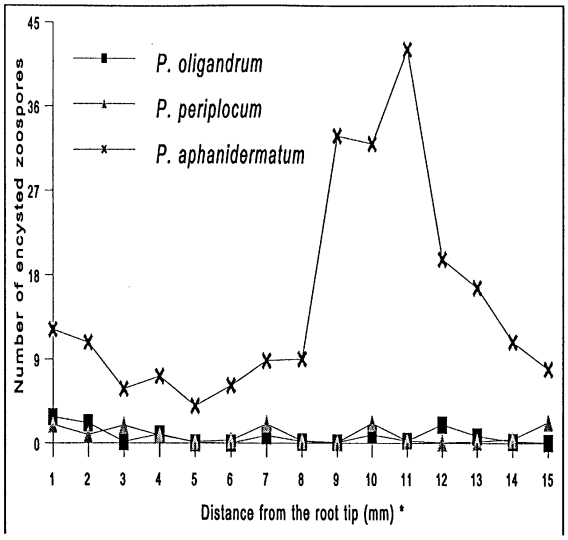

Figure 1. Accumulation of zoospore cysts of *P. oligandrum* (1004), *P. periplocum* (1048) and *P. aphanidermatum* (170) on cucumber roots. *) 0–1 mm = root tip. 1–8 mm = elongation zone. 8–15 mm = root hair zone.

Table 3. Root colonization index[1)] for cucumber seedlings inoculated with zoospores of mycoparasitic and pathogenic *Pythium* spp. using the selective medium method

Treatment	Days following inoculation			
	2	4	6	8
P. oligandrum	0.80^a	0.72^a	0.56^a	0.36^a
Pythium 'group F'	2.92^b	4.80^b	5.48^b	3.96^b
P. aphanidermatum	3.24^b	3.68^c	4.48^b	4.12^b

Values with the same letter, in each column, are not significantly different (P=5%).
[1)] The number of hyphae was determined with the average of five repetitions (5 root segments per repetition) and expressed according to the index: index 0 = no hyphae detected; index 1 = 1–20 hyphae per segment; index 2 = 21–40 hyphae per segment; index 3 = 41–60 hyphae per segment; index 4 = 61–80 hyphae per segment; index 5 = 81–100 hyphae per segment; index 6 = more than 100 hyphae per segment.

with *P. aphanidermatum*. Throughout the experiment, *P. aphanidermatum* severely suppressed plant growth.

Accumulation of zoospores of P. oligandrum, P. periplocum *and* P. aphanidermatum *to roots of cucumber and other plant species*

Large numbers of zoospores of *P. aphanidermatum* attached and encysted on the cucumber roots (Figure 1), but only a very low number of cysts of *P. oligandrum* and *P. periplocum* accumulated on roots, similar to the numbers on the capillary control tubes (data not presented). In contrast, larger number of cysts of *P. aphanidermatum* attached to the roots compared to the capillary control tubes (data not presented).

Considerable numbers of zoospores of *P. aphanidermatum* attached and encysted on all parts of the root but the root hair zone was clearly the most favoured zone of accumulation (Figure 1). Accumulation of zoospores of *P. periplocum* and *P. oligandrum* was not correlated to any root zone and occurred at a consistently low rate all along the root (Figure 1). Similar response patterns were observed in 5 other plant species. No difference was observed between the 2 *P. oligandrum* isolates.

Colonization of cucumber roots by P. oligandrum, Pythium *'group F' and* P. aphanidermatum

The number of hyphae found on the selective medium clearly showed that *P. oligandrum* was a very poor root colonizer compared to *Pythium* 'group F' and *P. aphanidermatum* (Table 3). The pathogenic *Pythium* species presented almost the same ability to colonize cucumber roots, since with the possible exception of the 4th day, no significant differences between the root colonization indexes of *Pythium* 'group F' and

P. aphanidermatum were recorded in the experimental period (Table 3).

Discussion

The results here reveal several strikingly different responses of cucumber seedlings, growing hydroponically, following inoculation with zoospores of mycoparasitic and pathogenic *Pythium* spp. For the first few days following inoculation, plants treated with zoospores of both pathogenic and the mycoparasitic *Pythium* spp., reacted negatively to the primary stages of root colonization, showing shorter roots compared with control plants. Likewise, Kratka et al. (1994) observed reduction of the dry weight of cucumber roots, during the first two days after inoculation with mycelium and oospore suspensions of *P. oligandrum* and *P. ultimum*. In our study, this reaction was less pronounced in plants inoculated with *P. oligandrum* than in plants inoculated with *Pythium* 'group F' and *P. aphanidermatum*. Of the 2 plant pathogenic species, *P. aphanidermatum* affected root elongation more than *Pythium* 'group F'.

Plants inoculated with *P. oligandrum* rapidly achieved the root length of the control plants, and by the 8th day after inoculation, *P. oligandrum* had a stimulating effect on the root elongation, since the inoculated plants had longer roots than those of the controls (Table 1). A similar effect on root elongation has been seen in roots of plants inoculated with growth promoting rhizobacteria (Schroth et al., 1984). While the stimulatory effect on root elongation by *P. oligandrum* reported here has not been previously reported, general plant growth promotion (Vesely, 1987; Cother and Gilbert, 1993; Kratka et al., 1994) has been found in seedlings treated with this fungus. Plant growth promoting effects have also been reported for several non-pathogenic *Pythium* species, including *P. vexans* and an unidentified *Pythium* sp. on wheat and ryegrass (Dewan and Sivisithamparam, 1988). Auxin is known to play an important role in plant growth regulation. According to Schroth et al. (1984), while high levels of auxin produced by a microorganism can influence plants negatively (e.g. by causing growth reduction), low levels may have a positive effect (e.g. by causing growth stimulation). In another study (Wulff, 1996), the level of auxin (indole-3-acetic acid) synthesized *in vitro* by *P. oligandrum* was very low compared to that produced by *P. aphanidermatum* and *Pythium* 'group F' isolates. The possible role of auxin synthesis by *P. oligandrum* in connection with growth stimulation seems worthy of further investigation.

Besides the absence of disease symptoms on roots inoculated with *P. oligandrum*, the fresh weight of the plants revealed no significant differences between the controls and plants inoculated with this fungus, indicating that *P. oligandrum* was not pathogenic to the cucumber plants. While *P. oligandrum* stimulated root elongation, it did not stimulate the growth of the plant in terms of the total fresh weight, in this experiment or, expressed as dry weight of the roots and upper parts of the plants, in another experiment (Wulff, 1996). In contrast, following inoculation with zoospores, both *Pythium* 'group F' and *P. aphanidermatum* reduced the growth of the cucumber roots, which showed disease symptoms. Of the two, *P. aphanidermatum* was found to be the most aggressive species in the hydroponic system used here. It caused generalized necrosis of the root system, inhibiting consistently and strongly root elongation, reducing plant growth and finally causing plant death. Likewise, in the study conducted by Favrin et al. (1988) with cucumber, *P. aphanidermatum* caused reduction of the root system (fewer lateral roots), inhibition of growth and plant death. The direct effect on root elongation seen here was not mentioned by Favrin et al. (1988), but in a different study (Larkin et al., 1995), inhibition of root elongation together with reduced branching were observed in alfalfa seedlings inoculated with other pathogenic *Pythium* spp.

Also our isolate of *Pythium* 'group F' was clearly pathogenic to cucumber, since it had a constant, negative influence on root length and plant growth, measured as fresh weight. The consistent inhibition of root extension during the course of the experiment, matches the findings of Wester et al. (1991), who observed that zoospores of their *Pythium* 'group F' isolate were strongly attracted to the elongation zone of the root. In the present study, *Pythium* 'group F' reduced significantly both root elongation and the fresh weight of the plant, showing it to be an aggressive pathogen of cucumber plants in hydroponic cultures, although as mentioned above, not as aggressive as the isolate of *P. aphanidermatum*. These results are also confirmed by ultrastructural studies conducted by Rey et al. (1996).

Considering the ability of the 3 *Pythium* species to colonize roots, it was found that *P. oligandrum* was hardly detected on the roots compared to *Pythium* 'group F' and *P. aphanidermatum*. The same results were obtained when detection was conducted by staining the hyphae of these *Pythium* species immunoenzymatically (Wulff, 1996). The poor root colonization

ability of *P. oligandrum*, reported here, has previously only been briefly mentioned. Thus, Martin and Hancock (1987) and McQuilken et al. (1990) noted that *P. oligandrum* was rarely isolated from cress and sugar beet roots when seeds were inoculated with oospores. However, in our study, despite its poor colonization ability, *P. oligandrum* stimulated root elongation. Also Cother and Gilbert (1993) found poor colonization of rice roots following treatment with an oospore suspension of *P. oligandrum*, despite the growth promoting effect observed on rice seedlings. Madsen (1996) compared the ability of *P. oligandrum* to colonize roots of diseased and non-diseased plants and found that a significantly higher number of roots from diseased plants where colonized by the mycoparasite.

The poor ability to colonize the roots showed by *P. oligandrum* matches very well with the low number of attached zoospores of *P. oligandrum* and *P. periplocum* found on the roots. The distribution of zoospores of the mycoparasitic species was fortuitous compared with that of the pathogen *P. aphanidermatum* and it seems that *P. oligandrum* and *P. periplocum* do not possess a mechanism for root recognition as shown by *P. aphanidermatum*, in the present study, and by *Pythium* 'group F', in a study conducted by Wester et al. (1991). This lack of a root recognition by the zoospores of the two mycoparasites was, in fact, directly observed under the microscope. Thus, no tactic response to the roots by zoospores of *P. oligandrum* and *P. periplocum* was observed. Instead, zoospores of the mycoparasitic species were seen to swim completely undirectionally and to encyst and adhere to any available surface, *i.e.* any part of the root, the plastic tank wall or the control capillary tubes.

Finally, taking practical biocontrol in hydroponic systems into account, we conclude from our results that it may prove difficult to establish *P. oligandrum* on young, healthy roots using zoospores as the inoculum source. However, establishment on roots of older plants might be more successful, because such roots are more likely to be colonized by other fungi from which *P. oligandrum* could, through mycoparasitism, obtain nutrients for growth, thus enabling it to protect the roots from pathogen attack.

References

Chérif M, Benhamou N and Bélanger RR (1991) Ultrastructural and cytochemical studies of fungal development and host reactions in cucumber plants infected with *Pythium ultimum*. Physiological and Molecular Plant Pathology 39: 353–375

Cother EJ and Gilbert RL (1993) Comparative pathogenicity of *Pythium* species associated with poor seedling establishment of rice in Southern Australia. Plant Pathology 42: 151–157

Dewan MM and Sivisithamparam K (1988) *Pythium* spp. in roots of wheat and rye-Grass in Western Australia and their effect on root rot caused by *Gaeumannomyces graminis* var. *tritici*. Soil Biology and Biochemistry 20: 801–808

Favrin RJ, Rahe JE and Mauza B (1988) *Pythium* spp. associated with crow root of cucumbers in British Columbia greenhouses. Plant Disease 72: 683–687

Funck-Jensen D and Hockenhull J (1983) The influence of some factors on theseverity of *Pythium* root rot of lettuce in soilless (hydroponic) growing systems. Acta Horticulturae 133: 129–136

Hockenhull J, Funck-Jensen D and Yudiarti T (1992) The use of *Pythium periplocum* to control damping-off of cucumber seedlings caused by *Pythium aphanidermatum*. In: Tjamos EC, Papavizas GC and Cook RJ (eds) Biological Control of Plant Diseases: Progress and Challenges forthe Future. (pp. 203–206) Plenum Press, New York

Jeffers SN and Martin SB (1986) Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Disease 70: 1038–1043

Jenkins SFJr and Averre CW (1983) Root diseases of vegetables in hydroponic culture systems in North Caroline greenhouses. Plant Disease 67: 968–970

Jones SW, Donaldson SP and Deacon JW (1991) Behaviour of zoospores andzoospore cysts in relation to root infection by *P. aphanidermatum*. New Phytologist 117: 289–301

Kratka J, Bergmanova E and Kudelova A (1994). Effect of *Pythium oligandrum* and *Pythium ultimum* on biochemical changes in cucumber (*Cucumis sativus* L.). Journal of Plant Disease and Protection 101: 406–413

Larkin RP, English JT and Mihail JD (1995) Effects of infection by *Pythium* spp. on root system morphology of alfalfa seedlings. Phytopathology 85: 430–435

Lutchmeah RS and Cooke RC (1985) Pelleting of seed with the antagonist *Pythium oligandrum* for biological control of damping-off. Plant Pathology 34: 528–531

Madsen AM, Robinson HL and Deacon JW (1995) Behaviour of zoospores cysts of the mycoparasite *Pythium oligandrum* in relation to their potential for biocontrol of plant pathogens. Mycological Research 99: 1417–1424

Madsen, AM (1996) Use of a metalaxyl tolerant *Pythium oligandrum* isolatefor selectively following its population in soil and its colonization of roots of diseased and non-diseased plants. In: Jensen DF, Jansson H-B and Tronsmo A (eds) Monitoring Antagonistic Fungi Deliberately Released into the Environment (pp39–48) Kluwer Academic Publishers, Dordrecht, Boston, London

Martin FN and Hancock JG (1987) The use of *Pythium oligandrum* for biological control of preemergence damping-off caused by *P. ultimum*. Phytopathology 77: 1013–1020

McQuilken MP, Whipps, JM and Cooke RC (1990) Control of damping-off in cress and sugar-beet by commercial seed-coating with *Pythium oligandrum*. Plant Pathology 39: 452–462

McQuilken MP, Whipps, JM and Cooke, RC (1992) Use of oospore formulations of *Pythium oligandrum* for biological control of *Pythium* damping-off in cress. Journal of Plant Pathology 135: 124–134

Plaats-Niterink van der AJ (1981) Monograph of the Genus *Pythium*. Centraalbureau voor Schimmel Cultures Baarn Rafin C and Tirilly Y (1995) Characteristics and pathogenicity of *Pythium* spp.

associated with root rot of tomatoes in soilless culture in Britanny (France). Plant Pathology 44: 779–785

Rahimian MK and Banihashemi Z (1979) A method for obtaining zoospores of *Pythium aphanidermatum* and their use in determining cucurbit seedling resistance to damping-off. Plant Disease Reporter 63: 658–661

Rey P, Benhamou N and Tirilly Y (1996) Ultrastructural and cytochemical studies of cucumber root infected by two *Pythium* species with different modes of pathogenicity. Physiological and Molecular Plant Pathology 49: 213–231

Rudemo M (1979) Statistik and sandsynlighedslære med biologiske anvendelser. Del 2. DSR forlag, Copenhagen

Salt GA (1979) The increasing interest in 'minor pathogens'. In: Schippers B.and Gams W (eds) Soil-Borne Plant Pathogens. (pp. 289–312) Academic Press, London

Schroth MN, Loper JE and Hildebrand DC (1984) Bacteria as biological control agents for plant disease. In: Klug MJ and Reddy CA (eds). Current Perspectives in Microbial Ecology. (pp. 362–369) American Society for Microbiology, Washington D.C.

Stanghellini ME and Rasmussen SL (1994) Hydroponics: a solution for zoosporic pathogens. Plant Disease 78: 1129–1138

Vesely D (1987) Biological control of damping-off by treating sugarbeet with a powdery preparation of the mycoparasitic *Pythium oligandrum* in large- scale field trials. In: Vancura V and Kuuc F (eds) Interrelationship between Microorganisms and Plant in Soil. (pp 445–449) International symposium, June 22–27, 1987. Academic of Sciences, Liblice, Czechoslovakia

Wester E, Jensen DF and Moller D (1991) Anatomical studies of cucumber roots inoculated with *Pythium* zoospores. In: Beemster ABR, Bollen GJ, Gerlagh M, Ruissen MA, Shippers B and Tempel A (eds) Biotic Interactions and Soil-Borne Diseases. (pp. 401–407) Proceedings of the First Conference of the European Foundation for Plant Pathology, Wageningen. Elsevier Science Publishers B.V., Amsterdam

Wulff, EG (1996) Studies of mycoparasitic and plant parasitic *Pythium* spp.: Root colonization, histopathology, growth stimulation and auxin production. M. Sc thesis. 99p. The Royal Veterinary and Agricultural University, Department of Plant Biology, Copenhagen