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Pathogenicity of *Pythium* species on cucumber in peat-sand, rockwool and hydroponics

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Abstract. *Pythium* spp. that cause damping-off of seedlings also can cause root rot of older plants and lead to yield reductions. This can be especially severe in soilless cultures where the fungus can spread easily with the nutrient solution. 39 *Pythium* isolates obtained from discolored roots were assayed for their ability to cause damping-off on cucumber seedlings in sand-peat and for their pathogenicity in soilless culture of cucumber in rockwool or hydroponic solution. Isolates of *Pythium aphanidermatum, P. irregulare, P. sylvaticum* and *P. ultimum* were highly pathogenic in sand-peat, but only *P. aphanidermatum* strains were pathogenic in soilless conditions and led to root decay, plant death in rockwool culture and growth reduction in hydroponic culture. One strain of *P. aphanidermatum* significantly reduced the yield of cucumber grown in rockwool under conditions similar to those of commercial cultures.

Introduction

Although *Pythium* spp. cause mainly damping-off diseases of seedlings of many plant species [Hendrix and Campbell, 1973; Van Der Plaats-Niterink, 1981], they also can cause severe root rot on mature plants such as sugarcane [Lee and Hoy, 1992], turfgrasses [Nelson and Craft, 1991], geranium [Chagnon and Bélanger, 1991] and tulips [Weststeijn, 1990] grown in soil. Root rot is especially important in hydroponics and rockwool cultures of tomato [Jenkins and Averre, 1983], cucumber [Chérif and Bélanger, 1992; Favrin et al., 1988; Jenkins and Averre, 1983; Stanghellini et al., 1988], spinach [Bates and Stanghellini, 1984; Gold and Stanghellini, 1985] and lettuce [Funck-Jensen and Hockenhull, 1983, Jenkins and Averre, 1983; Stanghellini and Kronland, 1986], in which the fungus can spread easily with the nutrient solution. In soilless culture of cucumber, symptoms produced by *Pythium* spp. are not always evident. In clear cases, roots show localized necrotic lesions that spread over the root system which decays. In other cases, the upper parts of plants may wilt during warm and sunny days even in the absence of obvious symptoms of necrotic lesions on the roots [Couteaudier and Lemanceau, 1989; Favrin et al. 1988; Stanghellini and Kronland, 1986] that appear uniformly discolored. Plant

infection by *Pythium* spp. may lead to yield reductions or death of plants. In some countries of western Europe cucumber growers are used to apply propamocarb (Schering) every 3 weeks to control yield reduction due to *Pythium* spp. In order to propose control procedures based on accurate experiments, it is necessary to determine which *Pythium* species are responsible for these yield reductions. *Pythium aphanidermatum* is highly pathogenic under soilless culture conditions, but other species can be isolated from cucumber roots, and their pathogenicity is still unclear.

The purpose of the present study was to collect many isolates of *Pythium* spp. form different origins and determine their pathogenicity on cucumber plants under different growing conditions. As it was impracticable to test all of the strains for their ability to induce yield reduction on mature plants, it was necessary to develop a test to evaluate the pathogenicity of *Pythium* spp. on young cucumber plants grown in rockwool or in hydroponic solution. Moreover, a standardized pathogenicity test was applied to characterize the ability of *Pythium* spp. to cause damping-off. Finally, one isolate of *Pythium* screened for its ability to induce death of plants in rockwool and to reduce growth of cucumber in hydroponic culture, was also tested for its ability to cause yield reduction in a cucumber culture under conditions analogous to those of a commercial crop.

Materials and methods

Isolates and cultural conditions. The isolates used in this study (Table 1) were obtained from discolored roots of cucumber or tomato plants in soilless culture with or without aerial symptoms. Infected root pieces were surface-disinfested in 1% NaOCl for 30 s and rinsed under sterile tap water for 15 min. Roots were cut into small piece (0.5 cm long) and plated onto malt agar (1%; Biokar Diagnostics, France) acidified to pH 5.5 by citric acid (0.25%). All cultures were transferred several times by hyphal tips on acidified malt agar. The absence of any bacterial contamination was checked in liquid peptone water before storage.

Pythium isolates were maintained on cornmeal agar (CMA, Difco Laboratories, Detroit) at room temperature in dark and subcultured every 4 months. Isolates were also stored in sterile, distilled water on hemp seeds at 4 °C for 1 year. Species were identified on CMA and V8-juice agar (20%, $^{v}/_{v}$) according to the key of Van der Plaats-Niterink [1981]. Two reference isolates of *P. intermedium* de Bary and *P. irregulare* Buisman were used as representative of species responsible for root rot in hydroponics [Stanghellini et al., 1988; Favrin et al., 1988] but they were not taken into account in the percentages presented in the study.

Inoculation with mycelium was used to compare the pathogenicity of isolates. For the damping-off test, isolates were grown for 48 h on malt

Table 1. Isolates of *Pythium* spp. characterized in this study

Isolate reference	Original[a] host	Species[b]	Cultural[c] conditions	Year
H1	Tomato	P. ultimum	Rockwool	1990
88P7		*P. flevoense* or group F		1988
88P8				
88P9				
88P10				
88P11				
88P12				
88P2			Nft	
88P6		nd		
PC1		*P. sylvaticum*		
88P4		*P.* group HS	Turf	
88P5				
PC3	(perfecto)	*P. flevoense* or group F	Nft	
PC5				
PC6				
PC10		*P.* group HS		
PC7			Rockwool	
PC8				
PC2		nd	Nft	
PC4				
PC12	(rondello)			1989
PC13				
PC11		*P. flevoense* or group F		
PC14			*	
PC15		nd	*	
PC16	(prisca)	*P.* group HS	Rockwool	
PC9		nd	Glasswool	1988
88P1	Cucumber	*P. intermedium*	Rockwool	
88P3		*P. aphanidermatum*		
OC1				1990
OC2				
OP3				
OP4				
N3T1			Nft	
N3T4				
N3T2		*P. flevoense* or group F		
N3DT2				
SFT2				
TAT2				
PIN 1		*P. intermedium*	Collection J. Mugnier	
PIR 1		*P. irregulare*	(Rhône-Poulenc)	

[a] ( ) = tomato varieties.

[b] Species were identified according to the key of Van der Plaats-Niterink (1981). Isolates belonging to *P. flevoense* or group F were not distinguished, because they were not tested with compatible isolates of *P. flevoense*. nd = not determined; isolates never produced sexual organs, hyphal swellings or zoospores.

[c] The strains were obtained from roots except isolates indicated by an * that were from nutrient solution. Nft = Nutrient film technique.

agar. For the soilless tests, the inoculation of the plants was made either by introduction of an entire cultured plate on CMA in the hydroponic solution or by introduction into the rockwool of ground mycelium grown for 5 days on malt liquid. Hyphae were washed three times in sterile water and ground in a mixer (Waring Blendor) for 2 min at high speed. Some fragments appeared empty of cytoplasm; others that were assumed to be able to give a colony were counted under the microscope with haemacytometer to determine the inoculum density. It has been verified that standardized grinding procedure give reproducible data. This procedure was necessary to assess the pathogenicity of *Pythium* strains that were not producing zoospores. To test the ability of *Pythium aphanidermatum* (Edson) Fitzp. to cause reductions in fruit yield, zoospores were used as inoculum. A 5 day-old culture on V8-juice agar was flooded with sterile water enriched with β-sitosterol (0.002%) to improve the production of zoospores; after 4 h, the sporangia that had formed released zoospores into the solution. Zoospore density was evaluated with a haemacytometer after immobilization of an aliquot of the suspension in a vortex mixer.

Damping-off test. The method proposed by Bouhot [1975] was applied to assess the ability of the isolates of *Pythium* to cause post-emergence damping-off of cucumber seedlings in soil. Seeds of cucumber (*Cucumis sativus* L. cv. Le Généreux) were sown (three seeds per pot) in sterile sand-peat mixture (20%/80%, $^{v}/_{v}$) and irrigated daily with tap water. After 5 days, when the cotyledons were open, 100 mg of oatmeal per pot were added around the hypocotyl of seedling with one 8 mm-diam plug of mycelium cut from a 48-h-old malt agar culture of *Pythium*. Light (16 h) and night (8 h) temperatures were 25 °C/23 °C before inoculation and 21 °C/18 °C after inoculation. Characteristic symptoms of damping-off appeared 5 days later and were evaluated after 6 days. There were four replicates of three plants per isolate in a randomized design. This experiment has been reproduced with similar results.

Root rot test in rockwool. To assess the pathogenicity of *Pythium* isolates on cucumber, plants were grown in rockwool (Grodan, The Netherlands). Seeds of cucumber (cv. 'Corona') were germinated in sterile Petri dishes on filter paper wetted with sterile water and after 24 h were sown in 9 ml rockwool plugs and watered daily with nutrient solution (Hydrokani; Hydro Azote spécialités; Vitrolles) (EC = 2; pH = 5). Each plant was inoculated 7 days after sowing when the first leaf was open with 9×19^6 propagules (corresponding to 1×10^6 mycelial fragments ml^{-1} rockwool). Number of diseased plants was assessed daily over a 3-week period. Light (16 h) and nigh (8 h) temperature was 28 °C/26 °C until the cotyledons open and then maintained at 25 °C/23 °C until the end of the experiment. The success of inoculation was checked by reisolation of the isolates from infected roots plated on acidified malt agar. Each isolate was applied to six replicates of

12 individual plants in a randomized design, and the experiment was repeated.

Root rot test in hydroponic culture. The pathogenicity of *Pythium* on cucumber plants in hydroponic culture was examined. After 10 days of culture in rockwool plugs as described above, young plants were transplanted to tanks (1 l) containing 900 ml of nutrient solution (Hydrokani; EC = 2; pH = 5) (one plant per tank). One week later, each tank was infested with a 5-day-old CMA culture of *Pythium*. Tanks of control plants received noninoculated CMA. The success of inoculation was monitored by reisolation as above. Two weeks after adding the inoculum, root rot was observed. Shoots and roots were then dried and weighted. Temperature conditions were as above. Three replicates per isolate were arranged in a randomized block design, and the experiment was repeated twice.

Effect of Pythium *on fruit yield.* Cucumber seeds (cv. Corona) were sown in 10 × 10 × 6.5-cm rockwool blocks (one plant per block) and watered daily with a nutrient solution (Hydrokani) maintained at pH 5.5 with a conductivity of 1.8 to 2.5 $mScm^2$. The temperature was as described in root rot test in rockwool. When the plants reached the sixth leaf stage (3 weeks after seedling), the blocks were transplanted to 10 × 15 × 6.5-cm rockwool pads (three plants per pad) on gutters (three pads per gutter) that return the drainage to a storage tank (30 L) so that the plants of each replicate and treatment (3 × 3 plants) were grown in an independent recirculating system. The composition of the nutrient solution was adjusted according to the stage of crop and to the analysis of the nutrient content of the solution in the rockwool.

An isolate of *P. aphanidermatum* (Edson) Fitzp. (OP4) pathogenic in the tests, was tested for its ability to reduce yield in cucumber grown under greenhouse conditions. Plants were either inoculated once at transplanting time or twice at transplanting time and 3 weeks later by applying 200 ml of a suspension of 3.25×10^3 zoospores ml^{-1} (corresponding to 6.5×10^5 zoospores per plant) uniformly to the surface of the block of rockwool. Plants were grown in five rows, each row corresponding to one block with infested (once or twice) and non infested plants in a randomized block design. The volume of drainage was assessed every 2 days, and the volume of nutrient solution consumed per plant was calculated. The experiment was maintained for 13 weeks after the transfer of the plants onto the rockwool pads. Throughout the experiment, marketable fruits with an average weight of 450 g were harvested, and the yield expressed as fruit weight per plant per week was calculated for each treatment. This experiment was conducted both under summer conditions from 16 April to 3 August and autumn conditions from 24 August to 16 November. These 2 periods differ mainly by the average of day length and maximum temperature

Statistical analysis. Data were analyzed by ANOVA after arcsin transformation for percentage data and means were separated by Duncan's multiple range test ($p \leq 0.05$).

Results

Damping-off test. In the damping-off test, the number of dead plants was scored 6 days following inoculation with *Pythium* and did not change after this time. Depending on strains, the percentages of dead plants varied between 0 to 100% (Table 2). Based on these percentages, the isolates were divided into three classes: 26% of the isolates belonged to class 1 (50 to 100% dead plants); 15% of the isolates belonged to class 2 (less than 50% dead plants); and 59% belonged to class 3 (no dead plants). The 2 reference strains of *P. intermedium* and *P. irregulare* determined respectively 75% and 17% of dead plants.

Root rot test in rockwool. Symptoms of disease (wilted and dead plants) appeared 6 days after inoculation. The number of dead plants increased during the next 2 weeks (data not shown) and subsequently did not change (Table 2). Two classes of isolates were distinguished based on pathogenicity. In class 1 (18% of the isolates), more than 65% of the plants died, whereas class 2 isolates (82% of the isolates) were non-pathogenic.

Root rot test in hydroponic culture. Two weeks after inoculation, the growth of plants, expressed as shoots and roots dry weight, was noted (Fig. 1). Two classes of isolates were defined with respect to growth reduction and presence of symptoms on roots compared with control plants. In class 1 (18% of the isolates), inoculated plants showed a significant growth reduction of roots and shoots and rooted roots in comparison with control plants. The means for reductions in root dry weights were 69% ± 7% and in shoot dry weights by 40% ± 8% respectively. In class 2 (82% of the isolates), inoculated plants grew as well as control plants and showed no decaying roots. Four isolates belonging to class 2 (plus the reference isolate of *P. intermedium* de Bary) induced a reduction of plant growth without inducing any obvious symptoms on the roots in the first experiment . However, this growth reduction was not consistent from one experiment to another.

Effect of Pythium *on fruit yield.* The experiments gave similar results whatever the season, thus only the results of the summer experiment are presented. The volume of nutrient solution consumed per plant per week is shown in Table 3 as a percentage of the control plants. From the first week of fruit production until the end of the experiment, inoculated plants showed reduced growth as revealed by a significant reduction of water

Table 2. Pythium isolates responsible for damping-off in soil and death of plants in rockwool plugs

Isolate reference	Percentage of dead plants[c]	
	Damping-off in soil[a]	In rockwool plugs[b]
88P3	100[a]	79[ab]
OC2	100[a]	79[ab]
OC1	91[ab]	62[b]
N3T4	89[abc]	79[ab]
OP3	87[abc]	81[ab]
OP4	84[abc]	91[a]
N3T1	68[bcd]	67[b]
H1	100[a]	0[c]
PC16	100[a]	
88P4	91[ab]	
PIN1	75[bcd]	
PC1	50[cde]	
PC7	39[de]	
PC10	17[e]	
P1R1	17[e]	
88P5	8[e]	
PC13	8[e]	
TAT2	8[e]	
Other isolates	0[e]	

[a] Percentage of dead cucumber seedlings grown in soil 6 days after inoculation with *Pythium*. 4 replicates of 3 plants were inoculated 5 days after sowing with a 8 mm plug of mycelium cut from a 48 hour old malt agar culture of *Pythium*.

[b] Percentage of dead plants of cucumber grown in rockwool plugs 3 weeks after inoculation with *Pythium*. 6 replicates of 12 plants were inoculated 7 days after sowing with 9×10^6 propagules of *Pythium*.

[c] Data were analysed after arcsin transformation; for each column, means followed by the same letter are not significantly different at $p = 0.05$ (Duncan's test).

absorption. Figure 2 shows that the cumulative yield, expressed as kg per plant, in the inoculated treatments was significantly lower than in the control. This difference in production was already significantly established after 3 weeks of production. Inoculating the plants twice with *Pythium* did not result in a significant reduction of yield compared to plants inoculated only once. In all inoculated treatments, plants showed rot of all the roots in contrast to white roots in the control but wilts of inoculated plants was never observed until the end of the experiment. Plants treated with an anti-*Pythium* fungicide (Propamocarb, 200 ml per plant at 0.25%; Schering) produced as much fruits as untreated plants, showing the absence of any pathogenic *Pythium* contamination in the untreated control plants (data not shown).

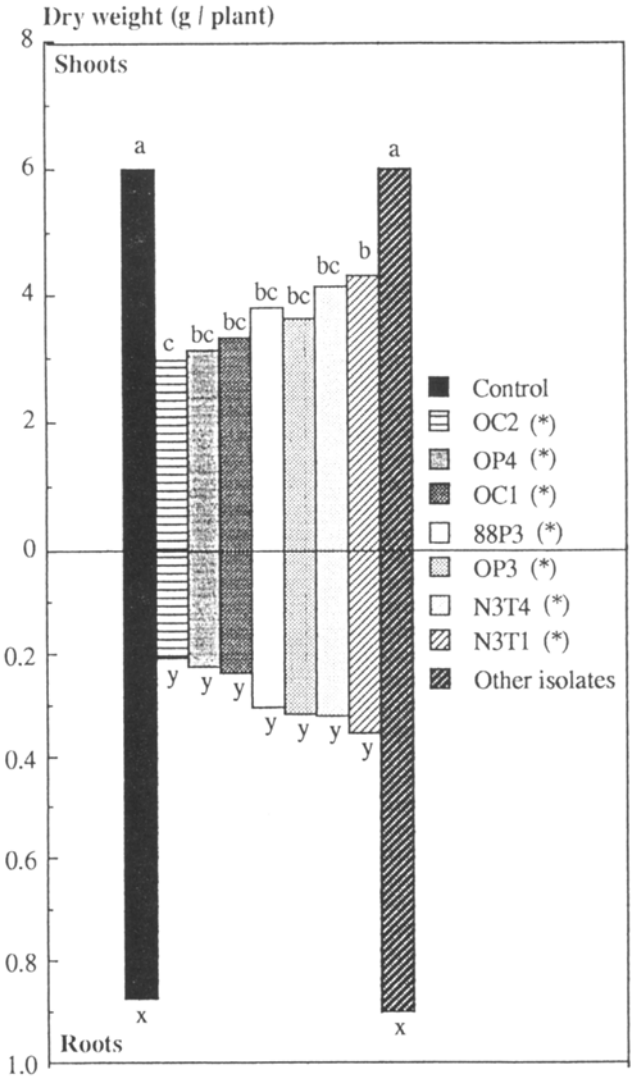

Fig. 1. Effect of *Pythium* on final biomass of cucumber plants in hydroponic culture 2 weeks after inoculation with *Pythium*. Plants were inoculated 17 days after sowing with a 5-day-old CMA culture of *Pythium*. Tanks of control plants received noninoculated CMA plates. * = root rot. Bars with the same letter are not significantly different at $p = 0.05$ (Duncan's test).

Discussion

Pythium spp. are responsible for root rot and yield reduction in several soilless crops such as tomato and cucumber [Chérif and Bélanger, 1992;

Table 3. Effect of *Pythium aphanidermatum* (isolate OP4) on volume of nutrient solution consumed by cucumber plants

	Weeks of fruit production[a]						
	0	1	2	3	4	5	6
Control	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]
OP4 (1×)	93.4[a]	78[b]	81.5[ab]	80[b]	86[b]	86[ab]	91[a]
OP4 (2×)		70[b]	66[b]	85[ab]	77[c]	78[b]	79[a]

[a] Values are given as percentages of control plants consumption (100%). Plants were inoculated once (1×; at transplanting time) or twice (2×; at transplanting time and 3 weeks later) with 200 ml of 6.5 10^5 zoospores of *P. aphanidermatum*. Week 1 corresponded to the first week of fruit production (= 3 weeks after the first inoculation with *Pythium*).

[b] Data were analysed after arcsin (x-0.5) transformation; for each column, means followed by the same letter are not significantly different at $p = 0.05$ (Duncan's test).

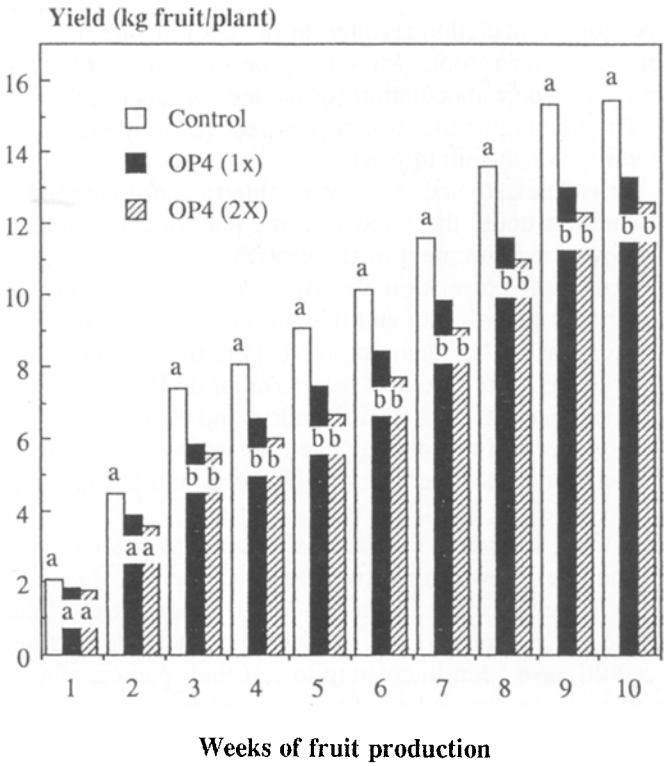

Fig. 2. Effect of *Pythium aphanidermatum* (strain OP4) on cucumber yield under commercial-like culture conditions. Plants were inoculated once (1×; at transplanting time) or twice (2×; at transplanting time and 3 weeks later) with 200 ml of 6.5 × 10^5 zoospores of *P aphanidermatum*. Week 1 corresponded to the first week of fruit production (= 3 weeks after the first inoculation with *Pythium*). Within a week, bars with the same letter are not significantly different at $p = 0.05$ (Duncan's test).

Favrin et al., 1988; Jenkins and Averre, 1983; Stanghellini et al., 1988]. These yield losses can occur even in the absence of any obvious root necrosis [Couteaudier and Lemanceau, 1989; Favrin et al., 1988; Stanghellini and Kronland, 1986]. *Pythium* spp. are consistently isolated in soilless culture of tomato and cucumber even from apparently healthy root systems. The pathogenicity of such unidentified *Pythium* isolates is still unclear, while the pathogenicity of easily identified species such as *P. aphanidermatum* or *P. ultimum* has been extensively studied [Bates and Stanghellini, 1984; Chérif and Bélanger, 1992; Favrin et al. 1988; Nelson and Craft, 1991; Weststeijn, 1990].

The purpose of our study was to characterize the pathogenicity of 39 isolates of *Pythium* obtained from roots of tomato and cucumber grown in different soilless conditions. Most of the isolates used in this study produced zoospores *in vitro* with the exception of 18 isolates. Therefore, we used mycelium as inoculum to enable the comparison of all the isolates. Whatever the type of inoculum used, mycelium in agar or propagules from ground mycelium, inoculation resulted in infection of the plants as shown by isolations made from roots. Moreover, the same types of symptoms were reproduced by zoospore inoculation for isolates producing this type of propagule. The ranking of the isolates for pathogenicity was similar whatever the type of inoculum used.

Among the isolates studied, 41% were able to cause damping-off of cucumber seedlings under the standardized conditions described by Bouhot [1975]. Moreover, measurement of the growth rate on malt agar (data not shown) confirmed the correlation already established [Messiaen et al., 1977] between fast growth rate and the ability to induced damping-off. The isolates responsible for damping-off belong to the species *P. aphanidermatum* (Edson) Fitzp., *P. intermedium* de Bary, *P. irregulare* Buisman, *P. sylvaticum* Campbell & Hendrix and *P. ultimum* Trow var. *ultimum*. These species have already been recognized as highly pathogenic on several plants under such conditions [Hendrix and Campbell, 1973; Van Der Plaats-Niterink, 1981].

On the contrary, only seven isolates, all from cucumber roots, were pathogenic on cucumber grown in rockwool, and all were *P. aphanidermatum* (Edson) Fitzp. Other species of *Pythium* tested in these experiments were not pathogenic on cucumber plants grown in rockwool or hydroponic culture. It would have been interesting to test their pathogenicity on tomato because most of these strains have been isolated from discolored tomato roots.

The experimental conditions chosen to perform the bioassay on young cucumber grown on rockwool plugs differed from the damping-off test in several ways: the inoculation was made 2 days later than in the damping-off test and the propagules were added to the substrate without any nutrients. It is well established that oat meal is necessary to induce damping-off and that plants become less susceptible to *Pythium* damping-

off a few days after emergence [Bouhot, 1980]. In fact, these conditions were chosen to avoid damping-off with the objective to detect, on young plants, strains of *Pythium* responsible for root damage and yield reduction on older plants. These experimental conditions seem to enable the distinction between isolates responsible for damping-off from isolates responsible for root decay. Indeed,the isolates detected as pathogenic in this soilless biotest also were the only ones able to reduce the growth of cucumber grown in hydroponic culture.

Three classes of isolates were defined based on their pathogenicity on cucumber in sand-peat and soilless conditions (Table 4). In class 1, the

Table 4. Comparison of the pathogenic behavior of Pythium isolates in different experiments

Species[a]	Number of isolates	Damping-off in soil[b]	Effect on plants in rockwool culture[c]	Effect on plants in hydroponic culture[d]
P. aphanidermatum	7	++	++	++*
P. ultimum	1	++	–	–
P. intermedium	1	++	–	+/–
P. group HS	1	++	–	+/–
	1	++	–	–
	1	+	–	+/–
	2	+	–	–
P. sylvaticum	1	+	–	–
P. irregulare	1	+	–	–
P. flevoense or group F	2	+	–	–
P. flevoense or group F	1	–	–	+/–
	13	–	–	–
Isolates nd	6	–	–	–
	1	–	–	+/–
P. group HS	1	–	–	–
P. intermedium	1	–	–	–

[a] Species were identified according to the key of Van der Plaats-Niterink (1981). Isolates belonging to *P. flevoense* or group F were not distinguished, because they were not tested with compatible isolates of *P. flevoense*. nd = not determined; isolates never produced sexual organs, hyphal swellings or zoospores.

[b] Percentages of dead cucumber seedlings grown in soil 6 days after inoculation with *Pythium*. Plants were inoculated 5 days after sowing with a 8 mm plug of mycelium cut from a 48 hour old malt agar culture of *Pythium*, according to the method proposed by Bouhot. Dead plants > 50% = ++; dead plants < 50% = +; no dead plants = –.

[c] Percentage of dead plants of cucumber grown in rockwool plugs 3 weeks after inoculation with *Pythium*. Plants were inoculated 7 days after sowing with 9×10^6 propagules of *Pythium*. Dead plants > 65% ++; no dead plants = –.

[d] Effect of *Pythium* on final biomass of cucumber plants in hydroponic culture 2 weeks after inoculation with *Pythium*. Plants were inoculated 17 days after sowing with a 5 day old CMA culture of *Pythium*. Tanks of control plants received noninoculated CMA plates. Growth reduction = ++; inconsistent behavior over different tests = +/–; root rot = *.

isolates were highly pathogenic and induced damping-off in sand-peat, death of plants in rockwool and growth reduction of plants with root rot in hydroponic culture. In class 2, isolates caused damping-off on plants but were not pathogenic in soilless conditions. In class 3, representing more than 50% of the isolates, isolates were not pathogenic whatever the cultural conditions; in this class, isolates belonging to *Pythium* group F are predominant.

This study confirmed the pathogenicity of *P. aphanidermatum* in soilless culture as mentioned by different authors [Bates and Stanghellini, 1984; Favrin et al., 1988). However, opposite to previous results [Chérif and Bélanger, 1992; Favrin et al., 1988; Jenkins and Averre, 1983; Stanghellini et al., 1988], the isolates of *P. irregulare* Buisman, *P. ultimum* Trow var. *ultimum* and *P. intermedium* de Bary used in this study, although inducing damping-off on cucumber seedlings, did not show any pathogenic activity on older plants grown on rockwool or in hydroponic solution. These results do not rule out the hypothesis that other *Pythium* species may be involved in yield reduction in soilless culture of cucumber. However, in all these studies, only one isolate of each species was used in pathogenicity tests. It is difficult to generalize to all the isolates belonging to a given species the behavior of the isolate studied. Moreover, the conditions of the tests described here may have been unfavorable for the expression of pathogenicity of other *Pythium* species. Temperature is known to be one of the most important factors for disease development due to *Pythium* spp. [Hendrix and Campbell, 1973] and may explained the differences observed between the behavior of isolates used in this study and in other studies. We controlled the environmental temperature of the tests to 25 °C, because temperature higher than optimal temperature of cucumber growth (22 °C) are usually observed in greenhouses; Stanghellini et al. (1988) employed a temperature of 20 °C to assess the pathogenicity of *P. intermedium*. Moreover, Chérif and Bélanger (1992) observed less severe symptoms due to *P. ultimum* under summer culture conditions where temperature averaged 25 °C than under spring culture conditions. Furthermore, it is clear that the physiology of the plants may influence greatly the behavior of these fungi that appear to function as 'minor pathogens' or 'deleterious microorganisms' according to the definitions proposed by Salt (1979) and Schippers et al. (1987). Indeed, five isolates used in the present study showed inconsistent behavior and caused a significant reduction of growth in only one of the experiments. These results are in agreement with observations that depending on growing conditions (pH, electric conductivity, . . .), cucumbers suffer or are unaffected by damage of *Pythium* [Couteaudier and Lemanceau, 1989; Zinnen, 1988].

One isolate of *P. aphanidermatum* highly pathogenic in pathogenicity tests produced symptoms of root rot on mature plants and induced a significant reduction in the fruit yield. This yield reduction was about 20% of the yield of non-infested plants. Growers usually use fungistatic

chemicals such as propamocarb (Schering) or metalaxyl (Ciba-Geigy) to control *Pythium*, but isolates of *Pythium* resistant to fungicides have been reported [Gold and Stanghellini, 1985; Price and Fox, 1986]. Stanghellini and colleagues experimented ultraviolet irradiation [1984] and filtration methods [1992] for controlling *Pythium* root rot on young plants in a hydroponic system, and recently, Chérif and Bélanger [1992] used potassium silicate in the nutrient solution to protect mature cucumber plants against *P. ultimum* Trow. Previous reports have shown successful control of *Pythium* damping-off by several microorganisms such as *Trichoderma* spp. [Sivan et al., 1984], nonpathogenic *Pythium* spp. [Martin and Hancock, 1987; Paulitz and Baker, 1987] and fluorescent *Pseudomonas* spp. [Callan et al., 1990; Howell and Stipanovic, 1980; Osburn et al., 1989; Parke et al., 1991; Weller and Cook, 1988; Weststeijn, 1990].

The biotests described in this paper will allow rapid screening for effective control methods based on the application of fungicides or biopesticides. Experimentation with old cucumber is both time and space consuming and does not allow the comparison of many treatments. The root rot pathogenicity test in rockwool can be applied at a large scale, and we are currently using it to screen for effective antagonists.

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