CHAPTER 3

PATHOGENICITY OF *PYTHIUM* SPECIES/GROUPS TO HYDROPONICALLY-GROWN BUTTER HEAD LETTUCE

Abstract

The pathogenicity of five *Pythium* species and representatives of three heterothallic groups to butter head lettuce was determined at 21°C and 28°C in three hydroponic systems, viz. static nutrient solution, hydroculture and a recirculating gravel system. Overall, *P. spinosum* was the most aggressive species at 21°C, followed by *P. irregulare* and *P.* group HS. *P. myriotylum* was the only species which consistently showed greater virulence at 28°C than at 21°C. No significant differences in virulence at the two temperatures were evident between *P. coloratum*, *P. diclinum*, and *P.* groups F and T. This is the first report describing the pathogenicity of the above species/groups to butter head lettuce in South Africa.

INTRODUCTION

Recirculating nutrient systems provide an ideal environment for spread of, and infection by, zoosporogenic plant pathogens (Chérif *et al.*, 1994; Stanghellini *et al.*, 2000). It is therefore not surprising that *Pythium* species are commonly associated with hydroponically-grown crops (Jenkins & Averre, 1983; Stanghellini *et al.*, 1984, 1996; Goldberg & Stanghellini, 1990; Moulin *et al.*, 1994; Buysens *et al.*, 1995; Wulff *et al.*, 1998) However, despite their prevalence, only a few *Pythium* species are highly virulent under hydroponic conditions, the most being considered as "minor pathogens" that reduce plant growth without causing obvious disease symptoms (Chérif *et al.*, 1997). Virulence of a species furthermore frequently depends on the strain involved (Jenkins & Averre, 1983) and on environmental conditions, particularly temperature (Hendrix & Campbell, 1973). Observations made during the present study showed that *Pythium* is associated with severe root rot and wilting of lettuce in commercial hydroponic

systems during the hot summer months.

The previous chapter (Chapter 2) indicated the presence of eight *Pythium* species and five heterothallic groups in hydroponica in South Africa. Isolates of some of the species/groups induced symptoms similar to those observed in commercial hydroponica when artificially inoculated into the hosts they have originally been isolated from. The purpose of the present study was to determine the pathogenicity and relative virulence of the most prevalent *Pythium* species/groups to butter head lettuce (*Lactuca sativa* L. var. *capitata* L.), the main target crop in this investigation.

MATERIALS AND METHODS

The *Pythium* species and groups listed in Table 1 were screened for pathogenicity in three separate experiments, viz. (i) static nutrient solution in controlled environment cabinets, (ii) aerated hydroculture system in a greenhouse, and (iii) recirculating gravel culture hydroponic system in a greenhouse. Limited space and facilities precluded inclusion of all the species/groups at the same time. The various experiments therefore had to be conducted twice, each time with a different group of isolates plus a control.

(i) Static nutrient solution

Three-week-old butter head lettuce *Lutetia* seedlings were transferred from steam-pasteurised growth medium (Canadian peat moss) to lidded 250 ml plastic cups, one seedling per cup. The cups contained a nutrient solution consisting of 0.45 g Agrasol® 'O 3:2:8 (Fleuron, P.O. Box 31245, Braamfontein, 2017), 0.3 g calcium nitrate monohydrate and 0.15 g Micromix® (Fleuron) l^{-1} tap water, with a pH of 7.0. Each seedling was supported by the lid of the cup, its roots submerged in the nutrient solution.

Twenty seedlings were inoculated with each of the *Pythium* isolates indicated in Table 1. Inoculum was prepared by blending a 5-day-old V8-juice agar Petri dish culture of each isolate for 15 seconds in 100 ml sterile distilled water in a Waring blender (Jenkins & Averre, 1983; Moulin *et al.*, 1994). Ten millilitres of inoculum suspension was added to each cup (Sanogo & Moorman, 1993), four days after transfer of the seedlings to the cups. Sterile blended V8-juice

agar served as control. Ten of the cups inoculated with each *Pythium* isolate were randomly arranged in a growth cabinet at 21°C and the other 10 in a growth cabinet at 28°C.

Seventeen days after inoculation the seedlings were removed from the cups, and their roots and shoots separated and weighed. Root rot was assessed according to a 0-4 scale (0 = healthy, 1 = 25%, 2 = 50%, 3 = 75%, 4 = 100% rottted). Five root segments, *ca.* 10 mm long, were excised from each seedling and plated on BNPRA selective medium (Roux & Botha, 1997). The identity of the *Pythium* species growing from each root segment after incubation for 3-6 days at 25°C was confirmed on water agar supplemented with 30 µg ml^{-1} ß-sitosterol (Botha & Coetzer, 1996).

Data were analysed statistically according to the GENSTAT 5 programme. Treatment differences were tested by means of one-way analysis of variance and Fisher's protected *t*-test was used to separate treatment means at 5% level of significance.

(ii) Hydroculture system

Plastic containers, 23 cm in diameter and with a capacity of 5 l, each fitted with a lid containing four 3cm-diameter holes spaced 9 cm apart, were filled with a nutrient solution consisting of 0.9 g Agrasol® 'O 3:2:8, 0.6 g calcium nitrate monohydrate and 0.3 g Micromix® l^{-1} tap water. Aeration was provided by a compressor supplying air through a 7-mm-diameter tube inserted through a hole in the lid of each container (Bates & Stanghellini, 1984). The nutrient solution was replaced on a weekly basis, and pH and electrical conductivity of the solution were maintained at 6.9 and 2.1 σ, respectively (Chérif et al., 1994).

A 4-week-old butter head lettuce seedling reared in steam-pasteurised Canadian peat moss was transplanted into each hole in the lid of each container, its roots submerged in the nutrient solution and the shoot supported by a strip of foam rubber. One week after transplanting, three containers with four plants in each were inoculated with one of the *Pythium* isolates indicated in Table 1. Inoculum was prepared from 5-day-old V8-juice agar Petri dish cultures. Two cultures per isolate were blended in 500 ml sterile distilled water. Each inoculum solution was divided in three and added to the nutrient solutions of three containers. Blended uncolonised V8-juice

agar served as control. Inoculation was repeated after two weeks. Plants were harvested three weeks after the first inoculation and processed as above.

(iii) Recirculating gravel system

The recirculating gravel system comprised four units, each with a 100 l reservoir feeding three troughs, 13 cm wide, 8 cm deep and 250 cm long, positioned at an incline of 1:13. The troughs were filled with previously unused, untreated 9.5mm-diameter granite chips. Nutrient solution with the same composition as in (ii) above was constantly circulated through the gravel in the troughs by means of an IDRA® 300 l h^{-1} submersible pump, returning to the reservoir by gravity flow. The nutrient solution was replaced once a week and the pH and electrical conductivity were maintained at 6.9 and 2.1 σ, respectively.

Sixteen four-leaf-stage butter head lettuce seedlings were transplanted from steam-pasteurised Canadian peat moss into each trough, with a 20 cm spacing between plants (Jenkins & Averre, 1983). One week after transplanting, each reservoir was inoculated with one of the *Pythium* isolates (Table 1). Inoculum was prepared from 5-day-old cultures on V8-juice agar in Petri dishes. Six cultures per isolate were blended in 500 ml sterile distilled water and the suspension added to a reservoir. Blended uncolonised V8-juice agar served as control. Plants were harvested three weeks after inoculation and processed as above.

RESULTS

All the *Pythium* species/groups caused significant root rot and reduced shoot and/or root development in at least one of the experiments (Tables 2-4). Wilting of aboveground parts was more evident at 28°C. Overall, the reduction in shoot growth resulting from infection with *Pythium* was 27.5% at 21°C and 18.8% at 28°C. The corresponding percentages for reduction in root growth was 25.5 and 27.9, whereas mean root rot rating at 21°C was 1.7 and 1.8 at 28°C. *P. spinosum* was the most aggressive species at 21°C, reducing shoot and root growth on average by 49.4% and 43.6%, respectively, compared to 11.9% and 22.9% at 28°C. Reduction in shoot growth due to inoculation with *P. spinosum* was significantly greater at 21°C than at 28°C in all three experiments, and reduction in root growth in two experiments. Root rot

ascribable to *P. spinosum* was almost similar at the two temperatures, the mean ratings being 2.1 at 21°C and 2.2 at 28°C.

P. irregulare and *P.* group HS also retarded plant growth to a greater extent at 21°C than at 28°C. At 21°C, inoculation with *P. irregulare* and *P.* group HS resulted in a mean reduction in shoot growth of 38.7% and 29.1%, respectively, and 34.0% and 29.5% in root growth. The corresponding percentages at 28 °C were 16.0, 13.5, 24,0 and 31.6. *P.* group HS reduced shoot growth significantly more at 21°C than at 28°C in two of the experiments, but no differences in root growth were evident between temperatures. With *P. irregulare*, shoot mass was significantly lower at 21 °C than at 28 °C in all three experiments, and root mass in two. As with *P. spinosum*, little or no differences in root rot were evident between temperatures (mean 1.6 for *P.* group HS at both temperatures, and 2.1 and 1.8 for *P. irregulare* at 21°C and 28°C, respectively). Although *P. spinosum*, *P. irregulare* and *P.* group HS generally were more virulent at 21°C, they also suppressed plant growth at 28°C in some of the experiments. Indeed, *P. irregulare* reduced root growth significantly more at 28°C than at 21°C in the recirculating gravel system.

The only species which consistently showed enhanced virulence at 28°C was *P. myriotylum*. On average, it reduced shoot and root growth at 28 °C by 29.4% and 38.6%, respectively, compared to 11.8% and 12.8% at 21°C. Mean root rot rating was 2.2 at 28°C and 1.5 at 21°C. The remaining species/groups did not differ much in aggressiveness between temperatures, although *P.* groups F and T tended to be somewhat more aggressive at 28°C, depending on the experiment.

In the absence of infection by *Pythium*, shoot mass of the lettuce seedlings at termination of the experiments was on average 42% higher at 21°C than at 28°C, and root mass 12%. However, plant vigour, particularly shoot growth, varied considerably between experiments. Mean shoot mass of control plants in static nutrient solution was 11.8 g at 21°C and 8.9 g (25% lower) at 28°C, compared to 159.7 g at 21 °C and 87.6 g (45% lower) at 28 °C in the recirculating gravel system. In the hydroculture system, shoot mass at 21 °C was 64.6 g at 21 °C and 70.3 g (9% higher) at 28°C. The overall reduction in shoot growth at 21°C as a result of infection with

Pythium was 27.6% in static nutrient solution, 30.4% in the hydroculture system and 24.6% in the recirculating gravel system, compared to 16.6%, 28.2% and 11.5%, respectively, at 28°C. The corresponding percentages reduction in root growth were 29.8, 29.1 and 17.6 at 21 °C, and 29.4, 29.3 and 25.1 at 28°C. Overall root rot rating at 21°C was 2.5 in static nutrient solution, 1.1 in the hydroculture system and 1.4 in the recirculating gravel system, and 3.1, 1.8 and 0.6, respectively, at 28°C.

The various *Pythium* species/groups could readily be isolated from the roots of plants with which they were inoculated. Some root necrosis, not due to infection by *Pythium*, was evident in control plants in all the experiments, particularly in static nutrient solution.

DISCUSSION

From the results presented above it is evident that all the *Pythium* species and groups evaluated in the study were pathogenic to butter head lettuce. This occurred regardless of whether the particular species or group had originally been isolated from lettuce or not. Indeed, the two most aggressive isolates, viz. *P. spinosum* at 21°C and *P. myriotylum* at 28°C, were both from other sources, though the *P. myriotylum* isolate originated from gravel substrate in a lettuce-growing hydroponicum. Two of the other non-lettuce root isolates, *P. coloratum* and *P. diclinum*, were also from hydroponica in which lettuce was grown.

As far as could be established, this is the first report of *P.* groups F, HS and T as pathogens of butter head lettuce, at least in South Africa. It is also the first time that Koch's postulates have been confirmed with *P. irregulare* on lettuce in South Africa, though the species is known as a pathogen of lettuce in other parts of the world (Ellis & Cox, 1951). Other species that have been reported to be pathogenic to lettuce elsewhere are *P. spinosum* (Ellis & Cox, 1951) and *P. myriotylum* (Jenkins & Averre, 1983). The latter is an interesting species, as far as the genus *Pythium* is concerned, in that it produces a toxin capable of causing leaf necrosis and stunting in tomato plants (Csinos & Hendrix, 1978). *P. myriotylum* also has an antagonistic relationship with *Rhizoctonia solani* J.G. Kühn (Garren, 1970), but acts synergistically with *Fusarium solani* (Mart.) Appel & Wollenw. in plant attack (Frank, 1972; García & Mitchell, 1975). These

attributes could contribute to *P. myriotylum* being one of the most important pathogens in hydroponica worldwide (Thompson & Labuschagne, 2001), particularly at high temperatures.

In general, temperature preferences of the various *Pythium* species/groups, as far as virulence is concerned, corresponded with what has been described in literature. Various reports refer to greater damage caused by *P. irregulare* and *P. spinosum* at lower temperatures (15-20°C), and by *P. myriotylum* at higher temperatures (Hendrix & Campbell, 1973; Hancock, 1991; Martin, 1995; Ben-Yephet & Nelson, 1999; Chellemi *et al.*, 2000). Extrapolated from growth rate (Van der Plaats-Niterink, 1981; Botha & Coetzer, 1996) and the results of the present study, *P. coloratum*, *P. diclinum* and *P.* group F can be classified as intermediate temperature pathogens, though the ability of *P.* group F to grow at >40 °C implies that it is also capable of causing damage at higher temperatures. With an optimal growth temperature of 30.5°C, and more damage caused to butter head lettuce at 28°C than at 21°C, *P.* group T should be regarded as a high-temperature pathogen. Results implicate *P.* group HS as a low-temperature pathogen of butter head lettuce, at least as far as its effect on shoot growth is concerned.

Temperatures in South African hydroponica vary considerably, depending on season and locality, but the minimum in winter and maximum in summer in the two lettuce-growing units surveyed in Chapter 2 are between 15-22°C and 30-36°C, respectively. Considering the diverse spectrum of *Pythium* species/groups with different temperature preferences present in these hydroponica, it is evident that the lettuce plants are at risk throughout the year. Nevertheless, the almost 1.5 times greater overall suppression of shoot growth at 21°C than at 28°C in the present study indicates that butter head lettuce is more susceptible to damage by the complex of *Pythium* species occurring in hydroponica at lower temperatures. This was not expected as butter head lettuce is a cool-weather crop (Morgan,, 1999), growing optimally at 12-21°C, and prone to heat stress that can aggravate injury by pathogens at temperatures above 25°C. It would thus appear if heat stress is subsidiary to the virulence of the *Pythium* species/groups present at a particular temperature in determining the extent of damage to the plant in commercial hydroponica. The issue nevertheless remains confounded. For instance, Stanghellini & Kronland (1986) reported yield reductions of 35-54% and 12-17% in lettuce infected with *Pythium dissotocum* Drechsler at 18°C and 28°C, respectively, and incidentally

also found uninfected lettuce to have a 29% higher shoot mass at 28°C than at 18°C. However, with spinach (*Spinacea oleracea* L.) which is also a cool-season crop, infection by *P. dissotocum* resulted in more stunting at 30°C than at 20°C (Bates & Stanghellini, 1984).

Besides the differences in temperature preferences, virulence of the various *Pythium* species/groups also varied considerably between systems. Overall, root rot rating and percentage reduction in plant mass were less in the recirculating gravel system than in static nutrient solution and the hydroculture system. It can be argued that the inoculum concentration in the gravel system was about seven and three times lower than in static nutrient solution and the hydroculture system, respectively, but more vigorous growth of the plants in the gravel system probably also contributed to their relative resistance. The poor growth of butter head lettuce in static nutrient solution was expected, considering the hypoxic environment and limited availability of nutrients. Nutrient supply, particularly the volume of nutrient solution available per plant, probably contributed to better growth in the gravel system than in the hydroculture system. Based on container capacity, each plant in the gravel system was exposed to a continuous supply of 2.08 l nutrient solution, 1.7 times more than the 1.25 l per plant in the hydroculture system. This ratio is reflected in the 1.8 times higher mass of control plants in the gravel system than in the hydroculture system, notwithstanding the fact that high levels of nutrients can predispose plants to infection by *Pythium* (Moorman, 1986; Gladstone & Moorman, 1989).

More effective pathogen dispersal in the hydroculture system could also have contributed to the increased severity of disease. In the gravel system, propagules have to be dispersed throughout the entire recycling process in order to infect all plants, whereas in the hydroculture system the entire root system of each plant is confined and constantly exposed to the pathogen (Jenkins & Averre, 1983). Nevertheless, the fact remains that growth conditions can have a marked effect on the virulence of a particular *Pythium* species (Zinnen, 1988).

P. group F, the species/group most commonly isolated from lettuce roots by far (Chapter 2), proved to be moderately virulent in static nutrient solution and the hydroculture system, but did not cause significant root rot or impediment of plant growth in the recirculating gravel system,

an environment which it seemed to prefer (Chapter 2). It is unlikely that this disparity could have been due to the different isolates of *P.* group F that were used. The isolate from wallop lettuce included in the recirculating gravel system was the same one evaluated in the hydroculture system, where it reduced shoot growth to approximately the same extent as the cos lettuce isolate in static nutrient solution, despite being introduced into the static nutrient solution at three times the concentration as in the hydroculture system. It is also unlikely that the lower initial inoculum concentration in the gravel system played a role, considering the 117% and 25% higher root rot rating in this system than in the hydroculture system at 21°C and 28°C, respectively.

Differences in virulence of *P.* group F in different substrates have been described before. Moulin *et al.* (1994) reported damping-off of cucumber (*Cucumis sativus* L.) in soil, inconsistent growth reduction in hydroponic culture, and no effect in rockwool culture, by *P.* group F or its homothallic counterpart, *Pythium flevoense* Van der Plaats-Niterink. Evidence indicates that oxygen concentration of the substrate could be a determining factor. Chérif *et al.* (1997) showed that tomato (*Lycopersicon esculentum* Mill.) plants inoculated with *P.* group F rapidly developed symptoms of infection and root decay in nutrient solution with moderate or low oxygen concentration, whereas highly oxygenated plants remained healthy and displayed significantly less root colonisation by the pathogen. A possible explanation for this phenomenon was that increases in lipoxygenase in tomato roots grown under oxygen stress and inoculated with *P.* group F could lead to degradation and disorganisation of membrane lipids, hence facilitating root colonisation by the pathogen and appearance of decay. Unlike *Pythium aphanidermatum* (Edson) Fitzp. for instance, *P.* group F also induces important defence reactions in cucumber plants, including formation of papillae and deposition of wall apposition and phenolic compounds (Rey *et al.*, 1996), but it is unclear if these defence mechanisms are still effective under poor aeration conditions (Chérif *et al.*, 1997). While the relatively hypoxic conditions in static nutrient solution in the present study could have aggravated disease, it is unlikely that hypoxia *per se* contributed to the differences in disease severity between the hydroculture and recirculating gravel systems. Results suggest that other stress factors may also have been involved.

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TABLE 1. ***Pythium*** **species/groups included in the present study**

Pythium sp./group	Hydroponicum[a]	Source[a]	Experiment[b]
P. coloratum		Growth medium[c]	(i)[¶], (ii)[¶], (iii)[¶]
P. diclinum		Run-off water	(i)[¶], (ii)[¶]
P. irregulare		Wallop lettuce	(i)[¶¶], (ii)[¶¶], (iii)[¶¶]
P. myriotylum		Gravel substrate	(i)[¶¶], (ii)[¶], (iii)[¶¶]
P. spinosum		Cucumber	(i)[¶], (ii)[¶¶], (iii)[¶]
P. group F		Cos lettuce	(i)[¶]
P. group F		Wallop lettuce	(ii)[¶¶], (iii)[¶]
P. group HS		Butter head lettuce	(i)[¶¶], (ii)[¶¶], (iii)[¶¶]
P. group T		Green oak lettuce	(i)[¶¶] , (ii)[¶]

[a] Refer to Chapter 2, Table 1.

[b] (i) Static nutrient solution, (ii) Hydroculture system, (iii) Recirculating gravel system.

[¶] Included in first run of experiment, [¶¶] Included in second run of experiment.

[c] Canadian peat moss growth medium for seedlings.

TABLE 2. **Effect of inoculation with *Pythium* species/groups on butter head lettuce seedlings grown at 21 and 28 °C in static nutrient solution in growth cabinets.**

Pythium sp./group	Reduction in shoot mass (%)[a]		Reduction in root mass (%)[a]		Root rot rating[b]	
	21°C	28°C	21°C	28°C	21°C	28°C
P. coloratum[c]	**16.3**	13.1	**16.2**	3.4	**2.9**	**2.2**
P. diclinum[c]	**34.4**	**23.2**	**28.5**	**35.1**	1.8	**3.5**
P. irregulare[d]	**41.3***	8.0	**36.7***	**24.2**	**2.8**	**2.8**
P. myriotylum[d]	11.8	**24.1***	7.3	**20.8***	**2.8**	**3.3**
P. spinosum[c]	**58.4***	8.7	**76.4***	**38.0**	**3.7**	**2.7**
P. group F[c]	**26.7**	**21.8**	**35.3**	**24.9**	**2.7**	**2.9**
P. group HS[d]	**28.9***	5.4	**31.2**	**38.6**	**2.8**	**3.4**
P. group T[d]	2.9	**28.6***	6.9	**30.5***	0.3	**3.6**

[a] Each value is the mean of 10 replicate cups with one plant in each, evaluated 17 days after inoculation and representing percentage reduction compared to the control; in each column, values printed in bold differ significantly from the control according to LS Means ($P \leq 0.05$);
* indicates a significantly greater reduction at the particular temperature within parameters according to Fisher's protected t-test ($P \leq 0.05$).

[b] Each value is the mean of 10 replicate cups with one plant in each, evaluated 17 days after inoculation; root rot was rated according to a scale 0 = healthy, 1 = 25%, 2 = 50%, 3 = 75% , 4 = 100% rotted; values printed in bold differ significantly from the control according to LS Means ($P \leq 0.05$).

[c] Included in first experiment.

[d] Included in second experiment.

TALBLE 3. Effect of inoculation with *Pythium* species/groups on butter head lettuce seedlings grown at 21 and 28 °C in a hydroculture system.

Pythium sp./group	Reduction in shoot mass (%)[a]		Reduction in root mass (%)[a]		Root rot rating[b]	
	21 °C	28 °C	21 °C	28 °C	21 °C	28 °C
P. coloratum[c]	28.6	**25.0**	23.9	**29.5**	**2.2**	1.3
P. diclinum[c]	36.3	**27.5**	23.5	**33.9**	**2.5**	1.8
P. irregulare[d]	**37.8***	**27.9**	**51.3***	17.2	**1.6**	**2.4**
P. myriotylum[c]	14.1	**39.7***	18.5	**29.9***	0.3	**2.4**
P. spinosum[d]	**49.9***	**23.7**	**25.5**	27.8	0.6	**3.7**
P. group F[d]	23.8	**34.9**	**30.1**	**49.3***	0.6	0.8
P. group HS[d]	**27.3**	**22.1**	**31.0**	25.0	**0.9**	0.8
P. group T[c]	25.5	**24.7**	28.7	21.9	0.4	0.8

[a]Each value is the mean of three replicate containers with four plants in each, evaluated three weeks after inoculation and representing percentage reduction compared to the control; in each column, values printed in bold differ significantly from the control according to LS Means ($P \leq 0.05$); * indicates a significantly greater reduction at the particular temperature within parameters. (according to Fisher's protected t-test ($P \leq 0.05$).

[b]Each value is the mean of three replicate containers with four plants in each, evaluated three weeks after inoculation; root rot was rated according to a scale 0 = healthy, 1 = 25%, 2 = 50%, 3 = 75%, 4 = 100% rotted; values printed in bold differ significantly from the control according to LS Means ($P \leq 0.05$).

[c] Included in first experiment.

[d] Included in second experiment.

TALBE 4. Effect of inoculation with *Pythium* species/groups on butter head lettuce seedlings grown at 21 and 28 °C in a recirculating gravel system.

Pythium sp./group	Reduction in shoot mass (%)[a]		Reduction in root mass (%)[a]		Root rot rating[b]	
	21 °C	28 °C	21 °C	28 °C	21 °C	28 °C
P. coloratum[c]	15.4	4.0	14.4	**15.9**	0.4	0.6
P. irregulare[d]	**36.9***	**12.2**	**14.1**	**30.7***	2.0	0.3
P. myriotylum[d]	9.4	**24.3***	**12.6**	**65.2***	1.3	0.9
P. spinosum[c]	**39.9***	3.3	28.9*	2.8	2.0	0.3
P. group F[c]	14.8	12.1	9.3	5.1	1.3	1.0
P. group HS[d]	**31.1***	**12.9**	**26.3**	**31.1**	1.1	0.6

[a] Each value is the mean of three troughs with 16 plants in each, evaluated three weeks after inoculation and representing percentage reduction compared to the control; in each column, values printed in bold differ significantly from the control according to LS Means ($P \leq 0.05$); * indicates a significantly greater reduction at the particular temperature within parameters. (according to Fisher's protected t-test ($P \leq 0.05$).

[b] Each value is the mean of three troughs with 16 plants in each, evaluated three weeks after inoculation; root rot was rated according to a scale 0 = healthy, 1 = 25%, 2 = 50%, 3 = 75% , 4 = 100% rotted.

[c] Included in first experiment.

[d] Included in second experiment.