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Trichoderma harzianum T39 and *T. virens* DAR 74290 as potential biological control agents for *Phytophthora erythroseptica*

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Abstract

Trichoderma harzianum isolate T39 and *T. virens* isolate DAR 74290 were evaluated as potential biological agents for control of pink rot of potato and root and stem rot of tomato caused by *Phytophthora erythroseptica*. Cell-free metabolites of *T. virens* DAR 74290 completely inhibited growth of *P. erythroseptica in vitro* and appeared to be fungicidal. *T. virens* DAR 74290 and Trichodex, a commercial formulation of *T. harzianum* T39, were tested for their ability to protect potato and tomato plants from disease caused by *P. erythroseptica* in glasshouse experiments. Trichodex and *T. virens* DAR 74290, alone and combined, reduced disease severity in shoots and roots of potatoes 10 weeks after inoculation with the pathogen. The yield of potatoes from plants treated with *P. erythroseptica* and *T. virens* DAR 74290 (mean of 12.9 g fresh weight/pot) was significantly greater than in controls inoculated with the pathogen alone (mean of 2.1 g/pot). Treatment with Trichodex alone increased the yield of tubers compared to the uninoculated controls. *T. virens* DAR 74290 increased the survival of tomato seedlings inoculated with the pathogen, and both this isolate and Trichodex decreased the severity of disease on tomato.

Introduction

Pink rot of potato, caused principally by *Phytophthora erythroseptica*, has been reported from the USA (Carroll and Sasser, 1974; Rowe and Schmitthenner, 1977), Europe (Lennard, 1980; Pethybridge, 1913), South America (Vargas and Nielsen, 1972), Iran (Anonymous, 1967) and Australia (MacNish, 1968; Wicks and Harding, 1996). Infected potato plants become chlorotic and wilt. Diseased tubers appear discoloured and, when cut open, the exposed tissue turns salmon pink and later black. Pink rot was most severe in water-logged soil and developed rapidly at 20–30 °C (Rowe and Nielsen, 1981; Vargas and Nielsen, 1972). Older plants were more susceptible than younger plants and wounding has been shown to hasten disease development (Vargas and Nielsen, 1972). Yield losses of 30% have been reported in south east South Australia, and further losses may occur in storage due to secondary bacterial infections (Wicks and Harding, 1996). *P. erythroseptica* has also been associated with diseases of other crops, including buck-eye rot (Stamps, 1978) and wilting and stunting of tomato (Gillings and Letham, 1989).

Control of *P. erythroseptica* in potatoes is achieved by avoidance of water-logged soil (Rowe and Nielsen, 1981; Wicks and Harding, 1996) and the application of fungicides such as metalaxyl (Mulrooney, 1982). However, insensitivity to metalaxyl has been reported among isolates of *P. erythroseptica* from the USA (Goodwin and McGrath, 1995). Biological agents could be an important component in control of *P. erythroseptica* if effective and if reliable formulations were readily available, and could be integrated

with chemical fungicides. The antagonistic activities of *Trichoderma* and *Gliocladium* species against plant pathogens have been studied extensively (Burgess and Hepworth, 1996; Chet, 1987; Elad et al., 1980; Harman et al., 1966; Howell, 1991; Lumsden and Locke, 1989; Papavizas, 1985). A number of commercial formulations, based mainly on *T. harzianum* and *T. virens* (formerly *Gliocladium virens*) are available for control of soilborne and foliar diseases of a range of horticultural crops (Harman et al., 1996; Lumsden et al., 1992; Samuels, 1996). *T. harzianum* isolate T39 is the active ingredient of Trichodex, which is reported to control *Botrytis* grey mould on a range of crops (Elad, 1994; O'Neill et al., 1996a,b). However, *T. harzianum* T39 failed to protect chickpea seed from *Botrytis cinerea*, perhaps due to the low temperatures that prevailed in the experiments (Burgess and Keane, 1997). Various species of *Trichoderma* have been evaluated for control of diseases caused by *Phytophthora* species, for example, *P. cactorum* in apple (Alexander, 1998; Roiger and Jeffers, 1991; Smith et al., 1990). Antagonism of *P. erythroseptica* by *Trichoderma* species *in vitro* has been reported (Okhovat et al., 1994) but there is no or little information on the efficacy of *Trichoderma* species against pink rot of potato and root and stem rot of tomato caused by *P. erythroseptica*.

The objective of this investigation was to evaluate the potential of *T. virens* isolate DAR 74290, *T. harzianum* isolate T39 and the commercial formulation, Trichodex, for biological control of *P. erythroseptica.*

Materials and methods

Fungal isolates

T. virens isolate DAR 74290 (formerly *G. virens*), which was isolated from capsicum in South Australia, had previously been shown to inhibit growth of *Sclerotium rolfsii in vitro* and to promote the survival of capsicum and wheat seedlings in infested potting mix (Na Lampang, 1994). *T. harzianum* T39 was isolated from Trichodex (Makhteshim Chemical Works, Be'er Sheva, Israel, supplied by Abbott Australasia Pty Ltd., New South Wales, Australia) with the supplier's permission. *Trichoderma* species were cultured on cornmeal agar (CMA, Oxoid). Four isolates of *P. erythroseptica*, $C_2$209, $C_2$211, $C_2$249 and $C_2$252, obtained from diseased potatoes in South Australia were maintained on CMA. All cultures were incubated at 25 °C in darkness unless stated otherwise.

Effects of Trichoderma *species on mycelial growth of* P. erythroseptica *in vitro*

Dual culture (Dennis and Webster, 1971b) and cellophane overlays (Dennis and Webster, 1971a) were used to observe the effects of the *Trichoderma* isolates on *P. erythroseptica*. All antagonist–pathogen combinations were examined on 10–15 ml of 1/5M32 agar medium (Sivasithamparam et al., 1979) in 9-cm Petri dishes, with four or five replicate plates per treatment. For dual cultures, mycelial plugs (5 mm in diameter), taken from actively growing, 3-day-old colonies of *P. erythroseptica* isolates $C_2$209, $C_2$211, $C_2$249 and $C_2$252 and *T. virens* DAR 74290 or *T. harzianum* T39, were placed 5 cm apart on the agar. Plugs of *P. erythroseptica* were applied 2 days prior to those of the *Trichoderma* isolates. Controls consisted of pure cultures. The experiment was performed twice; first *P. erythroseptica* was paired with *T. virens* and then with *T. virens* and *T. harzianum* and cultures were assessed 4 and 6 days after inoculation of the antagonist, respectively. For cellophane overlays, cellophane membranes (Australia Cellophane, Victoria), 9 cm in diameter, were boiled in distilled water, then interleaved with filter paper and autoclaved before being placed on the agar medium. One 5-mm diameter plug of *T. harzianum* or *T. virens* growing on 1/5M32 was placed on the centre of each cellophane membrane. For controls, a plug of sterile 1/5M32 agar was used instead of the antagonist. The cellophane membrane and adhering fungus, or agar plug, were removed after 1 or 2 days in the first two experiments and after 1 day in the third repetition. A plug of *P. erythroseptica* was then placed on the agar in the centre of the plate and incubated for 6 days. The surface area of the colonies was recorded daily, compared with controls and the percentage inhibition of growth was calculated. Where *P. erythroseptica* did not grow, the inoculum plug was transferred to fresh 1/5M32 to determine if the diffusible metabolites were fungicidal or fungistatic.

Effect of T. harzianum *T39 and* T. virens *DAR 74290 on development of pink rot in potato tubers*

Potato tubers were inoculated with various combinations of pathogen and antagonists in the laboratory,

using methods based on those used by Grisham et al. (1983) to assess pathogenicity of *P. erythroseptica*. Tubers of cvs Pontiac and Russett Burbank were surface disinfested by soaking in 0.5% sodium hypochlorite for 3 min then rinsed three times in sterile distilled water (SDW). A core, 8 mm in diameter and 25 mm deep, was removed aseptically from each tuber using a cork borer, and a 5-mm diameter plug of *P. erythroseptica* isolate $C_2$211 or of CMA alone (control) was placed in the hole. A plug of *T. harzianum* or *T. virens*, or of CMA alone (control), was placed on the top of the first plug. The core of potato tissue was replaced and the wound sealed with vaseline. There were three replicate tubers of each cv. per treatment. Tubers were placed individually in paper bags and incubated at 20 °C in the dark. Treatments were arranged in a completely randomised design and symptoms were recorded 8 days after inoculation. The extent of discolouration of the tuber surface was estimated as a percentage of total area (Grisham et al., 1983). Tubers were cut open and internal symptoms scored on a scale of 0–5 where 0 = no symptoms and 1–5 = increasing extent and intensity of pink discolouration of the cut tissue when exposed to air (Grisham et al., 1983). The experiment was repeated using *P. erythroseptica* isolate $C_2$249 and cv. Pontiac only, with plugs of the antagonist or CMA placed in the holes before those of the pathogen or CMA and with five replicate tubers per treatment.

Biological control of P. erythroseptica *on potato in glasshouse conditions*

The ability of *T. virens* DAR 74290 and Trichodex to reduce the incidence and severity of pink rot in potatoes grown in the glasshouse was investigated. Inoculum of the pathogen was prepared as follows. Rolled oats, 50 g in 50 ml distilled water, were autoclaved in polycarbonate containers (6.5 cm in diameter × 8 cm high; Disposable Products Ltd., South Australia) for 1 h at 121 °C on two successive days. *P. erythroseptica* isolates $C_2$209, $C_2$211, $C_2$249 and $C_2$252 were grown separately on CMA for 9 days and $\frac{1}{4}$ of the contents of one Petri plate were added to each container, mixed with the rolled oats and incubated at 25 °C for 30 days. Rolled oats infested with the different isolates of the pathogen were combined and blended in SDW to make a slurry. Inoculum of *T. virens* was prepared as above except that the rolled oats were incubated at 20 °C for 20 days. Treatments comprised: uninoculated control; *P. erythroseptica* alone; *T. virens* alone; Trichodex alone; *T. virens* plus Trichodex; *P. erythroseptica* plus *T. virens*; *P. erythroseptica* plus Trichodex; *P. erythroseptica* plus *T. virens* plus Trichodex. Inoculum was applied to pasteurised 'recycled soil' potting mix, consisting of used, composted potting mix (0.5 m^3), peat moss (0.1 m^3); blood meal (500 g); K_2PO_4 (200 g), agricultural lime (400 g), superphosphate (100 g), pH 6.5, in 20-cm diameter plastic pots. The following rates of inoculum were applied per kg potting mix: *P. erythroseptica*, 5 g infested oats; *T. virens* alone, 5 g infested oats; Trichodex alone, 2 g. Inoculum rates were halved in treatments involving both *T. virens* and Trichodex such that 2.5 g infested rolled oats and 1 g commercial formulation, respectively, were used per kg potting mix. The pathogen was applied 1 day before planting, and the antagonist on the day of planting tubers.

Tubers of cvs Pontiac and Russett Burbank of uniform size were surface-disinfested as described above and planted in treated potting mix, one tuber per pot. There were four replicates per treatment, arranged in a split plot design with potato cultivar as the main plots and treatments, the sub-plots. Plants were maintained in the glasshouse at 22–25 °C without supplementary lighting from October to December (spring–early summer). Pots were watered at 5-day intervals until emergence and daily thereafter, except that they were flooded for 3 days, with plastic bags placed around the pots to retain moisture, 30 days after planting, to enhance disease development. Fertiliser (NPK: 30 : 11 : 12), 15 g in 10 l water was applied, 250 ml per pot, 7 and 9 weeks after planting.

Wilting of potato plants was assessed 5 weeks after inoculation using the scale of Vargas and Nielsen (1972): 1 = healthy plant, 2 = 25% of leaves wilted, 3 = 50% of leaves wilted, 4 = 75% of leaves wilted, 5 = all leaves wilted. Samples of tissue were taken from the collar region of plants which had died or had discoloured or wilted shoots, and from one control plant of cv. Pontiac which had wilted slightly. Segments of tissue, 5 mm long, were surface-disinfested in 50% domestic bleach (0.5% available chlorine) for 3 min, rinsed three times in SDW and plated on the *Phytophthora*-selective medium of Ocana and Tsao (1966).

Plants were harvested 10 weeks after inoculation and the severity of disease on roots and stems was assessed

using a 1–5 scale modified from Nemec et al. (1996), such that 1 = roots and stems healthy and 5 = all roots and stems infected. Tubers from each pot were bulked together and fresh weight recorded.

Estimation of populations of pathogen and antagonists

Populations of pathogen and antagonists in the potting mix were estimated 30 days after planting and immediately before flooding. Potting mix (10 g) was removed from each pot, samples bulked for each treatment and cv., and air-dried. For each treatment, 1 g of potting mix was shaken in 199 ml of sterile tap water for 1 min and three 0.5 ml aliquots were spread on agar media. The medium of Ocana and Tsao (1966) was used to estimate the number of colony forming units (CFU) of *P. erythroseptica* per g potting mix. CFUs of *Trichoderma* species were estimated using a medium based on the *Trichoderma*-selective medium of Elad et al. (1981), comprising per l distilled water: glucose, 3 g; KCl, 0.15 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NH_4NO_3, 1 g; K_2HPO_4, 0.9 g; Difco BiTek agar, 15 g, with the following added after autoclaving: chloramphenicol, 250 mg; metalaxyl, 10 mg; pentachloronitrobenzene (Terraclor, Olin Chemicals, USA), 200 mg and rose bengal, 150 mg.

Biological control of P. erythroseptica *on tomato in glasshouse conditions*

Pasteurised potting mix in 10-cm-diameter plastic pots was inoculated with a mixture of the four isolates of *P. erythroseptica* alone, *P. erythroseptica* plus *T. virens* DAR 74290, *P. erythroseptica* plus Trichodex, prepared as above, or left uninoculated, and 10 seeds of tomato cv. Grosse Lisse (Grower's Pride, Hortico (Aust.) Pty Ltd.) were sown in each pot. There were four replicate pots per treatment. Plants were maintained in the glasshouse as above, except that they were set out in a completely randomised block design. Survival of seedlings was assessed at 10 day intervals but only data collected 30 days after inoculation were analysed. Surviving seedlings were removed from pots after 30 days and symptoms of disease caused by *P. erythroseptica* were recorded using a 1–5 scale modified from Nemec et al. (1996). *P. erythroseptica* was re-isolated from the diseased tomato plants as described above.

Statistical analysis

Data on percentage inhibition of radial growth, percentage surface area of tubers diseased and percentage plants or stems surviving were subjected to arcsin square root transformation before analysis. Data for disease severity and fresh weight of tubers were analysed directly. Analysis of variance was performed and means were separated using Duncan's Multiple Range Test at $P \leq 0.05$ (Little and Hills, 1978).

Results

Effects of Trichoderma *isolates on mycelial growth of* P. erythroseptica *in vitro*

T. harzianum T39 and *T. virens* DAR 74290 inhibited mycelial growth of *P. erythroseptica* in dual culture. The antagonists grew rapidly and quickly overgrew the pathogen. In most cases, there were no significant differences among isolates of *P. erythroseptica*; mycelial growth of *P. erythroseptica* was reduced by 49–71% and 49–54% by *T. virens* DAR 74290 and *T. harzianum* T39, respectively.

No growth was observed in *P. erythroseptica* cultures on medium previously overlaid with *T. virens*, regardless of whether *T. virens* had been incubated on the medium for 1 or 2 days. Furthermore, the plugs of *P. erythroseptica* failed to grow when transferred to fresh medium. *T. harzianum* T39 inhibited growth of the pathogen, in terms of colony area, by 14–22%.

Effect of T. harzianum *T39 and* T. virens *DAR 74290 on development of pink rot in potato tubers*

Uninoculated controls and tubers of Pontiac inoculated with *T. harzianum* T39 or *T. virens* DAR 74290 alone remained healthy (Table 1). Tubers of both Pontiac and Russet Burbank became extensively discoloured, both externally and internally, when inoculated with *P. erythroseptica* alone. There was no evidence of bacterial soft rot.

In general, disease on Pontiac tubers appeared to be more severe in experiment 1, when tubers were inoculated with *P. erythroseptica* isolate $C_2$211 followed by the antagonist than in experiment 2, when inoculated with the antagonist then *P. erythroseptica* isolate $C_2$249. In the first experiment, there was no significant difference in external or internal discolouration

Table 1. Disease symptoms on potato tubers inoculated with *P. erythroseptica*, *T. harzianum* T39 and *T. virens* DAR 74290

Treatment	External surface area discoloured (%)[a]		Disease severity[b]	
	Exp. 1[c]	Exp. 2[d]	Exp. 1[c]	Exp. 2[d]
Pontiac control	0 a	0 a	0 a	0 a
Pontiac + *T. virens*	—	0 a	—	0 a
Pontiac + *T. harzianum*	—	0 a	—	0 a
Pontiac + *P. erythroseptica*	53.3 c	42.6 c	4.3 c	4.6 c
Pontiac + *P. erythroseptica* + *T. virens*	43.3 c	27.8 b	3.0 c	2.3 b
Pontiac + *P. erythroseptica* + *T. harzianum*	53.3 c	40.6 c	3.6 c	2.8 b
Russet Burbank control	0 a	—	0 a	—
Russet Burbank + *P. erythroseptica*	40.0 c	—	3.6 c	—
Russet Burbank + *P. erythroseptica* + *T. virens*	16.6 b	—	1.0 ab	—
Russet Burbank + *P. erythroseptica* + *T. harzianum*	40.0 c	—	1.6 b	—

In Experiment 1 data are means of three replicates, in Experiment 2 data are means of five replicates. Means within columns followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range Test. — = treatment was not included.

[a]Data were subjected to arcsin square root transformation before analysis.

[b]Disease severity: 0 = no symptoms and 1–5 indicates increasing intensity of pink discolouration of cut tissue exposed to air (Gisham et al., 1983).

[c]*P. erythroseptica* isolate $C_2$211 was used.

[d]*P. erythroseptica* isolate $C_2$249 was used.

among Pontiac tubers inoculated with *P. erythroseptica* isolate $C_2$211 alone and in combination with *T. virens* or *T. harzianum.* In experiment 2, however, tubers of Pontiac inoculated with *P. erythroseptica* isolate $C_2$249 and *T. virens* showed significantly less external discolouration than those inoculated with the pathogen, alone or in the presence of *T. harzianum*. Pink rot was less severe in Pontiac tubers inoculated with both pathogen and antagonists than in those inoculated with the pathogen alone.

Russet Burbank was used in the first experiment only. External discolouration on tubers inoculated with *P. erythroseptica* isolate $C_2$211 and *T. virens* was 16.6% compared with 40% on those inoculated with the pathogen, alone or in the presence of *T. harzianum*, and the intensity of pink discolouration of tissue exposed to the air on tubers inoculated with the pathogen and antagonists was significantly less than in tubers inoculated with the pathogen alone.

Biological control of P. erythroseptica *on potato in glasshouse conditions*

Average populations of *T. harzianum* T39 and *T. virens* DAR 74290 in potting mix 30 days after inoculation ranged from 7.3×10^3 to 1.3×10^4 CFU g^{-1}, and 1×10^5 CFU g^{-1} in treatments receiving both forms of antagonist, while those of the pathogen were 1.7–2 $\times 10^3$ CFU g^{-1} potting mix (Table 2).

There were no significant differences in symptoms between cultivars, therefore data were combined and are presented in Table 3. Disease severity, after 5 weeks, in plants inoculated with the pathogen alone was significantly greater than in all other treatments. When symptoms were scored 10 weeks after inoculation, mean disease severity on roots and shoots of plants inoculated with the pathogen alone was 4.6 compared with 1.4 in the uninoculated control. Disease severity on plants inoculated with antagonists alone or in combination with the pathogen was significantly ($P \leq 0.05$) less than in plants inoculated with the pathogen alone, whereas these treatments did not differ significantly from one another nor from the uninoculated control. An exception was that plants inoculated with the pathogen, *T. virens* and Trichodex were more diseased than the uninoculated control. The percentage of stems surviving 10 weeks after inoculation was very variable and the antagonists had no significant effect. Although yields of tubers were very variable also, the average yield from plants treated with *P. erythroseptica* and *T. virens* was 12.9 g, significantly more than from plants inoculated with the pathogen alone (2.1 g). Trichodex alone increased the average yield of potatoes in the absence

Table 2. Populations of pathogen and antagonists (CFU g^{-1}) in potting mix 30 days after application

Treatment	*P. erythroseptica*	*T. harzianum* T39	*T. virens* DAR 74290	*Trichoderma* spp.
P. erythroseptica	1933 ± 339	—	—	—
P. erythroseptica + *T. virens*	1866 ± 262	—	11433 ± 1388	—
P. erythroseptica + Trichodex	1700 ± 81	13600 ± 616	—	—
P. erythroseptica + *T. virens* + Trichodex	1966 ± 47	—	—	100000 ± 244
T. virens	—	—	7333 ± 449	—
Trichodex	—	12833 ± 94	—	—

Values are means of three replicate plates ± SE.

Table 3. Effect of *T. virens* DAR 74290 and Trichodex on disease of potato cvs Pontiac and Russett Burbank caused by *P. erythroseptica*

Treatment	Disease severity[a]	Disease severity on roots and stems[b]	% stems surviving[c]	Yield (g per pot)
	5 weeks after inoculation	10 weeks after inoculation		
Uninoculated control	1.2 a	1.4 a	42 abc	6.8 ab
T. virens	1.4 a	1.9 ab	61 bc	9.2 abc
Trichodex	1.5 a	1.8 ab	42 abc	16.7 c
T. virens + Trichodex	1.3 a	1.6 ab	81 c	5.0 ab
P. erythroseptica	2.1 b	4.6 c	13 a	2.1 a
P. erythroseptica + *T. virens*	1.5 a	2.4 ab	53 abc	12.9 bc
P. erythroseptica + Trichodex	1.3 a	2.2 ab	53 abc	2.9 a
P. erythroseptica + *T. virens* + Trichodex	1.4 a	2.9 b	31 ab	6.3 ab

Data for Pontiac and Russet Burbank were combined and there were eight replicate pots per treatment. Numbers in each column followed by a common letter are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

[a]Disease severity: 1 = plant healthy; 2 = 25% of leaves wilted; 3 = 50% of leaves wilted; 4 = 75% of leaves wilted; 5 = all leaves wilted and plant dead (Vargas and Nielsen, 1972).

[b]Disease severity: 1 = plant healthy and 5 = all roots and stem infected (modified from Nemec et al., 1996).

[c]Data were subjected to arcsin square root transformation prior to analysis.

of *P. erythroseptica* but had no effect on fresh weight of tubers in pots inoculated with the pathogen.

P. erythroseptica was isolated from all inoculated plants showing symptoms but not from the one control plant of cv. Pontiac which had wilted.

Biological control of P. erythroseptica *on tomato in glasshouse conditions*

Survival of seedlings in pots treated with *P. erythroseptica* plus *T. virens* DAR 74290 was similar to that in the uninoculated control (Table 4). Disease severity in plants treated with the pathogen plus *T. virens* or Trichodex was 2.3, significantly ($P \leq 0.05$) less than that of plants inoculated with the pathogen alone (4.8).

Discussion

Cell-free metabolites produced by *T. harzianum* T39 reduced colony area by 14–22% whereas those of *T. virens* DAR 74290 completely inhibited growth of *P. erythroseptica* and appeared to be fungicidal. The *T. virens* isolate used in this study had previously been shown to produce gliotoxin *in vitro* (Na Lampang, 1994). Gliotoxin has been shown to be the major antibiotic inhibitory to *Pythium ultimum* and *Rhizoctonia solani* produced by a formulated strain of *T. virens* in soilless culture (Lumsden et al., 1992), while another antibiotic, viridin, was less active. Other strains tested produced gliovirin (Lumsden et al., 1992), an antibiotic reported by Howell (1991) to be associated with disease suppression by *T. virens* against damping off induced by *P. ultimum*. *T. virens* has also been shown to

Table 4. Effect of *T. virens* DAR 74290 and Trichodex on stem and root rot of tomato

Treatment	Seedlings surviving (%)[a]	Disease severity[b]
Uninoculated control	98 a	1.1 a
P. erythroseptica	75 c	4.8 b
P. erythroseptica + *T. virens*	93 ab	2.3 a
P. erythroseptica + Trichodex	80 bc	2.3 a

Data are means of four replicate pots, each sown with 10 seeds. Numbers within columns followed by a common letter are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

[a]Data were subjected to arcsin square root transformation before analysis.

[b]Disease severity: 1 = no symptoms and 5 = all roots and stems infected (based on Nemec et al., 1996).

produce endochitinase, which acted synergistically with gliotoxin to inhibit germination of conidia of *Botrytis cinerea* (Di Pietro et al., 1993). Isolates of *T. harzianum* are known to produce a range of antifungal antibiotics (Ghisalberti and Sivasithamparam, 1991) and enzymes (Lorito et al., 1994) whereas strain T39 is not identified as a producer of inhibitory compounds.

Although there were some differences in disease severity between tubers inoculated in the laboratory with the pathogen alone and in combination with *T. harzianum* T39 or *T. virens* DAR 74290 the antagonists did not provide consistent reduction of disease. The two experiments were not directly comparable, in that different isolates of the pathogen were used, cv. Russett Burbank was not used in the second experiment and the order of application of the pathogen and antagonists differed between experiments. The method of application may have been unsuitable for the evaluation of antagonists, as inoculation into wells in the tuber may have given the pathogen an advantage over the antagonist, or conditions within the potato tissue may not have been conducive to antagonism. For example, *Trichoderma* spp. are considered to be inhabitants of root surfaces (Dix, 1964) and it may be that the isolates used here would give better protection if applied to the tuber surface in the presence of the pathogen.

Inoculation of tubers of Pontiac and Russett Burbank in the glasshouse with all four isolates of *P. erythroseptica* combined resulted in severe disease of roots and stems 10 weeks after inoculation. Treatment of the potting mix with *T. virens* or Trichodex at the time of planting the tubers into infested potting mix reduced disease severity when assessed 10 weeks after inoculation. Yield of tubers, in terms of fresh weight, however, was very variable and only in plants inoculated with Trichodex alone or *T. virens* plus *P. erythroseptica*, was yield significantly greater than in controls inoculated with the pathogen alone. The effect of coating the tubers with the antagonists was not investigated here, however, Kay and Stewart (1994) found that *Trichoderma* species and *Chaetomium globosum* reduced the incidence of white rot of onion more effectively when applied as soil additives in a food base than as seed coatings or incorporated into alginate pellets.

T. virens was added to the potting mix as colonised rolled oats, a food base previously found to be suitable for this strain (Na Lampang, 1994), and *T. harzianum* was applied as a commercial formulation. While the population densities of antagonists and pathogen were not determined at the time of inoculation, approximately 10^4–10^5 CFU of the antagonists per g of potting mix were recovered 30 days after inoculation. These populations are close to the 10^5 CFU g^{-1} considered by Adams (1990) to be necessary to achieve control. However, younger mycelia of *T. virens* may have been more effective in protecting tubers from disease, as Lewis and Papavizas (1987) found that 3- and 8-day-old inoculum of *T. hamatum* in wheat bran inhibited *R. solani* in soil more effectively than did 15- and 40-day-old inoculum. The effect of age of inoculum and means of application on the ability of *T. harzianum* T39 and *T. virens* DAR 74290 to give consistent control pink rot of potato should be investigated. Similarly, the timing of the application of the antagonists and pathogen should be studied. In commercial crops, *P. erythroseptica* may be present as oospores in infested soil prior to planting, or may be introduced on infected tubers, however, the timing of infection in the field has not been determined. In this study, the antagonists were applied, at the time of planting, to soil which had been infested 1 day previously and it would be of value to examine the effects of infesting the soil both earlier than, and at the time of, planting. A biological control product which was effective when applied at the time of planting would be more likely to be accepted by growers than one which required additional cultivation. This

means of application would also reduce the need for long-term survival of the antagonists in the soil, which may be a limiting factor in the biological control of soil-borne fungal pathogens (Burgess and Hepworth, 1996; Deacon, 1991). Information on the timing of infection of potato by *P. erythroseptica* in the field is required to optimise the application of biological control agents.

The isolates of *P. erythroseptica* which induced pink rot in potato also caused root and stem rot of tomato. Similarly, Grisham et al. (1983) reported that *P. erythroseptica* isolated from potato in North and South America caused disease when inoculated into tomato fruit, whereas Gillings and Letham (1989) reported that *P. erythroseptica* isolated from tomato did not cause pink rot of wound-inoculated potato tubers. *T. virens* DAR 74290 provided some protection in terms of seedling survival, whereas disease severity was reduced by both *T. virens* and Trichodex compared to controls inoculated with the pathogen alone. Likewise, O'Neill et al. (1996a) reported that *T. harzianum* T39, applied as the commercial formulation, Trichodex, reduced the incidence of stem rot on wounded tomato stems inoculated with *Botrytis cinerea*.

In conclusion, *T. virens* DAR 74290 and *T. harzianum* T39 tested here reduced disease severity in potato plants and tomato seedlings in the glasshouse. Future research will involve studies of the mechanisms involved. These isolates warrant further investigation for their ability to control pink rot of potato, especially in commercial situations. An integrated approach using a combination of biocontrol agents and fungicides, such as metalaxyl, which does not appear to affect mycelial growth of these antagonists (Charoenwet et al., unpublished), may allow reduction in the amount of fungicide needed to suppress pink rot.

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