BIOLOGICAL CONTROL OF *SCLEROTINIA MINOR* IN LETTUCE USING *TRICHODERMA* SPECIES

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ABSTRACT

A *Trichoderma harzianum* isolate (C52) and a *Trichoderma viride* isolate (TV1), with known activity against *Sclerotinia* spp. in laboratory screens, were tested for their ability to control disease caused by *Sclerotinia minor* in lettuce crops in glasshouse trials (1996-1997). Different application methods of *T. harzianum* C52 were also tested, namely soil incorporation, pellet formation and a transplant root spore dip. In the first trial, *T. harzianum* soil incorporation significantly reduced disease incidence by 50% compared to the untreated control, and was comparable to a standard carbendazim fungicide treatment which gave 57% reduction in disease incidence. In the second trial, carbendazim and all three methods of *T. harzianum* application significantly reduced disease incidence by 55-78%, compared to the control and there were no significant differences between the treatments. The results suggest that *T. harzianum* (C52) has potential value as a biological control agent to control lettuce drop caused by *S. minor* in glasshouse systems.

Keywords: *Sclerotinia minor*, *Trichoderma harzianum*, *Trichoderma virens*, biological control, lettuce

INTRODUCTION

Sclerotinia spp. are serious pathogens of several important crops in New Zealand and regularly cause major losses in crops such as lettuce, carrots, brassicas and other vegetables. The main method of control currently being employed by growers against *Sclerotinia* diseases has been the use of chemicals. However, the increasing costs of soil fumigation, lack of suitable replacement for methyl-bromide and public concerns over repeated fungicide use for disease control has meant that alternative control methods, such as biological control are gaining interest with growers. Several fungi have been reported to have potential for the biological control of *Sclerotinia* spp. *Coniothyrium minitans* and *Sporidesmium sclerotivorum*, two specialised mycoparasites of *Sclerotinia* spp., have been reported to control disease in both glasshouse and field trials (Lynch and Ebben 1986; Budge and Whipps 1991; Fravel *et al.* 1992). *Trichoderma* spp. namely *T. harzianum* and *T. virens* (formally *Gliocladium virens*) have also been shown to attack both sclerotia and mycelium of *Sclerotinia* spp. (Tu 1980; Whipps and Budge 1990; Knudsen *et al.* 1991) and have been reported to give some disease control in field trials against sclerotia (Singh 1991; Ristaino *et al.* 1994; Šesan and Csépi 1995). This study reports on the effect of *T. harzianum* and *T. virens* isolates against disease caused by *S. minor* on lettuce. Different methods of *T. harzianum* application were also investigated.

METHODS

Fungi and inoculum production

T. harzianum isolate C52 and *T. virens* isolate TV1 were obtained from the Lincoln University Culture Collection, both originally isolated from soils in New Zealand.

Trichoderma spp. inoculum was prepared in kibbled maize and perlite for the soil incorporation method. One hundred ml of kibbled maize:perlite (15:85 v:v) moistened

with 20% distilled water was placed in 250 ml conical flasks and autoclaved. Flasks were then inoculated with 5 ml of spore suspension of *T. harzianum* C52 or *T. virens* TV1 (1×10^6 spores/ml) obtained by flooding 10-14 day old cultures grown on Potato Dextrose Agar (PDA; 39 g/litre; Gibco) and then incubated at 20°C in the dark for 3 weeks. The amount of inoculum in the maize:perlite was determined using a dilution technique described by Whipps *et al.* (1989). A 10 cm³ sample of the infested maize:perlite was ground to a fine dust using a mortar and pestle and added to 90 ml of sterile 0.01% agar. This suspension was then shaken for 10 min on a wrist action shaker, allowed to stand for a further 20 min prior to the preparation of a dilution series. For each dilution, 0.2 ml aliquots were spread onto three Czapek Dox Agar (CDA, 45.4 g/litre; Oxoid) plates containing aureomycin (18 mg/μl containing 93.5% chlortetracycline hydrochloride; Sigma Chemicals) and Triton X-100 (2 ml/litre; BDH Ltd. UK), and incubated at 20°C in the dark for 5 days. The number of *Trichoderma* colonies were counted visually and the number colony forming units (cfu)/cm³ of inoculum were calculated. For the first trial, the *T. harzianum* and *T. virens* inoculum contained 4×10^8 and 1×10^6 cfu/cm³ respectively. In the second trial, the *T. harzianum* inoculum contained 6×10^9 cfu/cm³.

T. harzianum C52 was also tested as a pellet formulation and transplant dip treatment. The pellet formulation was supplied by Agrimm Technologies Ltd., Christchurch. The pellets were a combination of dry *T. harzianum* spores, wetters and stickers (the formulation of which is proprietary information) adhered to the outside of non-viable barley grains. The amount of inoculum was determined by adding 1 g of pellet to 10 ml of 0.01% water agar and shaking at low speed using a wrist action shaker for 10 min and then a dilution series was made as described above for the maize:perlite inoculum. The pellet contained 1.5×10^4 cfu/g of pellet. For the transplant dip treatment, 10-14 day old cultures of *T. harzianum* C52 were flooded with a solution of 0.003% sodium alginate (Hopkins and Williams Ltd.) and the resulting spore suspension adjusted to give 8×10^7 spores/ml.

Glasshouse trial 1

Lettuce (*Lactuca sativa*, cv. Yateslake) seedlings were transplanted into 2 litre pots, half filled with potting compost (80% composted bark; 20% sand; 2 kg/m³ Osmocote Plus; 4 kg/m³ dolomite lime) and then half with field soil (Wakanui silt loam) which had previously been identified as being naturally infested with *Sclerotinia minor*. The potting compost was put into the pots first. There were five treatments, each of 20 plants: (i) Control, no treatment; (ii) single carbendazim spray (application rate of 3.75 ml/litre; Bavistan FL 500 g/litre) applied to run-off to lettuces at the time of transplanting; (iii) *T. harzianum* C52 pellet with 1 g pellet placed into the planting hole prior to lettuce transplanting; (iv) *T. harzianum* C52 soil incorporation, colonised kibbled maize:perlite incorporated (1% v:v) into *S. minor* infested soil and mixed thoroughly prior to lettuce transplanting; (v) *T. virens* soil incorporation, colonised kibbled maize:perlite incorporated as described above for *T. harzianum* (1% v:v). All plants were watered thoroughly and the treatments randomised throughout the glasshouse in a five replicate block design each containing four plants per treatment. The plants were assessed weekly for *S. minor* disease and scored as follows: 0 = Healthy; 1 = Wilting of lower leaves, early infection; 2 = Greater than two leaves infected and severe wilting; 3 = Dead. The trial ran from November 11th 1996 to January 20th 1997.

Glasshouse trial 2

The second trial was set up in a similar manner as that described above for glasshouse trial 1 using fresh *S. minor* infested soil gathered from the same field site. There were five treatments each with 36 plants: (i) Control, no treatment; (ii) single carbendazim spray at time of transplanting; (iii) *T. harzianum* C52 pellet; (iv) *T. harzianum* C52 colonised kibbled maize:perlite soil incorporation and (v) *T. harzianum* C52 transplant dip where lettuce roots were dipped in the spore suspension (8×10^7 spores/ml in 0.003% sodium alginate) for 20 seconds before transplanting. All treatments except (v) were set up as for Trial 1.

The plants were watered thoroughly and the treatments randomised throughout the glasshouse in a six replicate block design each containing six plants per treatment. The plants were assessed weekly for *S. minor* disease and scored visually as described above.

This trial ran from 28th February 1997 to 25th April 1997.

Statistical analysis

The mean number of diseased plants between treatments were compared for significant effects using LSD.

RESULTS

Glasshouse trial 1

At the first sign of *Sclerotinia* infection, diseased leaf material was sampled for presence of *S. minor* by plating (0.5 cm²) small sections of healthy and diseased tissue onto PDA containing aureomycin (18 µg/ml). In all cases *S. minor* was confirmed to be the causal agent. In all treatments, the first disease symptoms were observed at the four week assessment. It was observed that in the majority of cases *S. minor* infection led to the death of infected plants within 2-3 weeks. Therefore, plants were not scored using the disease severity index, only for the presence or absence of *S. minor* infection.

Disease incidence (%) in the control was 70%. Carbendazim and the *T. harzianum* C52 soil incorporation treatment significantly ($P < 0.05$) reduced disease incidence by 57.1% and 50% respectively compared to the untreated control (Table 1). These treatments were not significantly different ($P > 0.05$) from each other. There was no significant difference ($P > 0.05$) in the number of diseased plants in the *T. harzianum* C52 pellet and *T. virens* TV1 soil incorporation treatments in comparison to the control treatment.

TABLE 1: Effect of *Trichoderma* spp. treatments and carbendazim spray on the control of *S. minor* in lettuce in a glasshouse trial (Lincoln, 1996).

Treatment	Method of application	Mean disease incidence (%) ± SE	Percentage disease control ^a
Control		70 ± 5	0
Carbendazim	Foliar spray	30 ± 12 *	57
<i>T. harzianum</i> C52	Soil incorporation	35 ± 13 *	50
<i>T. harzianum</i> C52	Pellet	45 ± 12	36
<i>T. virens</i> TV1	Soil incorporation	60 ± 6	14

^a Percentage reduction in disease compared to the control.

* Mean values significantly different from the control at $P < 0.05$ as tested by LSD.

Glasshouse trial 2

The first diseased plants were observed in the control treatment at the two week assessment but in the *T. harzianum* pellet, transplant dip and soil incorporation treatments disease was not observed until the three, four and five week assessments respectively. The carbendazim treatment also delayed disease expression until the five week assessment. Percentage disease in the control was 50% at the end of the trial (8 weeks). The *T. harzianum* C52 soil incorporation, pellet and transplant dip and the carbendazim treatments significantly ($P < 0.01$) reduced disease incidence by 56%, 56%, 67% and 78% respectively compared to the untreated control (Table 2). There was no significant difference ($P > 0.05$) between the control achieved by any of these treatments.

TABLE 2: Effect of different *T. harzianum* C52 methods of application in comparison to carbendazim for control of *S. minor* in glasshouse lettuces (Lincoln 1997).

Treatment	Method of	Mean disease	Percentage
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	application	incidence (%) ± SE	disease control ^a
Control		50 ± 11	0
Carbendazim	Foliar spray	11 ± 7 *	78
<i>T. harzianum</i> C52	Transplant dip	17 ± 6 *	67
<i>T. harzianum</i> C52	Pellet	22 ± 7 *	56
<i>T. harzianum</i> C52	Soil incorporation	22 ± 7 *	56

^a Percentage reduction in disease compared to the control.

* Mean values significantly different from the control at $P < 0.01$ as tested by LSD.

DISCUSSION

The results of this study suggest that *T. harzianum* C52 has potential value as a biocontrol agent to control lettuce drop caused by *S. minor*, with levels of disease reduction comparable to that shown by carbendazim. Although there are several reports on the potential of *Trichoderma* spp. in controlling *Sclerotinia* disease based on laboratory and *in vitro* bioassays (Trutmann and Keane 1990; Whipps and Budge 1990; Knudsen *et al.* 1991), this paper is one of the few where a *Trichoderma* spp. has been used successfully in glasshouse trials to control *Sclerotinia* diseases. Singh (1991) reported control of *S. sclerotiorum* in peas by *T. harzianum* in both glasshouse and field trials. Using two different wheat bran formulations of *T. harzianum*, a mycelial and spore preparation, control was achieved in the field, with the mycelial preparation being the most effective. *T. viride*, as wheat grain formulations, was also reported by Gupta and Agarwala (1988) to give some control of *S. sclerotiorum* in cauliflower in glasshouse trials. Both *T. viride* and *T. harzianum* were also shown by Şesan and Csép (1995) to control *S. sclerotiorum* disease in both sunflower and soybean in field trials. *T. viride* was applied as dried biomass, with *T. harzianum* being a commercial biofungicide Trichodex 25WP (Maktheshim Agan Company, Israel).

Although there were no significant differences in control achieved by the different application methods of *T. harzianum* in the second trial, the pellet formulation gave lower disease control performing less well in trial 1 than in trial 2. This could be due to the higher disease pressure in trial 1 (70% disease) compared to trial 2 (50% disease), which suggests that some application treatments may only be effective under lower disease pressure compared to other methods. Similar observations have been reported in other biocontrol systems. For example, *C. minitans* was reported by Budge and Whipps (1991) only to be effective in controlling *S. sclerotiorum* disease in lettuce and celery under low to moderate disease pressure (30–50% infection). For practical purposes, the transplant dip method would provide a better system for delivery of the *T. harzianum* than incorporation of large volumes of colonised maize:perlite into a soil system. The former method would also be more acceptable to growers for control of *S. minor* since they could easily incorporate a dip treatment into their existing transplanting regime.

In conclusion, *T. harzianum* isolate C52 gave significant control of *S. minor* disease on lettuce in the glasshouse. Further glasshouse and field trials are planned in order to optimise delivery systems further to improve existing levels of control.

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