COMPATIBILITY OF THE BIOCONTROL AGENT *TRICHODERMA HARZIANUM* C52 WITH SELECTED FUNGICIDES

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ABSTRACT

Trichoderma harzianum C52 is an effective biocontrol agent of the onion white rot pathogen *Sclerotium cepivorum.* For this biocontrol agent to be integrated into an existing disease management programme, it must be compatible with the fungicides commonly used on onions. The sensitivity of *T. harzianum* spores to the field rate of eight fungicides commonly applied to onions was determined in an *in vitro* assay. Results indicate that *T. harzianum* was least sensitive to procymidone and captan and most sensitive to mancozeb, tebuconazole and thiram. A glasshouse pot trial confirmed that *T. harzianum* was sensitive to mancozeb but tolerant of captan. This research indicates that in furrow applications of *T. harzianum* would be compatible with a captan and/or benomyl seed treatment for control of other seedling diseases.
Keywords: *Sclerotium cepivorum*, *Trichoderma harzianum*, biocontrol, onion, fungicide sensitivity.

INTRODUCTION

Trichoderma harzianum C52 has been identified as a promising biocontrol agent of onion white rot disease caused by the soil-borne pathogen *Sclerotium cepivorum* Berk (Kay & Stewart 1994a). Under low to medium disease pressure, the biocontrol fungus gave good control of the disease (60-70%) when applied as a soil additive at planting time (McLean & Stewart 2000). However, under high disease pressure, efficacy of the biocontrol agent declined (30%), necessitating a combination of measures to obtain adequate disease control. There is an opportunity to integrate the use of *T. harzianum* C52 with reduced fungicide applications for white rot control. However, the biocontrol fungus must be compatible with the fungicides used to control white rot and other onion diseases.

The sensitivity of *T. harzianum* C52 to the fungicides routinely used on onions was determined in *in vitro* and glasshouse assays to determine its compatibility with fungicide applications as part of an integrated disease management programme.

METHODS

Test fungicides are listed in Table 1. Fungicides were prepared in distilled water and applied at the recommended field rate (Table 1).

In vitro spore sensitivity assay

A spore suspension was prepared by flooding 5 ml sterile water onto 10 day old *T. harzianum* C52 colonies grown on PDA in 9 cm diameter Petri dishes at 20°C in the light. The stock spore suspension was diluted to 1 x 10^5 spores/ml using potato dextrose broth to provide nutrients required for spore germination. Aliquots (0.5 ml) of spore suspension and each fungicide (0.5 ml) were added to 1.5 ml conical tubes, with sterile distilled water as

TABLE 1: Fungicide name, active ingredient, field rate and formulation of fungicides tested against *Trichoderma harzianum* (C52).

Fungicide product name	Active ingredient	Field rate: Foliar spray (ai/ha)	Field rate: Seed treatment (ai/kg seed)	Formulation
Benlate ®	benomyl	250 g	125 g	wettable powder
Sumisclex ® 25	procymidone	750 g	-	suspension concentrate
Sumisclex® WP	procymidone	-	5 g	wettable powder
Cereous	triadimenol	375 g	-	emulsifiable concentrate
Folicur® 430SC	tebuconazole	376 g	-	suspension concentrate
Orthocide® 48F	captan	6 kg	-	suspension concentrate
Orthocide 80W	captan	-	8 g	dust
Euparen® DF	dichlofluanid	1 kg	-	water dispersible granule
Thiram 40F	thiram	16 kg	-	suspension concentrate
Thiram technical	thiram	-	8 g	dust
Mancozeb 80W	mancozeb	1.6 kg	-	wettable powder

a no-fungicide treatment. Each spore/fungicide mix was replicated four times. The tubes were fastened to the side of a rotating arm in an oven and slowly rotated at 20-25°C. After 24 h, four samples were taken from each tube and germination was recorded for 50 spores per sample. Spores were considered germinated when germ tube length equalled spore diameter. Significant differences in spore germination between fungicides were determined using a one-way ANOVA with fungicide as the factor. Where ANOVA indicated a significant treatment effect, this was further explored using a Fisher's LSD test.

Glasshouse trial

Plastic pots (6 cm x 6 cm x 8 cm) were filled to within 2 cm of the top with Wakanui silt loam soil, and the soil moisture was adjusted to -0.3 bar. *Trichoderma harzianum* C52 was formulated as Trichoboost™ (conidia coated onto an inorganic based prill approximately 0.5–1 mm diameter) by Agrimm Technologies Ltd., Christchurch, New Zealand (9.5×10^6 spores/g product), and 0.05 g was added to each of six planting holes in each pot. Three pots (replicates) were prepared for each treatment. A single onion seed was placed into each planting hole (0.5 cm below the soil surface and equidistant from each other in a grid arrangement) and lightly covered with soil.

The glasshouse trial comprised 11 treatments. In the first five treatments, onion seeds were treated with one of the following fungicides before planting: captan, thiram, procymidone, benomyl or a combination of thiram, procymidone and benomyl (TPB) at the recommended field rate (Table 1). In four further treatments, uncoated onion seeds were planted as described above, and foliar sprays of dichlofluanid, mancozeb, procymidone and triadimenol were applied immediately after planting as soil drenches at the field rate (Table 1). The remaining two treatments were a TPB seed treatment combined with a soil drench of mancozeb (TPBM), and the *T. harzianum* formulation alone as a no-fungicide treatment.

Each pot was placed in a separate drip tray and randomly positioned in a glasshouse for the duration of the trial. Pots were watered as necessary. A teaspoon was used to collect a soil sample from one planting hole and the surrounding soil in each pot, before any fungicides were added (0) and 3, 12, 30 and 50 days after fungicide application. A colony forming unit (cfu) assay was performed on 1 g of the soil sample for each pot and aliquots (0.5 ml) from each dilution were pipetted and spread over the surface of four *Trichoderma* selective medium plates (McLean, 2001). Results

were analysed using a two-way ANOVA with fungicide and sample time as factors. Where ANOVA indicated a significant treatment effect, this was further explored using Fisher's LSD test.

RESULTS

In vitro spore sensitivity assay

The *T. harzianum* C52 spores in the no-fungicide treatment all germinated after 24 h incubation in potato dextrose broth. *Trichoderma harzianum* spores were least sensitive ($P<0.05$) to procymidone, which failed to inhibit spores from germinating (Table 2), and were also relatively insensitive to captan. *Trichoderma harzianum* spores were most sensitive to tebuconazole, thiram and mancozeb, at the rates used.

TABLE 2: Mean percentage inhibition of *Trichoderma harzianum* C52 spore germination when exposed to field rates of selected fungicides.

Fungicide	% Inhibition of spore germination	
procymidone	0	a[1]
captan	3.5	b
dichlofluanid	17.1	c
triadimenol	41.2	de
benomyl	58.3	f
mancozeb	100	g
tebuconazole	100	g
thiram	100	g

[1]Mean values followed by the same letter do not differ significantly ($P<0.05$).

Glasshouse trial

The *T. harzianum* C52 concentration in the no-fungicide and fungicide treatments prior to fungicide application (time 0) ranged from 1.4-7.8 x 10^4 cfu/g soil, with no difference ($P>0.05$) in cfu counts between treatments. Three days after fungicide spray application, the *T. harzianum* concentration in the mancozeb (2.2 x 10^3 cfu/g soil) and the TPBM (2.9 x 10^3 cfu/g soil) treatments was significantly lower ($P<0.05$) than the *T. harzianum* concentration in the no-fungicide and other treatments (Table 3). Twelve days after fungicide application, the *T. harzianum* concentration in all treatments, except the captan seed and procymidone soil drench treatments, were significantly lower ($P<0.05$) than the no-fungicide treatment. The *T. harzianum* concentration in the combined TPBM treatment was significantly less ($P<0.05$) than all other treatments except the mancozeb treatment. Thirty days after fungicide application, the *T. harzianum* concentration was similar for all treatments. Fifty days after initial fungicide application, the *T. harzianum* concentration in the dichlofluanid and procymidone soil drenches and the benomyl, thiram, and TPB seed treatments were significantly less ($P<0.05$) than the no-fungicide treatment. The *T. harzianum* concentration in the combined TPBM treatment was significantly less ($P<0.05$) than all other treatments.

DISCUSSION

The *in vitro* spore germination assay showed that *T. harzianum* C52 was highly sensitive to mancozeb, tebuconazole and thiram, less sensitive to benomyl, triadimenol and dichlofluanid, and relatively insensitive to procymidone and captan. However, the glasshouse results were less extreme with no single fungicide or combination of fungicides

TABLE 3: Mean number of *Trichoderma harzianum* C52 colony forming units per gram of soil at 3, 12, 30 and 50 days after fungicide application in the glasshouse trial.

Fungicide	Days after fungicide application							
	3		12		30		50	
No-fungicide	3.1×10^4	a[3]	6.5×10^5	a	6.8×10^5	a	3.1×10^5	a
thiram[1]	4.2×10^3	a	3.5×10^4	cd	3.8×10^4	a	4.2×10^4	c
procymidone[1]	4.7×10^3	a	3.9×10^4	cd	4.5×10^4	a	7.7×10^4	ab
captan[1]	3.8×10^4	a	1.3×10^5	ab	6.4×10^4	a	6.2×10^4	abc
benomyl[1]	1.2×10^4	a	3.5×10^4	cd	3.4×10^4	a	2.4×10^4	c
TPB[1]	3.4×10^4	a	2.8×10^4	cd	6.0×10^4	a	8.1×10^4	ab
procymidone[2]	6.5×10^3	a	9.8×10^4	ab	5.5×10^4	a	5.7×10^4	bc
triadimenol[2]	6.0×10^3	a	5.9×10^4	bc	2.3×10^5	a	4.0×10^4	c
mancozeb[2]	2.2×10^3	b	5.8×10^3	de	4.5×10^4	a	7.0×10^4	ab
dichlofluanid[2]	6.1×10^3	a	8.3×10^4	b	2.1×10^5	a	5.5×10^4	c
TPBM[1+2]	2.9×10^3	b	1.8×10^3	e	2.4×10^4	a	4.0×10^3	d

[1]Seed treatment [2]Foliar spray applied as a soil drench [3]Mean values followed by the same letter do not differ significantly within each column ($P<0.05$), n = 3.

completely suppressing the activity of *T. harzianum* C52 in the soil. The glasshouse results are a more realistic assessment of the compatibility of *T. harzianum* C52 with the various fungicides, since it is unlikely that the level of direct contact between fungus and fungicide observed in the *in vitro* assay would occur in the field environment given the strong buffering capacity of the soil. However, the *in vitro* results do allow us to better explain the trends detected in the glasshouse trial. For example, *Trichoderma* colonisation was lowest in the combination seed treatment plus mancozeb foliar spray (TPBM) and the single mancozeb treatment. This can be explained on the basis of the strong inhibition of *Trichoderma* spore germination exhibited by mancozeb and thiram. Similarly, there was almost no suppression of *Trichoderma* colonisation of the soil in the captan treatment, which was the fungicide that showed low inhibitory activity against spore germination in the *in vitro* assay. In many of the treatments, in particular the TPBM treatment, there was an initial decline in *Trichoderma* cfu counts and then a gradual recovery over time. It is possible that the fungicides reduced the germination capability of the initial spore inoculum but, subsequently, the germinated spores established and sporulated in the soil to bring the cfu counts back up to 10^4-10^5/g soil. A previous study showed that *T. harzianum* C52 mycelial growth was insensitive to thiram and mancozeb, which supports this hypothesis (Kay & Stewart 1994b).

Although further trials are needed to determine the sensitivity of *T. harzianum* to repeated fungicide applications, these preliminary results indicate that integrated control of onion white rot is possible. *Trichoderma harzianum* C52 could be applied at planting with captan and/or benomyl treated onion seed, the seed treatments providing control of other seedling diseases, but it may not be advisable to use thiram in combination with a *Trichoderma* treatment at planting time. If additional control of onion white rot is required, dichlofluanid or procymidone could be applied as foliar sprays towards the end of the growing season. This integration of fungicides and biological control agents may enable the number of fungicide sprays to be reduced, while still providing control of onion white rot.

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