Biological control of fusarium wilt of tomato by *Trichoderma* isolates

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Abstract This study investigated the ability of ten *Trichoderma* isolates to control the fusarium wilt pathogen of tomato, *Fusarium oxysporum* f. sp. *lycopersici*, as well as the effect of these isolates on tomato plant growth in the presence and absence of the pathogen. The isolates were obtained from the Lincoln Bio-Protection Research Centre Culture Collection and were inoculated into seed raising mix (0.5% w/w) in two glasshouse studies. Two *Trichoderma* isolates significantly ($P<0.05$) reduced tomato fusarium wilt incidence, as shown by 69% fewer plants with vascular discoloration. In the presence of the pathogen, one isolate significantly increased tomato plant growth by 50% or more. In the absence of the pathogen, none of the *Trichoderma* isolates consistently increased all plant growth parameters. The biocontrol mechanism of these *Trichoderma* isolates requires further investigation.

Keywords biological control agent, *Fusarium oxysporum* f. sp. *lycopersici*, plant growth promotion, systemic resistance, *Trichoderma* spp., fusarium wilt

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop in New Zealand with production valued at $100 million per annum, including $9 million of exports in 2014 (TomatoesNZ 2016). Several serious pathogens attack tomato plants. One of the major pathogens of both greenhouse and field grown tomatoes is the soil borne and host-specific pathogen, *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal agent of tomato wilt disease (Jarvis 1988). Chemical application to soil and resistant cultivars are the main approaches to control the disease (Fravel et al. 2003). However, fungicide application is often ineffective as the chemical may not reach the fungal propagules which are widely distributed in the soil (Campbell 1989). In addition, new races of the pathogen have overcome host resistance and discovery of new resistant varieties is expensive and difficult when no dominant gene is known (Fravel et al. 2003). Biological control, therefore, holds promise as a strategy for disease management. Biocontrol agents (BCAs) including fluorescent *Pseudomonas*, a non-pathogenic *Fusarium* strain, *Trichoderma harzianum* and *T. asperellum*, have been reported to provide control of fusarium wilt (Larkin and Fravel 1998; Cotxarrera et al. 2002; Yigit and Dikilitas 2007). In New Zealand, no BCA-specific fusarium wilt control is currently available, although the commercial product Trichoflow Nursery™ has a label claim for protective activity against *Fusarium* species (Anon. 2013).

One of the mechanisms involved in biocontrol

activity of antagonists is their ability to promote plant growth directly or indirectly. The direct effect occurs in the absence of the pathogen by means of phytostimulation, biofertilization and plant stress control (Lugtenberg and Kamilova 2009). Some BCAs improve the growth of the plant indirectly in the presence of the pathogen, reducing the negative impacts of the pathogen by using mechanisms such as antibiosis, induced systemic resistance and competition for nutrients and ecological sites (Lugtenberg and Kamilova 2009). These modes of action have been reported for *Trichoderma* spp. providing biocontrol against many plant pathogens (Howell 2003).

The objectives of this study were to evaluate the biocontrol efficiency of New Zealand isolates of *Trichoderma* against FOL, as well as to study the effect of these isolates on the growth promotion of tomato plants, both in the presence and absence of the pathogen.

MATERIALS AND METHODS

Fungal strains and inoculum preparation

Fusarium oxysporum f. sp. lycopersici (Sacc.) W.C. Snyder & H.N. Hansen 1940 (ICMP 5204, Landcare Research) was grown centrally on plates of potato dextrose agar (PDA) at 25°C in the dark for 7 days. Three plugs of FOL from the margin of the colony were added to a container containing 70 g wheat grain (dry-autoclaved) and 25 ml MYST broth (2.4 g malt extract, 2.13 g yeast extract, 10 g sucrose and 2.67 g tryptone per litre, Difco™). Containers were kept at 25°C for 3 weeks under constant light, before the infected wheat grain was dried in a laminar flow cabinet for 2 days, then ground to a powder using a coffee grinder. The powder was mixed with potting mix in the ratio of 5% w/w and the inoculated potting mix kept at room temperature for three days before being used for the pot experiment.

Ten *Trichoderma* isolates (Figure 1) from the Lincoln Bio-Protection Research Centre Culture Collection and a commercial product based on *Trichoderma* (Trichoflow Nursery™, Agrimm Technologies Ltd, New Zealand) were used in this study. All isolates, except LU540 (*T. virens*) and LU740 (*T. hamatum*), were *T. atroviride*. *Trichoderma* isolates were inoculated on PDA plates which were incubated at 22°C, unsealed for 7-10 days. *Trichoderma* conidia were harvested with 5 ml of half-strength PDB (Potato dextrose broth, DIFCO) from each plate and 2.5 ml of the conidial suspension was added to each of the containers with 100 g of sterilized peat/bran (50:50 v/v) and mixed well. Containers were incubated at 22°C under constant light for 2–3 weeks until conidiation developed. This inoculated peat/bran mixture was mixed with potting mix in the ratio of 0.5% w/w before sowing the seeds.

Pot experiment set up

A single tomato seed was sown at a depth of 1 cm in each cell (25 cm^3) of 12 multicellular trays containing potting mix either inoculated with each of the ten *Trichoderma* isolates, Trichoflow Nursery™, or non-inoculated. The potting mix (600 L Southland peat, 400 L pumice) contained the following fertilizer additions per cubic meter: 1.5 kg Osmocote Exact Mini (Everris International; containing 16% N, 3.5% P, 9.1% K), 5.0 kg dolomite lime (Golden Bay Dolomite, New Zealand), 2.0 kg agricultural lime (Oxford Lime Company), 1 kg HydroFOL (Everris International) and 1.0 kg Superphosphate (11% P; Ravensdown Cooperative, New Zealand). Emerged seedlings were grown under glasshouse conditions (20 ± 2°C) until they were 10 cm tall with two fully extended true leaves. Five seedlings from each of the treatments were randomly selected and then transplanted into 1 litre pots containing potting mix with or without FOL.

Assessment of pot experiment

One month after transplanting, the plants were uprooted carefully and separated into roots and shoots (above ground tissues). The percentage of plants with brown to black vascular discoloration and wilting symptoms, as visual evidence of fusarium wilt symptoms, was recorded. The roots were washed in running tap water and

scanned using a WinRhizo (Regent Instruments Inc., Canada) to determine their total length (Himmelbauer 2004). Stem thickness was measured using a Carbon Fiber Digital Calliper (resolution: 0.1 mm/0.01, accuracy: ± 0.2 mm/0.01; Fisher Scientific, Canada). The samples were then dried in an oven at 65°C for 2 days and the dry weight of each sample was recorded.

Experimental design and statistical analysis

The experiment was set up as a complete randomized block design with 6 replicates. Each replicate (block) contained 24 pots; one negative control (not inoculated with *Trichoderma* or FOL), one pathogen control (treated with FOL only), 11 treated with *Trichoderma* treatments only, and 11 treated with both *Trichoderma* and FOL (a total of 144 pots).

Statistical analysis was performed using GenStat software (16th Edition). Analysis of variance (ANOVA) was used to test the significance of the effects of *Trichoderma* treatments on fusarium wilt incidence and plant growth measurements. The effects of *Trichoderma* treatments on plant growth parameters in the absence of the pathogen were also determined. The least significant difference (LSD 5%) was used to compare the different treatments with each other. As there were no fusarium wilt symptoms on the seedlings that had not been inoculated with FOL (negative control and *Trichoderma* treatments), they were not included in the data analysis.

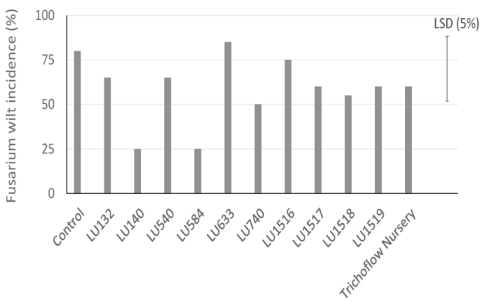

Figure 1 Effect of *Trichoderma* isolates and Trichoflow Nursery™ on fusarium wilt incidence (%) on tomato plants.

RESULTS

Fusarium wilt incidence

Eight of the *Trichoderma* isolates and Trichoflow Nursery™ did not reduce disease incidence, but LU140 and LU584 provided a 69% reduction ($P < 0.05$) compared with the pathogen control (Figure 1).

Plant growth promotion

Trichoderma isolates varied in their effect on tomato growth and ability to reduce the effect of FOL when subsequently applied in the pot experiment. There were statistically significant effects of *Trichoderma* treatments on plant growth parameters in the absence or presence of FOL inoculum (Table 1). In the absence of FOL, there was no consistent growth promotion response for any of the *Trichoderma* treatments. *Trichoderma* isolate LU132 significantly increased plant height and stem thickness compared with non-inoculated plants but had no significant effects on other plant growth parameters. Similarly, isolate LU740 increased stem thickness and root and shoot dry weight, but not plant height and root length. Isolates LU140, LU1517 and LU1518 had no effects on most plant growth parameters, apart from increasing shoot dry weight, root length and plant height, respectively (Table 1).

In the presence of FOL, *Trichoderma* isolate LU140 significantly increased all growth parameters compared with the pathogen control (Table 1). Isolate LU584 increased plant height and shoot dry weight, but had no significant effects on stem thickness, root length or dry weight compared with the pathogen control. Isolates LU132 and LU1517 increased only the root length, while Trichoflow Nursery™ increased the stem thickness and shoot dry weight (Table 1).

DISCUSSION

Two of the New Zealand *T. atroviride* isolates, LU140 and LU584, provided a significant reduction in FOL. They suppressed FOL by 69% compared with the control. These results are in agreement with previous studies (Cotxarrera et al. 2002; Larkin and Fravel 1998). Umashankar

Table 1. The effect of *Trichoderma* isolates on plant growth parameters

Treatment	Plant height to growing point (cm)[2]	Stem Thickness (mm)	Root Length (cm/plant)	Root Dry Weight (mg/plant)	Shoot Dry Weight (mg/plant)
Negative control[1]	12.7 a[3]	4.19 ab	474 a	55.0 ab	320 ab
LU132	17.6 c	4.56 d	639 ab	60.0 ab	375 bcd
LU140	13.7 ab	4.32 abcd	550 ab	79.5 bc	405 cde
LU540	13.8 ab	4.25 abc	656 ab	67.0 ab	380 bcd
LU584	15.5 bc	4.03 a	514 ab	61.5 ab	405 cde
LU633	14.0 ab	4.27 abcd	518 ab	65.0 ab	375 bcd
LU740	15.0 abc	4.52 cd	666 ab	97.0 c	460 e
LU1516	14.6 ab	4.35 bcd	584 ab	73.5 abc	355 bcd
LU1517	14.3 ab	4.36 bcd	750 b	79.0 bc	355 bcd
LU1518	15.8 bc	4.30 abcd	409 a	48.0 a	335 abc
LU1519	15.1 abc	4.29 abcd	561 ab	67.5 ab	260 a
Trichoflow Nursery™	15.0 abc	4.21 ab	509 ab	72.0 abc	425 de
df	**33**	**33**	**33**	**33**	**33**
F value	**0.131**	**0.105**	**0.388**	**0.067**	**0.002**
P value	**1.65**	**1.75**	**1.11**	**1.96**	**3.73**
Pathogen control[1]	6.5 ab	2.36 ab	135 a	14.0 abc	90 ab
LU132 + FOL	6.6 ab	2.59 abc	248 cd	19.5 abc	120 abc
LU140 + FOL	11.0 d	3.62 d	289 d	30.5 d	220 d
LU540 + FOL	6.9 ab	2.51 abc	204 abcd	18.3 abc	141 bc
LU584 + FOL	9.1 c	2.83 bc	215 abcd	22.0 abcd	145 c
LU633 + FOL	5.6 a	2.17 a	152 ab	18.3 abc	94 abc
LU740 + FOL	8.2 bc	2.81 bc	180 abc	24.5 cd	130 bc
LU1516 + FOL	6.4 ab	2.64 abc	150 ab	13.0 ab	75 a
LU1517 + FOL	8.3 bc	2.89 bc	238 bcd	17.0 abc	135 bc
LU1518 + FOL	6.8 ab	2.75 bc	222 abcd	16.5 abc	120 abc
LU1519 + FOL	7.6 bc	2.71 bc	141 a	12.0 a	130 bc
Trichoflow Nursery™	8.0 bc	2.92 c	226 abcd	23.0 bcd	145 c
df	**33**	**33**	**33**	**33**	**33**
F value	**<.001**	**0.002**	**0.029**	**0.049**	**0.001**
P value	**4.99**	**3.66**	**2.35**	**2.1**	**3.88**

[1] Negative control: Not inoculated with *Trichoderma* or *Fusarium oxysporum* f. sp. *lycopersici (*FOL); Positive control: treated with FOL only. [2] All plant growth parameters based on a mean of 30 plants.
[3] Means within the same column and section accompanied by the same letter are not significantly different according to an unrestricted LSD test at $P \leq 0.05$.

et al. (2010) reported that isolates of *Trichoderma*, *Pseudomonas*, and *Bacillus* isolated from different rhizosphere soils showed promise for controlling fusarium wilt and improving the growth and yield of tomato. In several studies, *T. harzianum* has also been reported to be effective against the fusarium wilt pathogen (Datnoff et al. 1995; Sivan et al. 1987; Srivastava et al. 2010).

T. atroviride isolates differed in their biocontrol activity against FOL in this study, which suggests bioactivity differences at the strain level. Differences in the biocontrol activity of *T. atroviride* strains against onion white rot have been previously reported (Harrison and Stewart 1988; Kay and Stewart 1994).

The spatial and temporal separation between the *Trichoderma* isolates and *Fusarium* challenge in the present study suggests that the reduction in disease incidence by these isolates was plant mediated, which concurs with a study conducted by Hoffland et al. (1996). Harman (2006) reported that one of the mechanisms for disease reduction caused by *Trichoderma* spp. is their ability to induce a potentiated state in the plant enabling it to become resistant to subsequent pathogen infection. The efficacy of the induced resistance varied according to the biocontrol strains. The isolates LU140 and LU584 in this study were shown to prime plants against pathogen attack more efficiently than other New Zealand *Trichoderma* isolates that were tested.

In the present study, some *Trichoderma* isolates enhanced tomato plant growth in the absence of the pathogen. The ability of *Trichoderma* isolate LU740 to increase tomato biomass in the absence of the pathogen suggests this isolate is likely to be able to influence the production of phytostimulators or phytohormones in the host, as suggested by Martínez-Medina et al. (2014). Isolate LU140 indirectly promoted tomato growth which appeared to be due to its biocontrol activity. FOL reduced all plant growth parameters, but in the presence of LU140 these negative effects were diminished. The negative effects of fusarium wilt disease result from disruption to plant growth by the blocking of xylem vessels, causing leaf senescence and reduced photosynthesis (Dimond 1995). Indirect effects of BCAs on the enhancement of plant growth have been noted frequently. However, the exact mechanisms by which they improve plant growth are not fully understood (Compant et al. 2005; Guo et al. 2004; Raj et al. 2003). Possible mechanisms include induction of systemic resistance (Hoitink et al. 2006), production of antibiotics to restrict the growth of the pathogen (Matarese et al. 2012), and competition with pathogens for nutrients or ecological niches (Sivan and Chet 1989). For example, Segarra et al. (2010) reported that *T. asperellum* protected tomato plants from fusarium wilt through competition for iron. Christopher et al. (2010) indicated that *T. virens* applied to tomato seed and soil indirectly promoted plant growth in the presence of the pathogen by inducing the expression of defence-related enzymes in tomato, which also suppressed the incidence of fusarium wilt disease.

Overall, this study identified *Trichoderma* isolates LU140 and LU584 as promising biocontrol agents against fusarium wilt in tomato. In comparison to the control, isolate LU140 also enhanced tomato growth in the presence of *Fusarium*, but in the absence of the pathogen it did not promote plant growth. The results support the idea that LU140 can indirectly promote the growth of the plant by preventing the deleterious effects of the pathogen and reducing the level of the disease. Spatial separation of the inoculation sites of *Trichoderma* and *Fusarium* suggests that LU140 induced a primed status in the plants in response to pathogen infection. However, further studies are needed to confirm that systemic resistance is induced by *Trichoderma* as a biological control mechanism against fusarium wilt of tomato.

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