

Evaluation of *Trichoderma harzianum* Strains to Control Crown and Root Rot of Greenhouse Fresh Market Tomatoes

N. Ozbay and S.E. Newman
Horticulture and Landscape Architecture
Colorado State University
Fort Collins, Colo. U.S.A.

W.M. Brown
Bioagricultural Sciences and Pest
Management
Colorado State University
Fort Collins, Colo., U.S.A.

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Abstract

Greenhouse tomato growers in the United States have few products available for chemical control of plant pathogens. Biological control of soilborne plant pathogens by antagonistic microorganisms is a potential alternative to the use of chemical pesticides during greenhouse production. Biological control experiments were conducted to test the effects of commercial and noncommercial strains of *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomato plants grown in two different hydroponic media, coir and rockwool. *Trichoderma harzianum* is a fungus that attacks a range of economically important phytopathogenic fungi. Tomato (*Lycopersicon esculentum* Mill., cultivar 'Caruso') plants were inoculated with *T. harzianum* strains (PlantShield™, T22 and T95) prior to challenge with the pathogen. They were applied into growing media prior to sowing and to roots at transplanting at two inocula densities, 10^6 or 10^7 conidia/ml. The results of this study demonstrated that *T. harzianum* strains, especially applied at transplanting, decreased disease incidence 79% for coir and 73% for rockwool, decreased disease severity 45% for coir and 48% for rockwool, and increased fruit yield 37% for coir and 25% for rockwool on tomato for *Fusarium* control.

INTRODUCTION

Fusarium crown and root rot (FCRR) caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker (FORL) is one of the most prevalent soil borne diseases of tomato. This disease occurs in both greenhouse and field worldwide and causes significant losses in tomato production. In closed systems, with recirculation of nutrient solution and rock wool as a growing medium, crown and root rot of tomato is a serious problem (Hartman and Fletcher, 1991; Rattink, 1992).

The use of *Fusarium*-resistant tomato cultivars can provide some degree of control of FCRR, but the occurrence and development of new pathogenic races is a continuing problem. Currently there are no commercially acceptable cultivars with adequate resistance to FORL (Jarvis, 1988; Jones et al., 1991; McGovern et al., 1993).

FCRR is generally controlled in tomato by pre-plant soil fumigation with methyl bromide (MBr). Tomatoes represent the largest single-crop use of MBr in the United States accounting for 25% of the total MBr use for soil fumigation (Anonymous, 1994). However, fumigation with MBr is expensive and not always an effective measure due to rapid colonization of growing media by FORL (Rowe et al., 1978; Gabor and Wiebe, 1997). In addition to other potential health, safety and environmental risks, concerns over the ozone-depleting properties of MBr have led to the phasing out of its use. Therefore, alternative control measures are necessary and need to be made available as soon as possible.

Biological control is an alternative to the use of chemical pesticides. Biological fungicides may act to suppress the population of the pathogenic organism through competition with pathogenic organisms, stimulate plant growth, which may allow plants to quickly outgrow any pathogen effects, or damage the pathogen by means of toxins produced (Cook, 2000; Gilreath, 2002). A variety of soil microorganisms have

demonstrated activity in the control of various soilborne plant pathogens, including *Fusarium* wilt pathogens. Of the fungi used for control of soilborne pathogens, various species of *Trichoderma* spp have received the most attention. *Trichoderma harzianum* is a fungal biocontrol agent that attacks a range of phytopathogenic fungi. *T. harzianum* alone or in combination with other *Trichoderma* species can be used in biological control of several plant diseases (Papavizas, 1985; Chet, 1987; Samuels 1996). It has been also shown to be effective in controlling *Fusarium* crown and root rot under greenhouse and field conditions. Although *Trichoderma* spp is ubiquitous, the type of the soil can affect growth, proliferation and effectiveness as biocontrol agent. Because soil ecology is complex, and with year-to-year fluctuations in climate conditions, treatments with microbials are often inconsistent (Quarles, 1993). The objective of this study was to evaluate the efficacy of existing biocontrol strains of *Trichoderma harzianum* for controlling FCRR of tomato under greenhouse conditions.

MATERIALS AND METHODS

The isolate of *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) used in this study was isolated from naturally infected tomato plants grown from a commercial tomato greenhouse. Samples collected from diseased plants were thoroughly washed with tap water; roots and crowns were removed. The crown and root pieces were surface sterilized by immersion for 2 min. in 3% bleach (sodium hypochlorite) solution followed by two rinses in sterile deionized water. The blotted tissue was placed on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI, U.S.A.) and incubated at 25°C for 5 days. Any developing fungus was isolated on new PDA plates, purified and identified according to Nelson (1983). Pathogenicity was confirmed for FORL in the test with tomato cv. 'Caruso'. A spore suspension (10^6 conidia + mycelial fragments/ml) was prepared by blending 14-day old cultures of FORL grown on PDA at 25°C with sterile distilled water and filtering the suspension through cheesecloth. Conidial densities in the suspension were determined by use of a hemacytometer under a light microscope. The Pathogen inoculum was added to rockwool blocks.

Three strains of biocontrol fungi were evaluated in this study. *T. harzianum* strain T95 (T95) (provided by Suzanne M. Nemeth, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, Colo., U.S.A.). *T. harzianum* Rifai strain KRL-AG2 (PlantShield™, 1×10^7 colony forming units/g as a wettable powder) was supplied by Bioworks Inc., Geneva, N.Y., U.S.A.) *T. harzianum* Rifai strain 1295-22 (T-22) was derived from 14-day old cultures grown on PDA plates incubated at 25°C. PlantShield™ and T22 are the same strain and the only difference in this study was the preparation of the strains for inoculum. T22 and T95 were maintained on PDA and kept at 4°C. (PlantShield™ was maintained in the container provided by the manufacturing company and kept at 4°C. Fungal inoculum of strain T22 and T95 was prepared by blending 2-week old PDA-grown cultures of the fungus with sterile distilled water, straining the suspension through cheesecloth. The *T. harzianum* strains T95 and T22 (10^6 and 10^7 conidia/ml) were inoculated either by soaking the rockwool cubes at sowing or by soaking the rockwool blocks at transplanting.

Experiments were carried out in the greenhouse facilities of the W.D. Holley Plant Environment Research Center, Colorado State University, Fort Collins, Colo., U.S.A. Greenhouse structural components and irrigation systems were surface sanitized prior to and between experiments with quaternary ammonia. The three *T. harzianum* strains were compared in their ability for controlling FORL attack to greenhouse tomato plants grown in two different soilless media. Seeds of tomato (*Lycopersicon esculentum* Mill., cv. Caruso), surface-sterilized by immersion in 1% bleach (sodium hypochlorite) for 30 min. and rinsed, were sown in rockwool cubes (4cm x 4 cm x 4cm) placed into a propagation flat and holes were filled with vermiculite. The tomato seedlings were transferred to rockwool blocks after two weeks. The seedlings were watered by hand on daily basis and complete nutrient solution was applied with each watering. The nutrient solution consisted of CHEM-GRO™ tomato formula (Hydro-Gardens, Colorado Springs, Colo.,

U.S.A), calcium nitrate and magnesium sulfate. Electrical conductivity of the solution was maintained between 1.5-2.0 mS/cm. Nutrient solution was adjusted to pH 6.2-6.5. Transplanting to slabs took place 5 weeks later. Each slab contained three tomato plants spaced about 30 cm apart. Each plant was irrigated by a single drip irrigation emitter after transplanting. Temperature was maintained at 18°C night and 25°C day. Tomato plants were trained to a single stem and supported by twine to an overhead wire. All lateral branches or suckers were removed when they are 3 cm to 7 cm long. The above cultural practices emulate commercial practices in Colorado.

Spore suspensions of *T. harzianum* strains T95 and T22 (10^6 and 10^7 conidia/ml), prepared as previously described, were inoculated once, either by soaking the rockwool cubes at sowing or by soaking the rockwool blocks at transplanting when the seedlings were 5 weeks old. PlantShield™ was applied once to rockwool cubes at sowing in the amount and concentration (0.5-1.0g/L) as per the label. The pathogen, *F. oxysporum* f.sp. *radicis-lycopersici*, was added to the rockwool blocks in inoculation level of 10^6 conidia/ml two weeks after transplanting. Plants were observed regularly for visible disease symptoms of the disease. In diseased tomato plants, symptoms worsened over the time, but none of the plants died. The populations of the biocontrol agents were not monitored over time. However, samples of drainage water (100ml) from each slab inoculated with the pathogen were collected three weeks after inoculation for determination of the presence of the pathogen in the effluent. FORL of tomato was evaluated 9 weeks after the inoculation of the pathogen for disease incidence and severity using a rating scale of 0 to 3 where 0=no disease and 3=50 to 100% internal necrosis of root system 10 to 15 cm up the stem from the crown (Datnoff et al, 1995). The mean percentage of severity for each numerical rating was used for estimating the differences between treatments. Yield was also recorded to measure response of the tomato plants to the biological control agents.

The design for efficacy tests was a factorial design with three replicates of each treatment. Rockwool and Coconut coir were the main plots. Each subplot consisted of a slab of growing medium (rock wool or coconut-fiber) with 91cm long x 18cm wide x 7.5cm deep encased in opaque plastic film. Three tomato seedlings were planted to each slab. Data were analyzed by analysis of variance (ANOVA) and the means were separated by using Fisher's LSD tests at alpha values of 0.05. Statistical analyses were conducted using the general linear models procedure of SAS Version 8e (SAS Institute Inc., Cary, NC, U.S.A.).

RESULTS AND DISCUSSION

Inoculation of tomato plants with FORL caused symptoms similar to natural infections described by Gabor and Wiebe (1997). The analysis of variance of data resulted in differences in treatment effects at $P \leq 0.05$. In this study we found all three strains of *T. harzianum* tested gave control of FCRR. In general, the treatments applied at transplanting resulted in more disease control than those applied at seeding. When *T. harzianum* strains were applied at transplanting, *Fusarium* crown and root rot incidence of greenhouse-grown tomatoes was reduced up to 79% in coir slabs and up to 73% in rockwool slabs (Table 1). The effects of the different treatments on disease severity 9 weeks after inoculation with the pathogen are illustrated in Table 2. *T. harzianum* strains reduced ($P \leq 0.005$) disease severity in tomato plants grown in coir and rockwool slabs (45%, 48% respectively) compared with untreated plants. Maximum disease control was obtained with T22 and T95 applied at transplanting. While increasing inoculum levels from 10^6 to 10^7 conidia/ml of biocontrol strains applied at transplanting generally resulted in an increase of disease control, increasing inoculum levels of biocontrol agents applied at seeding had no effect on the extent of protection.

Treatments that resulted in disease control also produced a yield increase ($P \leq 0.05$). The highest yield improvement was recorded in plots where *T. harzianum* strains have been applied at transplanting at the inoculum level of 10^7 conidia/ml. T22 and T95 increased fruit yield 37% for coir and 25% for rockwool on tomato control in the presence

of the pathogen with untreated control (Table 3). There was no fruit yield difference between rockwool and coir slabs (Table 4).

The objective of this study was to evaluate efficacy of existing biocontrol strains of *Trichoderma harzianum* including commercial formulations for controlling FCRR of tomato under greenhouse conditions. Our study showed that *T. harzianum* strains were effective in reducing disease incidence and severity of *Fusarium* crown and root rot of tomato under greenhouse conditions. The effect of *Trichoderma* on reduction of the crown and root rot disease and on yield of tomatoes has been investigated. When *T. harzianum* was applied as seed coating, crown and root rot incidence of greenhouse-grown tomatoes was reduced up to 80% by 75 days after sowing (Sivan et al., 1987). Van Steekelenburg (1991) showed that *T. harzianum* reduced the incidence and spread of *Fusarium* crown and root rot in rockwool-grown tomatoes.

T. harzianum strains increased yield in the presence of measurable disease. Reduction of disease by the use of *T. harzianum* strains had improved tomato yields between 6% and 37% in coir and between 2% and 25% in rockwool. However, they had no effect on yield in the absence of the disease compared with untreated and uninoculated control. This finding suggests that *T. harzianum* strains used in this experiment act only as biocontrol agents as reported by Lamb and Roskopf (2002). On the other hand, tomato plants grown in coconut coir slabs resulted in similar fruit yields to plants grown in rockwool slabs suggesting that coconut coir is a potential alternative to rockwool as growing medium for soilless tomato.

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Tables

Table 1. Effect of *Trichoderma harzianum* strains on incidence of *Fusarium* crown and root rot tomato plants grown in coir and rockwool slabs.

| Treatment | Disease incidence ¹ (%) | | | |
|--|------------------------------------|-----------------|----------|----|
| | Coir | | Rockwool | |
| <i>T. harzianum</i> T95 (10 ⁶ con./ml) at seeding + FORL. | 38.58 | bc ² | 44.31 | bc |
| <i>T. harzianum</i> T95 (10 ⁷ con./ml) at seeding + FORL | 38.37 | bc | 44.81 | b |
| <i>T. harzianum</i> T95 (10 ⁶ con./ml) at transplanting + FORL. | 38.17 | c | 22.97 | d |
| <i>T. harzianum</i> T95 (10 ⁷ con./ml) at transplanting + FORL | 19.41 | e | 19.58 | d |
| <i>T. harzianum</i> T22 (10 ⁶ con./ml) at seeding + FORL. | 43.03 | b | 37.98 | c |
| <i>T. harzianum</i> T22 (10 ⁷ con./ml) at seeding + FORL | 31.08 | d | 49.70 | b |
| <i>T. harzianum</i> T22 (10 ⁶ con./ml) at transplanting + FORL. | 15.72 | ef | 20.95 | d |
| <i>T. harzianum</i> T22 (10 ⁷ con./ml) at transplanting + FORL | 13.12 | f | 19.24 | d |
| PlantShield TM + FORL | 38.45 | bc | 44.15 | bc |
| <i>T. harzianum</i> T95 alone | 0.00 | g | 0.00 | e |
| <i>T. harzianum</i> T22 alone | 0.00 | g | 0.00 | e |
| PlantShield TM alone | 0.00 | g | 0.00 | e |
| FORL alone | 60.96 | a | 70.13 | a |
| Untreated control | 0.00 | g | 0.00 | e |

¹ Incidence was determined based on the number of plants exhibiting symptoms of FCRR.

² Numbers in a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's LSD test.

Table 2. Effect of *Trichoderma harzianum* strains on severity of *Fusarium* crown and root rot on tomato plants grown in coir and rockwool slabs.

| Treatment | Disease severity ¹ (0-3) | |
|--|-------------------------------------|----------|
| | Coir | Rockwool |
| <i>T. harzianum</i> T95 (10 ⁶ con./ml) at seeding + FORL. | 1.21 ab ² | 1.17 bc |
| <i>T. harzianum</i> T95 (10 ⁷ con./ml) at seeding + FORL | 1.16 abc | 0.99 d |
| <i>T. harzianum</i> T95 (10 ⁶ con./ml) at transplanting + FORL. | 1.10 bc | 1.02 cd |
| <i>T. harzianum</i> T95 (10 ⁷ con./ml) at transplanting + FORL | 0.99 cd | 0.79 e |
| <i>T. harzianum</i> T22 (10 ⁶ con./ml) at seeding + FORL. | 1.26 ab | 1.31 ab |
| <i>T. harzianum</i> T22 (10 ⁷ con./ml) at seeding + FORL | 1.23 ab | 1.32 ab |
| <i>T. harzianum</i> T22 (10 ⁶ con./ml) at transplanting + FORL. | 0.79 de | 0.88 de |
| <i>T. harzianum</i> T22 (10 ⁷ con./ml) at transplanting + FORL | 0.74 e | 0.81 e |
| PlantShield TM + FORL | 1.06 bc | 0.89 de |
| <i>T. harzianum</i> T95 alone | 0.00 f | 0.00 f |
| <i>T. harzianum</i> T22 alone | 0.00 f | 0.00 f |
| PlantShield TM alone | 0.00 f | 0.00 f |
| FORL alone | 1.34 a | 1.41 a |
| Untreated control | 0.00 f | 0.00 f |

¹ Disease severity was recorded 9 weeks after the inoculation of the pathogen, using a scale from 0 to 3 (see Materials and Methods).

² Numbers in a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's LSD test.

Table 3. Effect of *Trichoderma harzianum* strains on yield of tomato plants grown in coir and rockwool slabs.

| Treatment | Yield (kg/plant) | |
|--|------------------|----------|
| | Coir | Rockwool |
| <i>T. harzianum</i> T95 (10 ⁶ con./ml) at seeding + FORL. | 2.42 bc* | 2.22 abc |
| <i>T. harzianum</i> T95 (10 ⁷ con./ml) at seeding + FORL | 2.51 bc | 2.30 abc |
| <i>T. harzianum</i> T95 (10 ⁶ con./ml) at transplanting + FORL. | 2.75 abc | 2.27 abc |
| <i>T. harzianum</i> T95 (10 ⁷ con./ml) at transplanting + FORL | 2.31 bc | 2.75 abc |
| <i>T. harzianum</i> T22 (10 ⁶ con./ml) at seeding + FORL. | 2.04 c | 2.13 c |
| <i>T. harzianum</i> T22 (10 ⁷ con./ml) at seeding + FORL | 2.06 c | 2.19 bc |
| <i>T. harzianum</i> T22 (10 ⁶ con./ml) at transplanting + FORL. | 2.78 abc | 2.40 abc |
| <i>T. harzianum</i> T22 (10 ⁷ con./ml) at transplanting + FORL | 2.99 ab | 2.71 abc |
| PlantShield™ + FORL | 2.47 bc | 2.28 abc |
| <i>T. harzianum</i> T95 alone | 2.60 bc | 2.65 abc |
| <i>T. harzianum</i> T22 alone | 2.40 bc | 2.69 abc |
| PlantShield™ alone | 3.45 a | 3.04 ab |
| FORL alone | 2.17 c | 2.16 c |
| Untreated control | 2.73 abc | 3.06 a |

* Values of each column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's LSD test.

Table 4. Effect of growing media on tomato yield.

| Treatment | Yield (kg/plant) | Yield (t/ha) |
|--------------------|------------------|--------------|
| Coconut coir slabs | 2.9 a* | 171.5 a |
| Rockwool slabs | 2.8 a | 167.5 a |

* Values of each column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's LSD test.