*Phytopathologia Mediterranea* (2014) 53, 3, 502–514
DOI: 10.14601/Phytopathol_Mediterr-14476

RESEARCH PAPER

# *Trichoderma* strains suppress *Rhizoctonia* diseases and promote growth of potato

Emily HICKS[1], Damian BIENKOWSKI[1], Mark BRAITHWAITE[1], Kirstin McLEAN[1], Richard FALLOON[1,2] and Alison STEWART[1]

[1] Bio-Protection Research Centre, P.O. Box 85084, Lincoln University, Lincoln 7647, New Zealand
[2] New Zealand Institute for Plant & Food Research Limited, PB 4704, Christchurch, New Zealand

**Summary.** *Trichoderma* spp. from New Zealand soils were evaluated (in greenhouse experiments and one field trial) for *Rhizoctonia* disease suppression and plant growth promotion of potato plants. *Trichoderma virens* LU549, *T. atroviride* LU144 and *T. barbatum* LU1482 demonstrated the greatest suppression of cankers on potato plants; the percentage of diseased stolons was reduced by 41–46%, compared with the *R. solani* control. *Trichoderma virens* LU549 also increased average tuber weight by 210%, and *T. atroviride* LU144 by 146%, compared with the *R. solani* control (in which tuber formation was highly suppressed). In plant growth promotion pot trials, the greatest proportional increases for three plant growth parameters (compared with the untreated control) were elicited by: *T. harzianum* LU1491 (number of tubers), *T. barbatum* LU1489 (total tuber weight), and *Trichoderma* sp. 792 LU1483 (average tuber weight). All six of these strains were selected and evaluated in all combinations in a $2^6$ factorial greenhouse experiment. *Trichoderma atroviride* LU144 had positive impacts on several *Rhizoctonia* disease and plant growth parameters. Four of the strain combinations were subsequently tested in a field trial during the 2011/12 growing season, in which two *Trichoderma* strain combinations increased potato tuber yields. This research has shown potential for use of New Zealand *Trichoderma* strains to suppress *Rhizoctonia* diseases of potato and increase crop productivity.

**Key words:** biological control, combination.

## Introduction

Potato crops are economically important in New Zealand. They have an annual domestic value of $NZ 225.5 million, and an export value of $NZ 117.4 million (Aitken and Hewett, 2012). *Rhizoctonia solani* Kühn, is a soil-borne fungal plant pathogen which can cause serious loss in potato production worldwide (Banville *et al*., 1996). Symptoms of infection include lesions on below-ground shoots, stems and stolons, resulting in variations in numbers and size of tubers, and in some instances tuber malformations. Lesions can infect and destroy emerging shoots ('nipping'), delaying crop emergence. The fungus also forms sclerotia ('black scurf') on the surface of tubers (Banville *et al*., 1996) which are important inoculum sources, and can remain active in the soil for several years (Sherwood, 1970).

*Rhizoctonia* diseases on potato are commonly controlled using combinations of cultural and chemical disease management strategies to reduce soil-borne and tuber-borne inoculum. Cultural control methods include: crop rotation, reduced tillage and the use of certified (disease-free) seed potatoes. Chemical control of *R. solani* can effectively reduce tuber-borne inoculum (Hide and Cayley, 1982), but soil-borne inoculum is difficult to control with fungicides (Weinhold *et al*., 1982; Wilson *et al*., 2008b). Biological control could play an important role as an additional strategy for *R. solani* disease management. Commercial biological control products have

Corresponding author: E. Hicks
E-mail: emily.hicks@lincoln.ac.nz


ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm

been assessed for control of *R. solani* on potato with some success (Brewer and Larkin, 2005; Wilson *et al*., 2008a), although none effectively control stem canker and black scurf. None of these biological control products (or their active ingredients), are registered for use in New Zealand (Ministry for Primary Industries, 2013). The development of locally sourced organisms for biological control of *R. solani* would benefit the New Zealand potato industry, and may have wider application in potato production.

*Trichoderma* spp. are formulated and marketed as biological control agents (BCAs) for numerous plant pathogens, including *R. solani* (Whipps and Lumsden, 2001). The application of *Trichoderma* spp. has been associated with reduced *R. solani* diseases on crops including: bean, cotton, tomato, beet and potato (Elad *et al*., 1980; Beagle-Ristaino and Papavizas, 1985; Lewis and Papavizas, 1987; Grosch *et al*., 2006; Verma *et al*., 2007). Moreover, diverse *Trichoderma* spp. have been associated with increased crop productivity in species such as tobacco, tomato and radish (Windham *et al*., 1986; Gravel *et al*., 2007; Hoyos-Carvajal *et al*., 2009). Growth promotion by the application of *Trichoderma* spp. has not been extensively studied in potato, although there are reports of other plant-associated microorganisms promoting potato plant growth (Yao *et al*., 2002; Kumar *et al*., 2013).

The application of single microbial antagonists as BCAs can result in inconsistent disease control (Thilagavathi *et al*., 2007). In contrast, mixtures of microorganisms which have different modes of action can improve biocontrol efficacy and consistency over broad ranges of ecological and environmental conditions (Duffy *et al*., 1996; Srivastava *et al*., 2010; Stockwell *et al*., 2011).

The aim of the present study was to identify strains of *Trichoderma* spp. that could improve potato yields by suppressing *R. solani* diseases and/or promoting potato plant growth, when applied either as single strains or in combinations.

## Materials and methods

### Production of *Trichoderma* conidia

Fifty-three *Trichoderma* strains were selected from the Biocontrol Microbial Culture Collection (Bio-Protection Research Centre, Lincoln University), based on prior *in vitro* and *in vivo R. solani* suppression, or on their previous success in controlling plant diseases, including soil-borne plant pathogens in other biocontrol programmes. All strains were obtained from New Zealand soils.

*Trichoderma* strains were sub-cultured from -80°C glycerol stocks onto potato dextrose agar (PDA). Mycelial plugs (5 mm diam.) were used to inoculate Petri dishes containing PDA, or sterile plastic boxes with a 1 cm deep layer of sterile peat:wheat:water medium (1:1:1 v/v/v mixed). Media were incubated for 14 d (Petri dishes at 25°C, 12 h/12 h white light/dark; sterile plastic boxes in ambient laboratory conditions), and conidia were then harvested in sterile water. The concentrations of the conidial suspensions were determined using a haemocytometer, and adjusted as required.

### Production of *Rhizoctonia solani* inoculum

Two strains of *R. solani*, originally from potato tuber sclerotia (from Canterbury, New Zealand), were selected as pathogen inoculum. Strain Rs043-2, an AG 2-1 that produces strong canker symptoms (Bienkowski *et al*., 2010; Das *et al*., 2014), was used to inoculate greenhouse assays and the field trial. Strain Rs018-2, an AG 3 that is a prolific producer of black scurf (Das *et al*., 2014) was also used as inoculum in the field trial. Strains were maintained on sterilised barley seeds stored at 4°C. *Rhizoctonia solani* was cultured on PDA and incubated (25°C, in the dark) for 5 d. Five agar plugs (5 mm diam.) from the growing edge of a *R. solani* colony were used to inoculate sterile barley grain + V8 juice® (1:1 w/v) in each conical flask. Flasks were incubated (25°C, in the dark) for 14 d. The pathogen inoculum was applied at 0.1% w/v. For strain Rs043-2, this concentration of inoculum has been shown to cause intermediate levels of disease, approximately 60% incidence of stolon canker on plants grown from tissue-cultured plantlets, and approximately 85% of emerging shoots nipped on plants grown from minitubers (Bienkowski *et al*., 2010).

### Disease suppression and growth promotion pot trials

A total of eight greenhouse pot trial assays (PT1 to PT8) were used to test *Trichoderma* strains, either for suppression of *Rhizoctonia* canker symptoms or for growth promotion of potato plants (detailed in Table 1). Growth promotion assays (PT3 to PT7)

used tissue-cultured plantlets, and a growing medium of peat:pumice (3:2, v/v + fertiliser). All other assays used a growing medium of soil (silt loam):peat:pumice (7:3:2, v/v/v) + fertiliser and minitubers to more closely approximate field conditions (except PT1 which used tissue-cultured plantlets). In all assays, one potato plant was grown in each pot.

In disease suppression assays, for each treatment and for the *R. solani* experimental controls, *R. solani* infested barley + V8 medium was mixed through 9 L of growing medium. Growth promotion assays received no pathogen. Conidial suspensions of each *Trichoderma* strain ($1 \times 10^6$ conidia $g^{-1}$ of growing medium, Table 1) were evenly mixed through 9 L of growing medium. The negative/untreated control received 50 mL of water evenly mixed through 9 L of growing medium. In PT1, the fungicide Monceren® 250 FS (a.i. pencycuron 250 g $L^{-1}$, Bayer CropScience) was used as a treatment, and was applied to each pot (4.5 mL of Monceren® in 50 mL of water), equivalent to the recommended field in-furrow application rate of 5 kg a.i. $ha^{-1}$. For each assay, the treated growing medium was divided between ten (0.9 L capacity, 120 mm diam.) plastic pots with individual saucers.

Pot trial 2 (PT2) was used to validate *Trichoderma* strains which showed significant activity in previous assays and, for two of these strains, to evaluate the influence of application method and inoculation rate on suppression of *Rhizoctonia* diseases. For these two strains, 5 mL of a $2 \times 10^7$ conidia $mL^{-1}$ suspension of *Trichoderma* conidia was pipetted directly onto each minituber at planting, prior to covering with growing medium. Both of these strains were also tested at $1 \times 10^7$ and $1 \times 10^6$ conidia $g^{-1}$ of growing medium, as described in Table 1.

Tissue-cultured plantlets ('Russet Burbank', Aspara Pacific Ltd; 'Desiree' or 'Gladiator', Alex McDonald Merchants Ltd) were rinsed free of agar in running tap water before planting. One cultivar was not available for all of the pot trials. Potato minitubers (undersized for field planting, 'Desiree', Alex McDonald Merchants Ltd) which had been presprouted (2 weeks at 18°C, 12 h white light photope-

**Table 1.** Pot trial conditions for: *Rhizoctonia* disease suppression, potato plant growth promotion and *Trichoderma* strain combination.

| Assay Number | Potato propagule[a] | Pathogen inoculum rate | No. of *Trichoderma* isolates tested | *Trichoderma* conidia suspension concentration | Growing mix |
|---|---|---|---|---|---|
| Disease suppression trials | | | | | |
| PT1 | TC cv. 'Russet Burbank' | 1 g $L^{-1}$, 0.1% w/v | 22 | $1 \times 10^6$ conidia $g^{-1}$ of growing medium[b] | Soil-based |
| PT2 | MT cv. 'Desiree' | 1 g $L^{-1}$, 0.1% w/v | 3 | $1 \times 10^6$, or $1 \times 10^7$ conidia $g^{-1}$ of growing medium[b], or $2 \times 10^7$ conidia $mL^{-1}$ (5 mL) applied directly onto the tuber | Soil-based |
| Growth promotion trials | | | | | |
| PT3 | TC cv. 'Desiree' | nil | 38 | $1 \times 10^6$ conidia $g^{-1}$ of growing medium[b] | Peat:pumice |
| PT4 | TC cv. 'Gladiator' | nil | | | |
| PT5-7 | TC cv. 'Russet Burbank' | nil | | | |
| *Trichoderma* strain combination trial | | | | | |
| PT8 | MT cv. Russet Burbank | 1 g $L^{-1}$, 0.1% w/v | 6 | $1 \times 10^6$ conidia $g^{-1}$ of growing medium (per strain)[b] | Soil-based |

[a] TC, tissue-cultured plantlets, MT, minitubers.
[b] In 50 mL water, mixed through the growing medium.

riod) were planted at 30 mm depth. Pots were then arranged in randomised block designs (ten blocks, one replicate of each treatment per block) in a temperature-controlled greenhouse unit set to 16.5°C supplemented with full spectrum lighting (16 h light: 8 h dark). Pots were watered as required.

### *Trichoderma* strain combinations pot trial

Three *Trichoderma* strains previously shown to suppress *Rhizoctonia* disease symptoms (in PT1 and PT2), and three demonstrating growth promotion (in PT3 to PT7) were evaluated as combination treatments in a $2^6$ factorial pot trial (PT8). There were 63 treatments, with four negative controls, four *R. solani* controls and four fungicide treatments within each of four randomised blocks. For each treatment and for the *R. solani* controls, *R. solani* infested barley + V8 media was mixed through 9 L of growing medium. The negative control treatments had sterile barley + V8 medium (0.1% w/v). For each *Trichoderma* strain combination treatment, a suspension of conidia was prepared in 50 mL water and mixed through the growing media to provide a target concentration for each strain of $1 \times 10^6$ conidia $g^{-1}$ of soil mix. The four *R. solani* control treatments each received 50 mL of water. The treated growing medium was divided between four 0.9 L capacity pots with individual saucers.

### Pot trial harvest procedure

Plants were harvested after 7 weeks. Parameters measured for each plant in the disease suppression assays were: number of shoots (emerged or 'nipped'), number of stolons (total and diseased), number of tubers (normal or malformed), and weight of tubers. For the growth promotion assays, number of stolons, number of tubers, weight of tubers, and the dry weights of the shoots and roots were recorded for each plant.

### Field trial

Four combinations of *Trichoderma* strains were evaluated for their potential to suppress *Rhizoctonia* diseases and improve yield in a field trial. The trial was conducted over the summer 2011/12 at Southbridge, Canterbury, New Zealand. Treatments (three *R. solani* controls, one fungicide treatment (pencycuron), and four *Trichoderma* combination treatments) were replicated ten times in a randomised block design. Each plot consisted of one furrow, 2.4 m long, with spacing of 90 cm between rows. For each replicate, ten seed potatoes (red skinned cv. 'Desiree', Alex McDonald Merchants Ltd) were placed in the furrow at 20 cm intervals. The trial site was inoculated with two *R. solani* strains; Rs0432 (AG 2-1) and Rs018-2 (AG 3) mixed in a 1:1 ratio to achieve stem canker and tuber black scurf symptoms on plants. To inoculate the furrows, one *R. solani*-infested barley grain was placed between each tuber. Tubers were sprayed in-furrow with either a *Trichoderma* combination treatment ($1 \times 10^7$ conidia per potato tuber), or fungicide (pencycuron, Monceren® 250 FS, 20 L $ha^{-1}$) or water (*R. solani* controls). Furrows were immediately filled with soil to prevent desiccation of the applied conidia. *Trichoderma* combination treatments were applied at an equivalent rate to commercially available *Trichoderma* products (Agrimm Technologies Ltd, 2013). The trial was managed by the grower with standard commercial practice for fertiliser, herbicide and irrigation regimes, although no additional fungicides were applied to the trial site.

The trial was replicated twice. The first sampling (harvest 1) was carried out 4 weeks after planting, and the second (harvest 2) was at crop maturity (5 months after planting). At harvest 1, the number of shoots (emerged and nipped), and the number of stolons (total and diseased) were counted in each plot. At maturity (harvest 2), tubers in each plot were harvested and categorised by length into undersized (<45 mm), table (45–85 mm), oversized (>85 mm), and malformed (tubers which were not approximately oval), using standard industry categories, (Mr Kerry Hughes, Alex McDonald Merchants Ltd personal communication). The number and weight of the tubers from each plot were recorded. The table sized tubers were then individually scored for percentage of black scurf coverage (method modified from James, 1973) in the following categories: 0 = no black scurf, 1 = <1–5%, 5 = 5–10%, 10 = 10–15%, 15 = >15%. The mean percentage scurf coverage for each plot was calculated.

### Statistical analyses

Data from all assays and the field trial were analysed using analysis of variance, and the parameter means were compared using the generated unre-

stricted LSD at $P$=0.05. For the *Trichoderma* strain combination pot trial (PT8) the data were analysed both as for a $2^6$ factorial experiment and as 66 individual treatments using an unrestricted LSD test.

## Results

### *Rhizoctonia* disease control pot trial using plants from tissue-cultured plantlets in a soil-based mix (PT1)

Treatments which decreased ($P$<0.05) at least one plant disease parameter, or increased ($P$<0.05) at least one plant growth parameter, compared with the *R. solani* control are reported in Table 2. Pencycuron increased ($P$<0.05) the number of stolons (78%), and the total tuber weight (147%), and reduced ($P$<0.05) the percentage of diseased stolons (92%), compared with the *R. solani* control (Table 2).

Means of the 22 treatments for each parameter had the following ranges: number of stolons 3.3–5.6, diseased stolons 35.7–78.1%, total tuber weight 2.13–10.08 g and average tuber weight 0.81–4.68 g.

Strains *T. virens* LU549, *T. atroviride* LU144 and *T. barbatum* LU1482 decreased ($P$<0.05) the percentage of diseased stolons by 41–46% (Table 2). *Trichoderma atroviride* LU144 also increased ($P$<0.05) the average tuber weight by 146%. *Trichoderma virens* LU569 increased ($P$<0.05) the total tuber weight by 88% and average tuber weight by 210%. The reduction in percentage of diseased stolons was considered the most appropriate measure of disease control; therefore *T. virens* LU549, *T. atroviride* LU144 and *T. barbatum* LU1482 were selected for further evaluation.

### *Rhizoctonia* disease control pot trial using plants from minitubers in a soil-based mix (PT2)

The strains *T. virens* LU549, *T. atroviride* LU144 and *T. barbatum* LU1482 reduced ($P$<0.05) the number of stolons and increased the percentage of nipped shoots, irrespective of the method of application (Table 3). There was no statistically significant difference between the *R. solani* control and the treatment means for average tuber weight (average of group means: 1.20 g, data not shown). The average tuber weight for the negative control was 4.70 g.

### Growth promotion trials (PT3 to PT7)

Nine *Trichoderma* treatments increased ($P$<0.05) one or more plant growth parameter in at least one assay (Table 4). For each *Trichoderma* strain, the treatment mean was compared with the control mean within each pot trial. Three strains increased ($P$<0.05) the number of tubers: *T. harzianum* LU1491 by 75%, *T. rossicum* LU1492 by 60%, and *T. atroviride* LU132 by 43%. In addition, *T. harzianum* LU1491 also increased

**Table 2.** Means for four of the 22 *Trichoderma* treatments tested for suppression of *Rhizoctonia* disease on potato plants grown from tissue-cultured plantlets (PT1).

| Strain | No. of replicates | No. of stolons | Diseased stolons (%) | Total tuber weight (g) | Average tuber weight (g) |
|---|---|---|---|---|---|
| *R. solani* control | 40 | 4.05 | 66.2 | 4.08 | 1.51 |
| Pencycuron | 10 | 7.20[a] | 5.3[a] | 10.08[a] | 2.91 |
| Negative control | 10 | 4.90 | 0.2[a] | 6.05 | 2.43 |
| *T. virens* LU549 | 10 | 4.60 | 37.2[a] | 7.21 | 3.00 |
| *T. virens* LU569 | 10 | 4.30 | 74.4 | 7.69[a] | 4.68[a] |
| *T. atroviride* LU144 | 10 | 4.80 | 35.7[a] | 7.20 | 3.71[a] |
| *T. barbatum* LU1482 | 10 | 3.44 | 39.2[a] | 4.28 | 1.80 |
| LSD (5%) [40 vs 10 reps] | | 1.49 | 22.5 | 3.15 | 1.64 |
| LSD (5%) [10 vs 10 reps] | | 1.89 | 28.5 | 3.99 | 2.07 |

[a] Means (per plant) that are different to the *R. solani* control, using an unrestricted LSD ($P$<0.05).

**Table 3.** Means of *Trichoderma* treatments tested for suppression of *Rhizoctonia* disease on potato plants grown from minitubers (PT2).

| Treatment [a] | Application (conidia mL$^{1}$ soil mix) | No. of replicates | Emerging shoots nipped (%) | No. of stolons | Diseased stolons (%) | Total tuber weight (g) |
|---|---|---|---|---|---|---|
| *R. solani* control | - | 30 | 10.3 | 12.2 | 77.4 | 9.82 |
| Negative control | - | 10 | 0.0 | 8.5[d] | 0.8[c] | 21.60[c] |
| LU144 | $1 \times 10^6$ | 10 | 31.7[d] | 7.5[d] | 74.0 | 6.70 |
| LU549 | $1 \times 10^6$ | 10 | 40.5[d] | 6.9[d] | 80.8 | 3.31[d] |
| LU549 | $1 \times 10^7$ | 10 | 25.0 | 8.3[d] | 75.0 | 4.68[d] |
| LU549 | $1 \times 10^{6}$ [b] | 10 | 49.6[d] | 4.6[d] | 81.1 | 2.76[d] |
| LU1482 | $1 \times 10^6$ | 10 | 33.8[d] | 5.7[d] | 80.7 | 6.03 |
| LU1482 | $1 \times 10^7$ | 10 | 11.7 | 11.7 | 74.7 | 8.17 |
| LU1482 | $1 \times 10^{6}$ [b] | 10 | 54.1[d] | 3.5[d] | 51.1[c] | 5.08[d] |
| LSD (5%) [30 vs 10 reps] | | | 19.0 | 3.2 | 14.7 | 4.33 |
| LSD (5%) [10 vs 10 reps] | | | 23.3 | 3.9 | 18.1 | 5.30 |

[a] There was no pencycuron control in this experiment.
[b] Applied directly onto the minituber.
[c] Treatment means (per plant) are significantly different to the *R. solani* control (positive effect), using an unrestricted LSD ($P$<0.05).
[d] Indicates treatment means (per plant) that are significantly different to the *R. solani* control (negative effect), using an unrestricted LSD ($P$<0.05).

the number of stolons by 45%. *Trichoderma barbatum* LU1489 increased ($P$<0.05) total tuber weight 265%, and *T. rossicum* LU1492 by 79%. *Trichoderma* sp. 792 LU1483 (undescribed species) increased ($P$<0.05) average tuber weight, by 129%, and *T. crassum* LU555 by 45%. *Trichoderma* sp. 792 has been recorded only in New Zealand (unpublished data).

*Trichoderma harzianum* LU1491, *T. barbatum* LU1489 and *Trichoderma* sp. 792 LU1483 gave the greatest proportional increases for each of three plant growth parameters (number of tubers, total tuber weight, and average tuber weight, respectively) compared with the control, and so were considered to have the greatest potential to promote potato plant growth.

### *Trichoderma* strain combination trial (PT8)

Results for ten *Trichoderma* strain combinations (of 63) which significantly reduced *Rhizoctonia* disease expression or increased potato plant growth parameters are reported in Table 5. The means for all 63 treatments ranged from: nipped shoots 0–50.9%, number of stolons 2.3–9.5, diseased stolons 17.50–83.30%, healthy stolon tips 2.25–8.75, total tuber weight 0.05–4.13 g, and average tuber weight 0.07–0.81 g ($\log_{10}$ back-transformed). Compared with the *R. solani* control, the fungicide control (pencycuron) decreased ($P$<0.05) the percentage of diseased stolons by 89%, and increased ($P$<0.05) the number of healthy stolon tips by 108%, the total tuber weight by 769% and the average tuber weight by 145% (Table 5).

Eight of the ten *Trichoderma* combinations reduced ($P$<0.05) three or more disease parameters and/or increased three or more plant growth parameters ($P$<0.05), compared with the *R. solani* control. Combination 'A' (*T. virens* LU549, *T. atroviride* LU144, *T. harzianum* 1489, *T. harzianum* 1491) reduced the percentage of diseased stolons by 58% and combination 'C' (*T. virens* LU549, *T. atroviride* LU144, *T. harzianum* 1489) reduced this parameter by 69%, compared with the *R. solani* control (statistically equivalent to the reduction achieved with pencycuron, Table 5). Moreover, combination 'A' reduced the percentage of nipped stolon tips by 73%, and combination 'C' reduced the percentage of nipped stolon tips by 78%, and reduced the disease score by 67%.

**Table 4.** *Trichoderma* strains which increased one or more mean potato plant growth parameter for plants grown from tissue-cultured plantlets in a series of greenhouse assays (PT3 to PT7).

| Pot trial | Treatment | No. of replicates | No. of stolons | No. of tubers [a] | Tuber weight (g) [a] | Average tuber weight (g)[b] | Shoot dry weight (g) | Root dry weight (g) |
|---|---|---|---|---|---|---|---|---|
| PT3 | Control | 20 | 2.4 | 0.8 (1.3) | 0.29 (0.20) | -1.44 (0.04) | 0.80 | 0.10 |
| | *T. barbatum* LU1489 | 10 | 3.3 | 1.1 (1.6) | 0.62[c] (0.73) | -1.18 (0.07) | 0.77 | 0.15 |
| | *T. harzianum* LU1522 | 10 | 5.3[c] | 1.4 (3.1) | 0.41 (0.25) | -1.28 (0.05) | 0.77 | 0.13 |
| | LSD (5%)[10 vs 10 reps] | | 2.4 | 0.7 | 0.38 | 0.61 | 0.34 | 0.06 |
| | LSD (5%)[20 vs 10 reps] | | 2.1 | 0.6 | 0.33 | 0.53 | 0.30 | 0.05 |
| PT4 | Control | 10 | 6.8 | 1.8 | 0.47 (0.44) | -0.86 (0.14) | 1.61 | 0.15 |
| | *Trichoderma* sp. *792* LU1483 | 10 | 5.1 | 1.0 | 0.37 (0.37) | -0.50[c] (0.32) | 1.41 | 0.11 |
| | LSD (5%)[10 vs 10 reps] | 10 | 2.3 | 1.5 | 0.43 | 0.36 | 0.40 | 0.05 |
| PT5 | Control | 50 | 2.2 | 2.0 | 3.39 | 1.74 | 1.20 | 0.20 |
| | *T. harzianum* LU1491 | 10 | 3.2[c] | 3.5[c] | 4.41 | 1.42 | 1.32 | 0.22 |
| | *T. rossicum* LU1492 | 10 | 2.2 | 3.2[c] | 6.08[c] | 2.17 | 1.32 | 0.20 |
| | *T. harzianum* LU1493 | 10 | 2.9 | 2.7 | 5.05 | 1.94 | 1.40 | 0.27[c] |
| | *T. harzianum* LU1495 | 10 | 3.4[c] | 2.9 | 3.28 | 1.13 | 1.63[c] | 0.26 |
| | LSD (5%)[10 vs. 10 reps] | | 1.2 | 1.5 | 2.61 | 1.48 | 0.37 | 0.09 |
| | LSD (5%)[50 vs. 10 reps] | | 0.9 | 1.2 | 2.02 | 1.15 | 0.29 | 0.07 |
| PT6 | Control | 40 | 3.4 | 3.7 | 8.87 | 3.09 | 1.13 | 0.22 |
| | *T. atroviride* LU132 | 10 | 3.7 | 5.3[c] | 6.58 | 1.64 | 1.33 | 0.26 |
| | *T. harzianum* LU1522 | 10 | 2.9 | 2.5 | 7.42 | 3.16 | 1.04 | 0.18 |
| | LSD (5%)[10 vs 10 reps] | | 1.2 | 1.8 | 3.16 | 1.90 | 0.35 | 0.08 |
| | LSD (5%)[40 vs 10 reps] | | 1.0 | 1.4 | 2.50 | 1.50 | 0.28 | 0.06 |
| PT7 | Control | 50 | 7.1 | 5.4 | 17.37 | 3.68 | 4.17 | 0.65 |
| | *T. crassum* LU555 | 10 | 7.4 | 5.3 | 18.46 | 5.32[c] | 4.04 | 0.63 |
| | LSD (5%)[10 vs 10 reps] | | 2.3 | 1.9 | 4.93 | 1.90 | 0.50 | 0.10 |
| | LSD (5%)[50 vs 10 reps] | | 1.8 | 1.5 | 3.82 | 1.47 | 0.39 | 0.08 |

[a] Square root transformed means (per plant) are presented with raw means in parentheses.
[b] $Log_{10}$ transformed means (per plant) are presented with back-transformed means in parentheses.
[c] Treatment means (per plant) that are significantly different to the *R. solani* control, using an unrestricted LSD ($P<0.05$).

Combination 'B' (*T. atroviride* LU144, *T. harzianum* 1489, *T. harzianum* 1491) and combination 'D' (*T. barbatum* LU1482, *T. virens* 549, *T. atroviride* LU144, *T. harzianum* 1489) increased three or more growth parameters ($P<0.05$) compared with the *R. solani* control. In particular, combination 'B' increased total tu-

**Table 5.** Treatment means of ten of the 63 *Trichoderma* treatments tested for suppression of *Rhizoctonia* canker symptoms and enhanced potato plant yield parameters in a greenhouse trial (PT8).

| Treatment | Combination | No. of Replicates | Shoots Nipped (%) | No. of Stolons | Diseased Stolons (%) | Healthy Stolon Tips (Number) | Total Tuber Weight (g) | Average Tuber Weight (g)[a] |
|---|---|---|---|---|---|---|---|---|
| *R. solani* control | | 16 | 4.2 | 5.1 | 56.58 | 3.87 | 0.92 | -0.71 (0.19) |
| Pencycuron | | 16 | 0.0 | 6.3 | 6.52[b] | 8.06[b] | 8.00[b] | 0.32[b] (2.10) |
| Negative control | | 16 | 0.0 | 5.9 | 7.80[b] | 7.25[b] | 6.17[b] | 0.15[b] (1.41) |
| LU549 + 144 + 1489 + 1491 | A | 4 | 35.4[c] | 4.5 | 23.96[b] | 4.50 | 1.21 | -0.48 (0.33) |
| LU144 + 1489 + 1491 | B | 4 | 0.0 | 8.0 | 67.10 | 6.50 | 4.13[b] | -0.34 (0.45) |
| LU549 + 144 + 1489 | C | 4 | 16.7 | 5.8 | 17.50[b] | 5.50 | 0.66 | -0.85 (0.14) |
| LU1482 + 549 + 144 + 1489 | D | 4 | 0.0 | 7.8 | 56.11 | 7.75[b] | 3.23[b] | -0.23 (0.58) |
| LU1482 + 144 + 1483 + 1489 | E | 4 | 8.3 | 9.0[b] | 40.71 | 7.75[b] | 1.34 | -0.72 (0.19) |
| LU1482 + 144 + 1491 | F | 4 | 0.0 | 8.3[b] | 70.94 | 7.25[b] | 2.44 | -0.49 (0.32) |
| LU549 + 144 + 1483 + 1491 | G | 4 | 8.3 | 5.5 | 61.40 | 5.25 | 3.50[b] | -0.17[b] (0.68) |
| LU1482 + 549 + 144 + 1483 + 1491 | H | 4 | 0.0 | 9.5[b] | 45.57 | 8.75[b] | 2.99 | -0.48 (0.33) |
| LU1482 + 144 + 1489 + 1491 | I | 4 | 16.7 | 9.0[b] | 40.40 | 8.75[b] | 0.81 | -0.84 0.14) |
| LU1482 + 549 + 144 + 1489 + 1491 | J | 4 | 0.0 | 7.8 | 41.36 | 7.25[b] | 3.79[b] | -0.21[b] (0.61) |
| LSD (5%) [4 vs 4 reps] | | | 32.8 | 3.8 | 41.20 | 3.99 | 2.72 | 0.62 |
| LSD (5%) [16 vs 4 reps] | | | 25.9 | 3.0 | 32.57 | 3.15 | 2.15 | 0.49 |

[a] $Log_{10}$ transformed means (per plant) are presented with back-transformed means in parentheses.
[b] Indicates treatment means (per plant) that are significantly different (positive effect) to the *R. solani* control, using an unrestricted LSD ($P<0.05$).
[c] Indicates treatment means (per plant) that are significantly different (negative effect) to the *R. solani* control, using an unrestricted LSD ($P<0.05$).

**Table 6.** Mean disease and plant parameters for potato plants treated with *Trichoderma atroviride* LU144 ($2^6$ factorial analysis of results from PT8).

| Treatment[a] | Treatment presence (+) or absence (-) | Total shoots nipped (%) | Diseased stolons (%) | No. of stolons | Healthy stolon tips (number) | No. of tubers | Total tuber weight (g) |
|---|---|---|---|---|---|---|---|
| *R. solani* control | | 4.2 | 56.6 | 5.1 | 3.9 | 2.6 | 0.9 |
| Negative control | | 0.0 | 7.8 | 5.9 | 7.3 | 3.2 | 6.2 |
| Pencycuron | | 0.0 | 6.5 | 6.3 | 8.1 | 4.0 | 8.0 |
| *T. atroviride* LU144 | + | 10.9 | 48.4 | 7.0 | 6.1 | 4.0 | 1.9 |
| | - | 19.4 | 54.7 | 5.3 | 4.4 | 3.2 | 1.5 |
| *P*-value[b] | | 0.01 | 0.10 | <0.001 | <0.001 | 0.01 | 0.07 |

[a] Experimental and fungicide control means are presented for explanatory purposes.
[b] The *P*-value designates the significance of the difference between the means of groups with (+) or without (-) LU144.

ber weight by 349% and combination 'D' increased this parameter by 251%. Combination 'D' also increased the number of healthy stolon tips by 100%, to an amount equivalent to plants treated with pencycuron. Combination 'B' also increased the number of tubers by 89%, and combination 'D' increased this parameter by 112% and the shoot dry weight by 39% (data not shown).

Individual strain effects were analysed as a $2^6$ factorial. Of the six strains, only *T. atroviride* LU144 had a statistically significant positive effect on any of the parameters measured (Table 6). This strain reduced the percentage of total shoots nipped ($P$=0.01), and increased the number of stolons ($P$<0.001), the number of healthy stolon tips ($P$<0.001) and the number of tubers ($P$=0.01, Table 6). All ten combinations presented in Table 5 include the strain *T. atroviride* LU144. There were no other significant interaction effects.

### Field trial

At the first harvest, 45% of shoots were nipped and 28% of stolons were diseased in the *R. solani* control treatment. The fungicide treatment (pencycuron) reduced ($P$<0.05) the percentage of nipped shoots by 54% and increased ($P$<0.05) plant dry weight by 73% compared with the *R. solani* control (Table 7). No treatments affected the number of diseased stolons, and the *Trichoderma* strain combinations did not significantly reduce disease of any of the assessed variables (Table 7).

At harvest 2, the average black scurf score for all treatments was 1.2%, with no differences between group means (data not shown). Pencycuron increased ($P$<0.05) the weight of oversized tubers by 32% and the total tuber weight by 13% compared with the *R. solani* control (Table 8). Pencycuron also decreased ($P$<0.05) the weight of undersized tubers by 26% compared with the *R. solani* control (Table 8), and increased the number of oversized tubers by 40% (data not shown). Combinations 'A' (*T. virens* LU549, *T. atroviride* LU144, *T. barbatum* LU1489, *T. harzianum* LU1491) and 'C' (*T. virens* LU549, *T. atroviride* LU144, *T. barbatum* LU1489) increased ($P$<0.05) the total tuber weights by 15% and 13% respectively, compared with the *R. solani* control (Table 8). Combination 'C' also increased the number of oversized tubers by 28% (data not shown).

## Discussion

Biological control is an alternative strategy which may provide effective and sustainable control of *R. solani* on potato or other host crops (Larkin and Brewer, 2005). There are no biological products registered for control of *R. solani* on potato in New Zealand. In this study, the *Trichoderma* combination treatments which demonstrated disease suppression under greenhouse conditions also increased total tuber weight in the field.

Individual *Trichoderma* strains provided variable control of *R. solani* diseases in the pot assays. In trials

**Table 7.** Mean disease and potato plant parameters (4 weeks after planting, harvest 1) from a field trial where four *Trichoderma* strain combinations and fungicide were tested for suppression of *Rhizoctonia* diseases.

| Treatment | Combination | No. of replicates | Shoots nipped (%) | Diseased stolons (%) | Plant DW (g) |
|---|---|---|---|---|---|
| *R. solani* control | | 30 | 44.54 | 27.92 | 44.70 |
| Pencycuron | | 10 | 24.05[a] | 25.64 | 77.43[a] |
| LU549 + 144 + 1489 + 1491 | A | 10 | 42.11 | 29.45 | 46.60 |
| LU144 + 1489 + 1491 | B | 10 | 44.57 | 31.22 | 47.40 |
| LU549 + 144 + 1489 | C | 10 | 40.51 | 34.30 | 51.40 |
| LU1482 + 549 + 144 + 1489 | D | 10 | 42.18 | 34.97 | 52.60 |
| LSD (5%)[10 vs 10 reps] | | | 16.85 | 12.61 | 11.60 |
| LSD (5%)[30 vs 10 reps] | | | 13.76 | 10.30 | 9.47 |

[a] Treatment means (per plot) are significantly different (positive effect) to the *R. solani* control, using an unrestricted LSD ($P<0.05$).

**Table 8.** Mean potato yield parameters (at crop maturity, harvest 2) from plots in a field trial where four *Trichoderma* strain combinations and fungicide were tested for suppression of *Rhizoctonia* diseases and plant growth promotion.

| Treatment | Combination | No. of replicates | Undersized tubers (kg) | Table tubers (kg) | Deformed tubers (kg) | Oversized tubers (kg) | Marketable (table + oversized, kg) | Total tuber weight (kg) |
|---|---|---|---|---|---|---|---|---|
| *R. solani* control | | 30 | 0.38 | 4.09 | 1.42 | 4.97 | 9.05 | 10.85 |
| Pencycuron | | 10 | 0.28[a] | 4.14 | 1.51 | 6.58[a] | 10.72[a] | 12.50[a] |
| LU549 + 144 + 1489 + 1491 | A | 10 | 0.35 | 4.44 | 1.69 | 5.78 | 10.21 | 12.25[a] |
| LU144 + 1489 + 1491 | B | 10 | 0.37 | 4.26 | 1.68 | 4.86 | 9.12 | 11.17 |
| LU549 + 144 + 1489 | C | 10 | 0.40 | 4.04 | 1.75 | 6.09 | 10.13 | 12.28[a] |
| LU1482 + 549 + 144 + 1489 | D | 10 | 0.34 | 3.94 | 2.45[b] | 4.06 | 8.00 | 10.78 |
| LSD (5%)[10 vs 10 reps] | | | 0.12 | 1.17 | 1.09 | 1.40 | 1.68 | 1.41 |
| LSD (5%)[30 vs 10 reps] | | | 0.10 | 0.95 | 0.89 | 1.14 | 1.37 | 1.15 |

[a] Indicates treatment means (per plot) that are significantly different (positive effect) to the *R. solani* control, using an unrestricted LSD ($P<0.05$).

[b] Indicates treatment means (per plot) that are significantly different (negative effect) to the R. solani control, using an unrestricted LSD ($P<0.05$).

where minitubers were planted, there was a trend for individual *Trichoderma* strains to increase the number of nipped emerging shoots (a negative effect). In contrast, plants grown from tissue-cultured plantlets (which are not susceptible to shoot nipping at emergence) were more responsive to the *Trichoderma* treatments. All three potato cultivars used were susceptible to *Rhizoctonia* diseases, however the use of three different host cultivars in different experiments may have contributed to variability of biocontrol.

There are many examples of microbial combinations having synergistic disease control effects (Duffy

*et al*., 1996; Brewer and Larkin, 2005; Srivastava *et al*., 2010; Jain *et al*., 2011) and on plant growth promotion (Rudresh *et al*., 2005). Therefore, we tested a hypothesis that combining disease control strains with growth promoting ones could enhance biocontrol. No examples of application of multiple *Trichoderma* spp. for the control of *R. solani* have been reported, despite the well-known collective range of bioactive traits within the *Trichoderma* genus (Benitez *et al*., 2004).

In this work, the *Trichoderma* strain combination treatments provided the greatest disease suppression or increase in plant growth parameters, and all combinations included *T. atroviride* LU144. Strain *T. atroviride* LU144 gave inconsistent disease suppression when applied alone. However, when in a combination with other strains *T. atroviride* LU144 gave, overall, a statistically significant ($P<0.05$) positive effect on potato shoot nipping, number of stolons, number of symptomless stolon tips and number of tubers. These results indicate that *T. atroviride* LU144 should be considered for further evaluation as a biological control agent for suppression of *Rhizoctonia* diseases of potato.

In the field, shoot and stolon disease were severe at harvest 1 and none of the four combinations of *Trichoderma* strains significantly reduced early disease symptoms. In contrast, at crop maturity (harvest 2), these high disease levels did not lead to high black scurf severity on the potato tubers; the average scurf score was 1.2%. This low level of black scurf on the tubers could be explained by environmental conditions or the timing of the harvest at crop maturity. Other studies have also shown that increased stem and stolon damage is not consistently correlated with black scurf or yield of progeny tubers (Simons and Gilligan, 1997).

At crop maturity the total tuber weights resulting from the *Trichoderma* strain combinations 'A' and 'C' were significantly greater than the *R. solani* control treatment, and were equivalent to the fungicide treatment. As an example, *Trichoderma* combination 'A' offers a potential increased financial gain of gross value of $NZ 3500 per hectare [based on average 2012 market value, calculated as total import and export sales value over total tonnage produced in NZ, (Aitken and Hewett, 2012)]. The increase in total tuber weight resulting from combination 'A' is mostly attributed to a 13% increase in marketable tuber weight, which equates to 6 t $ha^{-1}$ more than the *R. solani* control.

As reported previously, three of the *Trichoderma* strains used in combination treatments in the field were selected from the disease suppression pot assays for their ability to reduce incidence and severity of stolon disease. It is therefore possible that treatment of potato tubers with combinations 'A' or 'C' caused yield increases by reducing diseased stolons during the growing season (between 4 weeks after planting and crop maturity). Other studies have reported that treatment with *Trichoderma* was required to reduce disease severity towards the end of the growing season in the field (Wilson *et al*., 2008a). When applied in-furrow alone, or in combination with a fungicide seed tuber treatment, *T. harzianum* increased the proportion of marketable-sized tubers, and decreased the incidence of black scurf on marketable progeny tubers (Wilson *et al*., 2008a). Based on the lack of control of shoot nipping in greenhouse assays and in the field, coupled with repeated demonstration of reductions in stolon disease symptoms in greenhouse assays, we suggest that the *Trichoderma* strains require a period of establishment before they are able to provide disease suppression later in the potato growing season. *Trichoderma* spp. have also been shown to promote plant root development, which is often associated with increased plant yield and biomass (Harman *et al*., 2004). The yield increase measured in the present study may have resulted either from suppression of *Rhizoctonia* diseases (between 4 weeks after planting and crop maturity), or from direct interactions between the *Trichoderma* strains and the potato plants, for example from hormone regulation or nutrient acquisition (Hermosa *et al*., 2012); or possibly from a combination of both these effects. The contribution of both mechanisms needs to be more fully explored to achieve maximum potential from these biocontrol combinations.

In the present study, *Trichoderma* strains were screened using potato plants, for *Rhizoctonia* disease suppression and improved tuber yield. Strain *T. atroviride* LU144 showed potential as a biocontrol agent as part of a combination treatment. Furthermore, this research has demonstrated that two different combinations of *Trichoderma* spp. ('A' and 'C') increased potato plant yield in the field in one growing season. Further research will be conducted to assess the potential of *T. atroviride* LU144 as a commercial product for biological control.

## Acknowledgements

This research was funded by the Ministry of Business, Innovation and Employment, New Zealand, (contract number LINX0804). We thank Mr Robin Oakley (Oakleys Premium Fresh Vegetables, Ltd) for allowing us to use his land for our field trial and also for managing the trial. We also thank Mr David Saville for statistical advice.

## Literature cited

Agrimm Technologies Ltd, 2013. *Tenet® bio-fungicide granule*. http://www.tricho.com/pdfs/Label%20Tenet.pdf

Aitken A.G. and E.W. Hewett, 2012. *Fresh Facts*. http://www.freshfacts.co.nz

Banville G.J., D.E. Carling and B.E. Otrysko, 1996. Rhizoctonia disease on potato. In: *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (B. Sneh, S. JabajiHare, S. Neate and G. Dijst, ed.), Kluwer Academic Publishers, The Netherlands, 321–330.

Beagle-Ristaino J.E. and G.C. Papavizas, 1985. Biological-control of Rhizoctonia stem canker and black scurf of potato. *Phytopathology* 75, 560–564.

Benitez T., A.M. Rincon, M.C. Limon and A.C. Codon, 2004. Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology* 7(4), 249–260.

Bienkowski D., A. Stewart, R.E. Falloon, M. Braithwaite, L.L. Loguercio and E. Hicks, 2010. A disease assay for *Rhizoctonia solani* on potato (*Solanum tuberosum*). *New Zealand Plant Protection Society (Inc.)* 133–137.

Brewer M.T. and R.P. Larkin, 2005. Efficacy of several potential biocontrol organisms against *Rhizoctonia solani* on potato. *Crop Protection* 24, 939–950.

Das S., F.A. Shah, R.C. Butler, R.E. Falloon, A. Stewart, S. Raikar and A.R. Pitman, 2014. Genetic variability and pathogenicity of *Rhizoctonia solani* associated with black scurf of potato in New Zealand. *Plant Pathology* 63, 651–666.

Duffy B.K., A. Simon and D.M. Weller, 1996. Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. *Phytopathology* 86(2), 188–194.

Elad Y., I. Chet and J. Katan, 1980. *Trichoderma harzianum* - biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology* 70(2), 119–121.

Gravel V., H. Antoun and R.J. Tweddell, 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: Possible role of indole acetic acid (IAA). *Soil Biology & Biochemistry* 39, 1968–1977.

Grosch R., K. Scherwinski, J. Lottmann and G. Berg, 2006. Fungal antagonists of the plant pathogen *Rhizoctonia solani*: selection, control efficacy and influence on the indigenous microbial community. *Mycological Research* 110, 1464–1474.

Harman G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito, 2004. *Trichoderma* species - Opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology* 2(1), 43–56.

Hermosa R., A. Viterbo, I. Chet and E. Monte, 2012. Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology* 158, 17–25.

Hide G.A. and G.R. Cayley, 1982. Chemical techniques for control of stem canker and black scurf (*Rhizoctonia solani*) disease of potatoes. *Annals of Applied Biology* 100(1), 105–116.

Hoyos-Carvajal L., S. Orduz and J. Bissett, 2009. Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*. *Biological Control* 51(3), 409–416.

Jain A., S. Singh, B. Kumar Sarma and H. Bahadur Singh, 2011. Microbial consortium-mediated reprogramming of defence network in pea to enhance tolerance against *Sclerotinia sclerotiorum*. *Journal of Applied Microbiology* 112, 537–550.

James W.C., 1973. Assessing severity of plant diseases. Black scurf of potatoes. In: *Canadex. Potato Diseases*, Agriculture Canada, Ottawa, Ontario, 258–630.

Kumar S.S., M.R.K. Rao, R.D. Kumar, P. Sachin and C.S. Prasad, 2013. Biocontrol by plant growth promoting rhizobacteria against black scurf and stem canker disease of potato caused by *Rhizoctonia solani*. *Archives of Phytopathology and Plant Protection* 46(4), 487–502.

Larkin R. and M. Brewer, 2005. Effects of biological amendments on soil microbiology and soilborne potato diseases in different cropping systems. *Phytopathology* 95(6), S56–S56.

Lewis J.A. and G.C. Papavizas, 1987. Reduction of inoculum of *Rhizoctonia solani* in soil by germlings of *Trichoderma hamatum*. *Soil Biology & Biochemistry*, 19(2), 195–201.

Ministry for Primary Industries, 2013. *ACVM register*. https://eatsafe.nzfsa.govt.nz/web/public/acvm-register

Rudresh D.L., M.K. Shivaprakash and R.D. Prasad, 2005. Effect of combined application of *Rhizobium*, phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer arietinum* L.). *Applied Soil Ecology* 28(2), 139–146.

Sherwood R.T., 1970. Physiology of *Rhizoctonia solani*. In: *Rhizoctonia solani, Biology and Pathology.* (J.R. Parmeter Jr., ed.), University California Press, Berkeley, CA, USA, 69–92.

Simons S.A. and C.A. Gilligan, 1997. Relationships between stem canker, stolon canker, black scurf (*Rhizoctonia solani*) and yield of potato (*Solanum tuberosum*) under different agronomic conditions. *Plant Pathology* 46(5), 651–658.

Srivastava R., A. Khalid, U.S. Singh and A.K. Sharma, 2010. Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. *Biological Control* 53 (1), 24–31.

Stockwell V.O., K.B. Johnson, D. Sugar and J.E. Loper, 2011. Mechanistically compatible mixtures of bacterial antagonists improve biological control of fire blight of pear. *Phytopathology* 101(1), 113–123.

Thilagavathi R., D. Saravanakumar, N. Ragupathi and R. Samiyappan, 2007. A combination of biocontrol agents improves the management of dry root rot (*Macrophomina phaseolina*) in greengram. *Phytopathologia Mediterranea* 46, 157–167.

Verma M., S.K. Brar, R.D. Tyagi, R.Y. Surampalli and J.R. Valero, 2007. Antagonistic fungi, *Trichoderma* spp.: Panoply

of biological control. *Biochemical Engineering Journal* 37(1), 1–20.

Weinhold A.R., T. Bowman and D.H. Hall, 1982. Rhizoctonia disease of potato - effect on yield and control by seed tuber treatment. *Plant Disease* 66(9), 815–818.

Whipps J.M. and R.D. Lumsden, 2001. Commercial use of fungi as plant disease biological control agents: status and prospects. In: *Fungal Biocontrol Agents: Progress, Problems and Potential* (C.J. T. Butt, N. Magan ed.), CABI Publishing, Wallingford, UK, 9–22.

Wilson P.S., P.M. Ahvenniemi, M.J. Lehtonen, M. Kukkonen, H. Rita and J.P.T. Valkonen, 2008a. Biological and chemical control and their combined use to control different stages of the Rhizoctonia disease complex on potato through the growing season. *Annals of Applied Biology,* 153(3), 307–320.

Wilson P.S., E.O. Ketola, P.M. Ahvenniemi, M.J. Lehtonen and J.P.T. Valkonen, 2008b. Dynamics of soilborne *Rhizoctonia solani* in the presence of *Trichoderma harzianum*: effects on stem canker, black scurf and progeny tubers of potato. *Plant Pathology* 57(1), 152–161.

Windham M.T., Y. Elad and R. Baker, 1986. A mechanism for increased plant-growth induced by *Trichoderma* spp. *Phytopathology* 76(5), 518–521.

Yao M.K., R.J. Tweddell and H. Desilets, 2002. Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by *Rhizoctonia solani*. *Mycorrhiza* 12(5), 235–242.

*Accepted for publication: June 22, 2014*
*Published online: December 22, 2014*