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Increased bulb yield following seed coating of radish (*Raphanus sativus* L.) with selected isolates of *Trichoderma* species in soil naturally infested with *Rhizoctonia solani*

Jaeseung Lee[1], Diwakar R.W. Kandula[1]*, John G. Hampton[1,2] and Rainer Hofmann[2]

[1]*Bio-Protection Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, New Zealand*
[2]*Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647, New Zealand*

**Corresponding author:* diwakar.kandula@lincoln.ac.nz

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Abstract Red radish (*Raphanus sativus*) is highly susceptible to the soil-borne fungus *Rhizoctonia solani*, which can cause severe crop losses. In a glasshouse experiment, untreated seeds of radish cvs. French Breakfast and Red Round were grown in potting mix where *R. solani* inoculated wheat-bran was added at rates of 0.25, 0.5 and 1.0 g per 100 g potting mix. Seedling emergence was reduced by one third and two thirds respectively by the two higher inoculum rates, and final plant numbers were ca. 20%, 50% and 80% less than in the uninoculated control.

The ability of T*richoderma* spp. to increase radish yields by limiting the damage caused by *R. solani* has long been known but has not been evaluated in New Zealand. Inoculum of each of four *Trichoderma* spp. isolates LU132 (*T. atroviride*), LU785 (*T. hamatum*), LU1437 (*T. harzianum*) and LU1358 (*T. polysporum*) was prepared in sterile wheat-bran and 0.5 g wheat-bran was added per 100 g potting mix. In a second glasshouse experiment, *R. solani* (0.25 g inoculated wheat-bran) was added per 100 g potting mix before untreated seeds of both radish cultivars were sown. Potting mix without either *R. solani* or *Trichoderma* served as the control. Maximum seedling emergence did not differ among the treatments for cv. French Breakfast, but was increased by the presence of either isolate LU132 (*T. atroviride*) or LU1358 (*T. polysporum*) for cv. Red Round. The presence of isolate LU1347 (*T. harzianum*) in the potting mix significantly increased plant survival in both cultivars. Each of the four *Trichoderma* isolates reduced the percentage of diseased plants with isolate LU132 (*T. atroviride*) providing the strongest response.

In a third glasshouse experiment, *Trichoderma* treated seeds, thiram fungicide treated and untreated seeds of both radish cultivars were sown in naturally *R. solani* infected soil. The same treatments were used in a field trial at a site known to be infected by *R. solani*. In the third glasshouse experiment, seed treatment with *Trichoderma* isolates LU1347 (*T. harzianum*), LU1358 (*T. polysporum*) and LU785 (*T. hamatum*) significantly increased bulb fresh weight in cv. Red Round, but no treatments increased bulb fresh weight in cv. French Breakfast. In the field experiment, bulb yield for the thiram seed treatment did not differ from that of the untreated control. However, seed treatment with isolate LU785 (*T. hamatum*) increased subsequent bulb yield by 96% for both cultivars, and seed treatment with isolate LU132 (*T. atroviride*) or isolate LU1358 (*T. polysporum*) also significantly increased bulb yield (by 85% and 60% respectively) in cv. French Breakfast. A possible explanation for this result was sought by undertaking a fourth glasshouse experiment for radish cv. Red Round only. In this experiment, all four *Trichoderma* spp. isolates more than doubled bulb yield by producing not only a greater number of bulbs but also larger bulbs than the untreated control. *Trichoderma* seed coating may provide an alternative to fungicide seed treatment for radish production.

Keywords radish, *Trichoderma* seed coating, *Rhizoctonia solani*, bulb yield

INTRODUCTION

Radish (*Raphanus sativus* L.), a member of the Brassicaceae family, is an annual broadleaf root vegetable crop grown in both temperate and tropical regions of the world (George 2009). Many types are cultivated but red radish, which originated in Europe (Kole 2007), is popular in New Zealand and fifteen different cultivars are currently available (Lee 2018). Of these, Red Round and French Breakfast are popular because of their crisp, crunchy texture and their flavour, the former being mild and the latter peppery (Hallinan 2015). Vegetable radish is grown year-round in New Zealand, either in glasshouse production or outdoors. Radishes are normally harvested 40-50 days after sowing (Egmont Seeds 2017).

The soil-borne pathogen *Rhizoctonia solani* Kühn is common in New Zealand (Sneh et al. 2004) and can cause establishment problems in brassica crops including radish (Rimmer et al. 2007; Sherf & MacNab 1986). Symptoms include root girdling and vascular discolouration, brown-red rot on twisted (wire) stems, and seedling damping-off (Rimmer et al. 2007). Control involves treating seeds with products that contain the fungicides thiram, iprodione and/or metalaxyl-M (J. McKay pers. comm. 2019), although the effectiveness of these products in reducing the impact of *R. solani* on radish in New Zealand has not been reported. Biological control offers a potential alternative to chemical control. The ability of *Trichoderma* spp. (*T. hamatum* and *T. harzianum*) to reduce the damage to radish caused by *Rhizoctonia solani* has long been known (Harman et al. 1980; Henis et al. 1978; Mihuta-Grimm & Rowe 1986) but has not been evaluated in New Zealand. Also, Askew & Laing (1994) found that either *T. polysporum, T. hamatum* or *T. harzianum* treatment of cucumber seeds provided effective control of *R. solani* in South African studies. In New Zealand, Kandula et al. (2015) reported than an isolate of *T. atroviride* provided the strongest activity against *R. solani* attacking perennial ryegrass (*Lolium perenne* L.).

The aims of the experiments in this study were to test the hypotheses that: (a) In the absence of seed treatment, *R. solani* will reduce radish production by negatively affecting seedling emergence and growth; and (b) treatment of radish seeds with *Trichoderma* spp. will promote subsequent plant growth and provide control of *R. solani*.

MATERIALS AND METHODS

Radish seed lots

Seeds of two red radish cultivars, Red Round (F_1 hybrid) and French Breakfast (open pollinated) were used in this study. The former was supplied by South Pacific Seeds (NZ) Ltd and the latter purchased from Egmont Seeds Ltd. Cv. Red Round had a germination of 86% while cv. French Breakfast had a germination of 91% when a standard laboratory germination test method was used (Lee 2018).

Trichoderma isolates

Screening

Lee (2018) initially screened 21 isolates from eight *Trichoderma* species from the Bio-Protection Research Centre's "Trichobank" for their ability to provide biocontrol of *R. solani* using the methods described by Kandula et al. (2015). From these, four isolates, LU132 (*T. atroviride*), LU1347 (*T. harzianum*), LU1358 (*T. polysporum*) and LU785 (*T. hamatum*) were selected for validation in three glasshouse experiments and a field experiment. These four Trichoderma species varied in their ability to produce conidia, and root colonisation was observed as a dominant factor at a given concentration rather than concentration of spores added to the seed/soil.

Trichoderma isolates were grown in sterile wheat bran for three weeks using the method described by Kandula et al. (2015).

For Glasshouse Experiment 2, this medium was used for inoculating pots.

Seed coating

In the third and fourth glasshouse experiments and the field experiment, spores were harvested by washing using sterile water, filtering through a double layer of Myra cloth (pore size 22-25 µm; Merck KGaA, Germany), pouring into a 50 mL Falcon® tube and centrifuging at 3500 rpm, at 21°C for 10 min before pouring off the water from the tube. Sterile water (0.5 mL) was then added to each tube which was then vortexed for 10 min, following which 20 g seed of each cultivar (approximately 2000 seeds) was mixed into each *Trichoderma* isolate tube, which was then vortexed for 1 min. Then 0.28 µL polymer (polyselect '100' series, Germains Seed Technology, UK) was added to each tube which was again vortexed for 1 min. *Trichoderma* spore concentration after seed coating was measured by placing 10 coated seeds in 10 mL sterile water, mixing in a flask shaker and preparing a dilution of 10^{-2} before counting spores using a Neuman-Boyer haemocytometer. For cv. French Breakfast, *Trichoderma* spore concentrations ranged between 2.0×10^6 and 4.5×10^6 while for cv. Red Round they ranged between 1.1×10^6 and 4.5×10^6 (Lee 2018).

All the treated seeds were air dried in a laminar flow cabinet for 2 h, then placed in a sterile lidded container and stored at 4°C until sowing.

Seed coating with thiram

Separately, 0.3 g thiram granules (Thiram Fungus Control D.F. Granules-400g/L thiram, Kiwicare Corporation Ltd. Christchurch, New Zealand) was mixed with sterile water in a Falcon® tube which was vortexed for 20 sec before 20 g seed was added to the tube and vortexed for 1 min. Finally 0.28 µL polymer was added and vortexed for 1 min.

All the treated seeds were air dried in a laminar flow cabinet for 2 h, then placed in a sterile lidded container and stored at 4°C until sowing.

Glasshouse Experiment 1

A colony of *R. solani* (strain RS 73/LU8003) from the Lincoln University microbial culture collection was used to prepare a wheat-bran based inoculum using the method of Kandula et al. (2015). Growth media (composted bark 400 L, pumice 100 L, Osmocote 1,500 g [16N:35P:10k], and horticultural lime 500 g) was obtained from the Lincoln University Nursery and 3.5 kg placed into each of 48 sterilised 41.5 cm

x 30 cm x 6.5 cm trays. Four *R. solani* inoculum rates were then prepared: nil, 0.25 g, 0.5 g and 1.0 g inoculated wheat-bran per 100 g growth medium. A further 1 kg of each of these four inoculated mixes was each added to twelve trays (a total of 48 trays).

In March 2017, untreated seeds of radish cv. French Breakfast (24 trays) and Red Round (24 trays) were sown by hand at a depth of 1.2 cm in rows spaced 3-4 cm apart and with approximately 2.3 cm between seeds in the row. Thirty seeds were sown per tray. The sown trays were placed in a glasshouse maintained at an average temperature of 17±2°C at Lincoln University and watered as required. For each cultivar there were therefore four *R. solani* inoculum rate treatments (Table 1) each with six replicates arranged in a randomised block design.

Seedling emergence was recorded daily until no further increase was recorded at nine days after sowing (DAS). At 33DAS, the number of plants in each tray was recorded and each examined for visible symptoms of *R. solani* infection, primarily wire stem and leaf lesions (Rimmer et al., 2007). These results were expressed as the percentage of diseased plants. Each plant was then carefully removed from the tray and washed to remove growth medium residue from the roots. Fresh and dry weights were then recorded using a Mettler Toledo PB 3003-5 delta range balance, the latter after oven drying plants at 65°C for 3 days.

Glasshouse Experiment 2

Eighty-four 1.5 L sterilised pots were each filled with 1.25 L of the growth medium described for Glasshouse Experiment 1. For all pots except the nil control, *R. solani* inoculated wheat-bran (0.25 g per 100 g growth medium) was added. This inoculum rate was chosen based on the results from Glasshouse Experiment 1 as Kandula et al. (2015) suggested using an inoculum rate that would result in approximately 30-50% of plants with *R. solani* symptoms. Each of the four *Trichoderma* isolates was added to 14 pots at a rate of 0.5 g inoculated wheat-bran per 100 g growth medium. The treatments were therefore a nil control, a *R. solani* only control, and each of the four Trichoderma isolates plus *R. solani* (Table 2).

Seeds of the two radish cultivars were sown by hand at 10 seeds per pot in May 2017. For each treatment there were seven replicates arranged in a randomised block design. The experiment was conducted in the same glasshouse as described for Glasshouse Experiment 1. Average temperature was 16±2°C.

Seedling emergence was recorded daily until no further increase was recorded at 10DAS. At 56DAS, the number of plants per pot was recorded, the number subtracted from the maximum seedling emergence, and expressed as percentage plant survival. The number of diseased plants was also recorded and results also expressed as a percentage.

Glasshouse Experiment 3

This experiment was conducted in a glasshouse maintained at an average temperature of 17±2°C at Lincoln University, in August 2017. Field soil naturally infested with *R. solani* was mixed with pumice in a 4:1 ratio (Kandula et al. 2015), sieved to 2 mm and used as the growth medium. One and a half litre pots were filled with 1.25 L of the growth medium. A total of 48 pots was prepared for each radish cultivar – the four *Trichoderma* isolate coated seed treatments, the thiram-treated seeds and an untreated control, each with eight replicates.

For each treatment and replicate, 10 seeds spaced 3–3.5 cm apart were placed into each pot and covered with growing medium to a depth of 1 cm. Pots were arranged in a randomised block design and watered as required. Seedling emergence was counted every day for ten days. At 20 DAS, leaf chlorophyll was measured on ten randomly chosen mature leaves (> 30 mm x 30 mm) per pot using a single-photon avalanche diode (SPAD) chlorophyll meter (Model 502 plus, Konica Minolta Inc., Japan). At 33 DAS, all the plants from each pot were carefully extracted from the growing medium and washed to remove soil residue from the roots. The number of plants per pot and number of leaves per plant were counted, and leaf area measured using a LI-3100C area meter (LI-COR Biosciences USA). The number of diseased plants was also recorded. Plants were then divided into shoots and bulbs and fresh weight of each recorded.

Field Experiment

Following Glasshouse Experiment 3, a field experiment was conducted using the same two radish cultivars at the Lincoln University Research Farm to test the initial findings on a larger scale. The soil was a Templeton silt-loam and the previous crop was pasture. The soil at this site is naturally infested with *R. solani* (Kandula et al. 2015). A seed bed was prepared using conventional methods and seeds were sown on 4 October 2017 at a sowing rate of 15 kg/ha using a seed cone drill (Hans-Ulrich Hege 901) at a depth of 2 cm in rows spaced 15 cm apart. The six seed treatments were as described for Glasshouse Experiment 3. Plot size was 1 x 3 m. The trial design was a randomised block with eight blocks and 12 treatments (6 seed treatments x 2 cultivars) per block. Plots were irrigated as required using a reel-gun spray which delivered 10 mm water per application. Slug bait pellets (Westminster brand) containing 1.5 g/kg metaldehyde were applied at 3 kg/ha to all plots at 13 DAS and the insecticide Attack (Nufarm, Australia; 1 L pirimiphos-methyl and permethrin in 700 L water/ha) applied at 34 DAS to control leaf miner (*Scaptomyza flava*).

Seedling emergence was recorded by counting all seedlings in a 30 x 60 cm quadrat each day to 12 DAS, and again at 43 DAS to determine post-emergence losses. All the plants were extracted from two 1-m lengths of row using a spade and then washed. The number of plants with visible *R. solani* symptoms (primarily wire stem and leaf lesions), and fresh bulb weight were recorded.

Glasshouse Experiment 4

As a result of the field trial, a further glasshouse experiment was conducted in August 2021 using a method similar to that described for Glasshouse Experiment 3. The differences were that: only one cultivar, Red Round (new seed-lot with 98% germination) was used; pots were 4 litres filled with 3.5 L growth medium; and there were six replicates of each treatment. Seedling emergence was recorded 10 DAS, and

at 43 DAS, all the plants from each pot were counted before being carefully extracted from the growing medium. The tops were cut off and discarded, while the bulbs were washed to remove soil, blotted with a paper towel to remove water, and fresh weight was recorded. Bulbs were then sorted into four categories: large (>30 mm); medium (<30 - >20mm); small (<20 mm); or no bulb, and data were expressed as a percentage of the total bulb weight for each category.

Statistical analysis

Data were analysed using randomised block analysis of variance (ANOVA). The least significant difference (LSD) test at P<0.05 was used to further investigate the difference of mean values among the treatments. All data analyses were carried out using Genstat software (18th edition, VSN International, Hemel Hempstead, UK).

RESULTS

Glasshouse Experiment 1

In the absence of *R. solani*, maximum mean seedling emergence was 28/30 for cv. French Breakfast and 26/30 for cv. Red Round (Table 1). Emergence for the 0.25 g/100 g wheat bran *R. solani* inoculum rate did not differ from that of the control for either cultivar, but the two higher inoculum rates significantly decreased seedling emergence. By 33DAS, some post-emergence plant death had occurred in all the four treatments but more plants died in trays inoculated with *R. solani* at all three rates than the control (Table 1). All the *R. solani* treatments resulted in diseased plants, with the percentage increasing as inoculum rate increased. On a per tray basis, the two higher *R. solani* inoculum rates significantly reduced plant fresh and dry weight (Table 1), but there were no significant differences in fresh or dry weight per plant (data not presented).

Glasshouse Experiment 2

For both radish cultivars, maximum seedling emergence was not reduced when grown in *R. solani* inoculated soil. In cv. French Breakfast, emergence did not differ among the treatments, but for cv. Red Round, coating seed with either LU132 (*T. atroviride*) or LU1358 (*T. polysporum*) increased seedling emergence over that of the *R. solani* control (Table 2). At 56 DAS, plant survival for cv. French Breakfast did not differ among the treatments except for LU1347 (*T. harzianum*) for which there was a significant increase (Table 2). For cv. Red Round, plant survival in the nil control and LU1347 (*T. harzianum*) treatments was significantly greater than that of the *R. solani* control. For both cultivars, the nil control had no diseased plants and all the *Trichoderma* treatments had a significantly lower percentage of diseased plants than the *R. solani* control (Table 2).

Glasshouse Experiment 3

Seedling emergence did not further increase after eight DAS for either radish cultivar (data not presented). At 8 DAS, seed treatment with LU132 (*T. atroviride*), LU1347 (*T. harzianum*) and LU1358 (*T. polysporum*) had significantly (P<0.05) increased the number of seedlings that emerged for cv. French Breakfast but not for cv. Red Round (Table 3). Some post-emergence plant death had occurred by 33 DAS, and the incidence of post-emergence plant death was significantly lower in cv. French Breakfast seed treated with either LU132 (*T. atroviride*) or LU1358 (*T. polysporum*) than for the untreated control. There was no significant difference in the number of plants at 33 DAS between thiram-treated seed and untreated seed. For cv. Red Round, post-emergence plant death was lower for seed treated with either LU132 (*T. atroviride*), LU 1347 (*T. harzianum*) or thiram than the untreated control (Table 3) and these treatments had between 15 and 24% more plants than the control.

Table 1 Effect of *Rhizoctonia solani* inoculum rate on radish seedling emergence, final plant number and plant weight (Glasshouse Experiment 1).

Cultivar	*Rhizoctonia solani* inoculum[1]	Maximum seedling emergence at 9 DAS[2]	Final plant number at 33 DAS[2]	Diseased plants (%)	Plant weight (g/tray)	
					Fresh	Dry
French Breakfast	0.00	28	27	0	170	13.8
	0.25	25	21*	23	143	12.9
	0.50	18*	13*	34	73*	5.0*
	1.00	10*	3*	87	18*	0.9*
Red Round	0.00	26	25	0	242	14.9
	0.25	22	21*	31	162	10.4
	0.50	18*	11*	60	51*	26*
	1.00	9*	5*	88	9*	0.5*
LSD P<0.05		4.6	3.6	-	56.4	4.74

[1] grams of inoculated wheat-bran per 100 g potting mix; [2]days after sowing (DAS);
* significantly different values at P<0.05

Table 2 Effect of *Trichoderma* isolates on radish seedling emergence, plant survival and plants with symptoms of *Rhizoctonia solani* infection when grown in *Rhizoctonia solani* inoculated potting mix (Glasshouse Experiment 2).

Cultivar	Treatment	Maximum seedling emergence at 10 DAS[1]	Plant survival at 56 DAS[1] (%)	Diseased plants (%)
French Breakfast	Nil control	9.3	84	0
	Rhizoctonia solani control[2]	9.1	78	44
	LU132 (*T. atroviride)*	9.5	86	10*
	LU1347 (*T. harzianum*)	8.8	99*	20*
	LU1358 (*T. polysporum*)	9.4	88	14*
	LU785 (*T. hamatum*)	9.2	87	19*
LSD P<0.05		0.78	10.5	4.2
Red Round	Nil control	8.7	92*	0
	Rhizoctonia solani control[2]	8.5	79	31
	LU132 (*T. atroviride)*	9.5*	86	2*
	LU1347 (*T. harzianum*)	8.8	91*	12*
	LU1358 (*T. polysporum*)	9.5*	86	8*
	LU785 (*T. hamatum*)	9.4	84	22*
LSD P<0.05		0.82	9.2	4.7

[1] DAS - days after sowing [2]0.25 g inoculated wheat bran per 100 g potting mix;
* significantly different from the *Rhizoctonia solani* control at P<0.05.

For both cultivars, all the seed treatments increased the leaf chlorophyll content and shoot fresh weight (Table 3). In cv. French Breakfast, all seed treatments except LU132 (*T. atroviride*) increased leaf number and all but LU132 (*T. atroviride*) or LU1358 (*T. polysporum*) increased leaf area, but none of the treatments increased bulb fresh weight. For cv. Red Round, all the treatments increased leaf number and area, and LU1347 (*T. harzianum*), LU1358 (*T. polysporum*) and LU785 (*T. hamatum*) increased (P<0.05) bulb fresh weight (Table 3).

Table 3 Effect of radish seed treatment on plant performance when sown in soil naturally infested with *Rhizoctonia solani* (Glasshouse Experiment 3).

Seed treatment	Seedlings emerged at 8 DAS[1] (10 seeds sown)	Leaf chlorophyll content at 20 DAS[1] (SPAD value)	No. of plants/ pot at 33 DAS[1]	Leaves per plant at 33 DAS[1]		Fresh weight per plant at 33 DAS[1] (g)	
				No.	Area (cm^2)	Shoot	Bulb
cv. French Breakfast							
Untreated seed	8.7	22.9	7.79	3.06	36.3	1.67	1.38
LU132 (*T. atroviride*)	9.6*	27.6*	9.43*	3.63	46.0	2.89*	2.04
LU1347 (*T. harzianum*)	9.1*	28.6*	8.57	4.02*	67.0*	3.70*	2.08
LU1358 (*T. polysporum)*	9.0*	25.9*	9.00*	3.94*	56.6	3.36*	1.29
LU785 (*T. hamatum*)	8.6	28.6*	8.57	4.01*	65.5*	3.79*	2.08
Thiram	8.7	29.6*	8.57	4.30*	73.6*	4.17*	2.16
LSD P<0.05	0.14	2.9	1.12	0.49	24.6	1.07	1.13
cv. Red Round							
Untreated seed	8.0	23.8	7.29	3.59	29.3	1.71	2.23
LU132 (*T. atroviride*)	8.4	30.8*	8.43*	4.60*	59.2*	3.43*	3.28
LU1347 (*T. harzianum*)	9.0	32.8*	9.00*	4.50*	61.8*	3.65*	4.01*
LU1358 (*T. polysporum*)	8.4	32.4*	8.29	4.98*	67.3*	3.77*	4.62*
LU785 (*T. hamatum*)	7.1	32.7*	7.00	4.88*	66.1*	3.64*	5.25*
Thiram	8.6	31.1*	8.71*	4.36*	61.2*	3.51*	3.77*
LSD P<0.05	1.05	4.3	1.1	0.66	18.28	0.99	1.35

[1] days after sowing (DAS); *significantly different values at P<0.05.

At 33 DAS, the number of *R. solani* infected plants averaged 1.1 for cv. French Breakfast and 2.2 for cv. Red Round. None of the treatments differed from the respective controls for infected plants so these data are not presented.

Field experiment

Neither maximum seedling emergence nor post-emergence plant death differed between the untreated seed and any of the seed treatments in either cultivar (Table 4). However, for cv. French Breakfast the percentage of diseased plants at 43 DAS was significantly (P<0.05) lower than the control for all the seed treatments while, for cv. Red Round, seed treatment with either LU1358 (*T. polysporum*) or thiram resulted in fewer diseased plants (Table 4). In this experiment, the bulb yield in control plots for cvs. French Breakfast and Red Round were 389 g/m^2 (3.9 t/ha), and 457 g/m^2 (4.6 t/ha) respectively. Fresh bulb yield was nearly doubled by use of LU785 (*T. hamatum*) as a seed treatment for both cultivars (Table 4). Seed treatment with either LU132 (*T. atroviride*) or LU1358 (*T. polysporum*) also significantly (P<0.05) increased fresh bulb yield for cv. French Breakfast. Although not always significant, the *Trichoderma* treatments produced increases in fresh bulb weight ranging from 29% to 97%, whereas the thiram seed treatment did not increase fresh bulb yield (Table 4).

Glasshouse Experiment 4

No differences in seedling emergence were recorded at 10 DAS (Table 5), but some post-emergence seedling death subsequently occurred because, at 43 DAS, final plant numbers had reduced. Final plant number was significantly higher for seed treated with isolate LU1358 (*T. polysporum*) than all the other treatments (Table 5).

Total bulb weight per pot was significantly increased by all the five seed treatments over that of the untreated seed (Table 5). These increases were between two and three times the untreated seed bulb weight. This was explained by the percentage of bulbs within each of the four size categories; nearly 30% of the plants in the untreated seed control failed to form a bulb (Figure 1), and only 45% of the bulbs that were produced were in the large and medium size categories (Table 5). This compared with over 80% for plants from seed treated with isolate LU132 (*T. atroviride*), LU1347 (*T. harzianum*) or LU785 (*T. hamatum*). Within each bulb-size category, seed treatment with LU1347 (*T. harzianum*), LU1358 (*T. polysporum*) or LU785 (*T. hamatum*) resulted in a significantly greater (P<0.05) percentage of large sized bulbs. Also, seed treatment with LU132 (*T. atroviride*) resulted in a significantly greater (P<0.05) percentage of medium sized bulbs, seed treatment with LU785 (*T. hamatum*) led to a significantly smaller (P<0.05) percentage of small sized bulbs, and seed treatment with LU132 (*T. atroviride*), LU1347 (*T. harzianum*) or LU785 (*T. hamatum*) resulted in a significantly lower (P<0.05) number of plants that failed to form a bulb. Plants from seeds treated with thiram did not differ from the untreated control for the percentage of bulbs in any of the four size categories (Table 5).

DISCUSSION

Radish seedling emergence and growth

The first hypothesis for this study was that, in the absence of seed treatment, the presence of *R. solani* in soil would reduce radish production by negatively affecting seedling emergence and plant growth. This hypothesis was partially

Table 4 Field Experiment with soil naturally infested with *Rhizoctonia solani*. Effect of radish seed treatment on seedling number (12 DAS), post-emergence plant mortality (43 DAS), percentage diseased plants (with symptoms on leaf and/or hypocotyl), and bulb yield.

Treatment	Seedlings/m^2	Post-emergence plant mortality/m^2	Diseased plants (%)	Bulb yield (g/m^2)
cv. French Breakfast				
Untreated seed	98	21	14.7	389
LU132 (*T. atroviride*)	90	12	8.1*	714*
LU1347 (*T. harzianum*)	103	18	8.7*	503
LU1358 (*T. polysporum*)	103	22	8.1*	621*
LU785 (*T. hamatum*)	98	12	7.1*	762*
Thiram	85	11	4.4*	445
LSD P<0.05	25.4	15.2	4.92	162.0
cv. Red Round				
Untreated seed	75	7	11.3	457
LU132 (*T. atroviride*)	87	14	8.8	745
LU1347 (*T. harzianum*)	73	5	6.9	696
LU1358 (*T. polysporum*)	93	5	6.2*	724
LU785 (*T. hamatum*)	80	6	10.0	899*
Thiram	74	3	3.1*	471
LSD P<0.05	20.5	7.8	4.52	326.3

*significantly different values at P<0.05.

Table 5 Effect of seed treatment of radish (cv. Red Round) on plant performance when sown in soil naturally infested with *Rhizoctonia solani* (Glasshouse Experiment 4).

Seed treatment	Seedlings emerged at 10 DAS[1] (10 seeds sown)	Final plant No at 43 DAS[1]	Bulb size at 43 DAS [1] (Percentage in each category)				Total bulb weight at 43 DAS [1] (g/pot)
			Large	Medium	Small	No bulb	
Untreated seed	9.3	8.7	19.8	25.2	26.5	28.5	59.7
LU132 (*T. atroviride*)	9.2	8.2	34.5	45.1*	12.9	7.4*	158.1*
LU1347 (*T. harzianum*)	9.3	9.0	46.4*	36.3	11.0	6.3*	144.9*
LU1358 (*T. polysporum*)	9.8	9.7*	36.1*	37.8	14.1	12.0	143.4*
LU785 (*T. hamatum*)	9.3	8.7	47.5*	41.3	7.5*	3.7*	179.4*
Thiram	9.2	8.2	29.1	39.5	20.2	11.1	92.7*
LSD P<0.05	n/s	0.99	16.07	16.36	16.71	17.59	31.66

[1] days after sowing (DAS); *significantly different values at P<0.05.

supported in the first glasshouse experiment where the addition of *R. solani* inoculum to the growth medium significantly reduced seedling emergence of both cultivars at the two higher inoculum rates used. *R. solani* attack can result in seed death and severe damage to seedling roots pre-emergence such that emergence is prevented (Rimmer et al. 2007). In the second glasshouse experiment, *R. solani* did not reduce seedling emergence, but this was expected as the inoculum rate used (0.25g per 100g growth medium) did not reduce emergence in the first glasshouse experiment. In field soil naturally infested with *R. solani*, radish seedling emergence from untreated seed did not differ from that of *Trichoderma* or thiram treated seeds, with the one exception of cv. French Breakfast in the third glasshouse experiment. This suggests that the inoculum present in the field soil was insufficient to reduce seedling emergence, but this inoculum level was not recorded.

Post-emergence, *R. solani* can cause wire stem (Rimmer et al. 2007) which, when severe, results in seedling death. This situation occurred in both radish cultivars in the first glasshouse experiment, where the post-emergence losses of radish increased significantly as the *R. solani* inoculum rate increased. This also occurred in cv. Red Round but not cv. French Breakfast in the second glasshouse experiment.

Whether *R. solani* infection reduced growth of plants grown from untreated seed could not be proven conclusively. On a per unit area (i.e. per tray) in the first glasshouse experiment, the two highest rates of *R. solani* inoculum significantly reduced plant fresh and dry weight of both radish cultivars, but individual plant fresh and dry weights did not differ. This counter-intuitive result was due to a competition effect; in the untreated seed control, 25-27 plants per tray were competing for space and resources, c.f. only 3-5 plants per tray at the highest *R. solani* inoculum rate so the latter were bigger individual plants. When grown in soil containing *R. solani* in the third glasshouse experiment, plants from both cultivars grown from untreated seed had fewer and smaller leaves and tended to have smaller shoot and bulb fresh weight than those of plants grown from thiram or *Trichoderma* treated seeds, although for the latter, there were some differences amongst the isolates.

***Radish plant growth and control of* R. solani**

The second hypothesis was that treatment of radish seeds would promote subsequent plant growth and provide control of *R. solani.* In the third glasshouse experiment, shoot growth was promoted by all the *Trichoderma* treatments for both cultivars while bulb yield was increased in cv. Red Round only by some of the *Trichoderma* isolates. In the field, bulb yield was significantly increased by three of the *Trichoderma* isolates for cv. French Breakfast, but only by one isolate for cv. Red Round. However in the fourth glasshouse experiment bulb yield of cv. Red Round was almost tripled because nearly all the plants produced a bulb, and the majority of these bulbs were in the medium/large size category unlike the plants grown from untreated seed where nearly 30% of plants failed to form a bulb. *R. solani* is known to reduce radish bulb yield by interfering with nutrient supply from both above- and below-ground plant tissues because of stem damage at the soil surface (Rimmer et al. 2007; Figure 1).

None of the *Trichoderma* treatments provided complete control of *R. solani.* However, for both cultivars in the second glasshouse experiment, these treatments all significantly reduced the percentage of diseased plants, a result also obtained in the field for cv. French Breakfast, but not cv. Red Round where only LU1358 (*T. polysporum*) provided a significant reduction. Whether the bulb-yield response for the *Trichoderma* treatments recorded was a result of either *R. solani* control, or of growth promotion, or a combination of both cannot be conclusively determined from these results. However, the results from the fourth glasshouse experiment strongly suggest that the *Trichoderma* treatments were able to reduce *R. solani* stem damage, allowing the plants to produce more bulbs.

All the four *Trichoderma* species used in this research have been reported previously to provide biocontrol against *R. solani.* Mihuta-Grimm & Rowe (1986) obtained positive results from fluid drilling cultures of *T. hamatum* in field trials but not with *T. hamatum* coated seed. Henis et al. (1978) reported beneficial effects from adding cultures of *T. harzianum* to soil infected with *R. solani.* Askew & Laing (1994) found that cucumber seeds coated with either

Figure 1 Radish bulb size of cv. Red Round from Glasshouse Experiment 4. Bulbs from untreated seed (left) and LU1358 (*Trichoderma polysporum*) treated seed (right) at harvest (43 days after sowing).

T. polysporum, T. hamatum or *T. harzianum* provided effective control of *Rhizoctonia solani* in initial experiments but results from further trials showed inoculum cultured on barley to be more effective than seed coating. Kandula et al. (2015) found that treatment of perennial ryegrass seeds with LU132 (*T. atroviride*) significantly increased seedling emergence in *R. solani* infested soil. Except for the fourth glasshouse experiment, the variability in responses to *Trichoderma* seed treatment among the other four experiments meant that it was not possible to consistently quantify all beneficial effects from the seed treatments. A similar result was also reported by Kandula et al. (2015) for perennial ryegrass. However, Mihuta-Grimm & Rowe (1986) reported significant reductions in the disease index of an unspecified radish cultivar when a culture of *T. hamatum* was applied directly to *R. solani* infested soil. The results obtained in the current study may be due to unreported differences in radish cultivar tolerance of *R. solani*, and/or uneven distribution of *R. solani* in the field soil used.

In Glasshouse Experiment 3 where all of the seed treatments increased shoot fresh weight in both cultivars, they also significantly increased leaf chlorophyll content. Chlorophyll content is a significant factor in plant growth (Kibe et al. 2017). Some *Trichoderma* species produce volatile organic compounds, such as 6-pentyl-2H-pyran-2-one (6 PP), and the auxin indole-3-acetic acid, which are known to promote leaf chlorophyll content (Garnia-Vergera et al. 2016; Nieto-Jacobo et al. 2017). LU132 (*T. atroviride*) produces 6 PP (Nieto-Jacobo et al. 2017), but whether the other three *Trichoderma* strains used also do so is not known. However these results are overall similar to many others reporting plant growth promotion following *Trichoderma* application (e.g. reviews by Harman 2005; Nieto-Jacobo et al. 2017). In the field experiment, *Trichoderma* seed treatments had no impact on radish seedling emergence, unlike the reports of Henis et al. (1978) and Kandula et al. (2015). However, *R. solani* was present in the soil, because at 43DAS, 15% of cv. French Breakfast and 11% of cv. Red Round plants in the control for each cultivar had *R. solani* symptoms, and as previously reported (Henis et al. 1978; Mihuta-Grimm & Rowe 1986), *Trichoderma* seed treatments reduced the percentage of infected plants. With over 80% of the plants in the control plots showing no apparent symptoms of *R. solani* infection, the low bulb yields recorded were unexpected. Radish is normally harvested between 40-50DAS (Egmont Seeds 2017), and in this trial the bulb yields in the control plots for cvs. French Breakfast and Red Round were 389 g/m^2 (3.9 t/ha), and 457 g/m^2 (4.6 t/ha) respectively, less than half the expected yield (Pervez et al. 2003). *Trichoderma* seed treatments resulted in bulb yield increases, with LU785 (*T. hamatum*) doubling yield in both cultivars.

The fourth glasshouse experiment was therefore conducted on cv. Red Round in an attempt to try to explain the bulb yield increases recorded in the field. The results suggest that failure to form a bulb by plants in the untreated control may have been a factor (although this information was not recorded in the field experiment). Also, the treated seeds produced a greater bulb yield because they produced more larger bulbs than the control. Possible reasons for this have already been discussed.

Thiram seed treatment was included in this study because it is registered for control of damping off diseases in New Zealand (Holden 2020). In the field experiment, this treatment significantly reduced the percentage of diseased plants in both cultivars, but had no impact on bulb yield, unlike the *Trichoderma* seed treatments. In the fourth glasshouse experiment, plants grown from thiram-treated seed produced a 75% greater bulb yield than the control, but bulb size in each category did not differ from that of the control. This result suggests that thiram was providing some control of *R. solani*, but also supports the suggestion that the *Trichoderma* treatment bulb yield increases were additionally effective because of plant growth promotion.

CONCLUSIONS

This study supported the hypothesis that, in the absence of seed treatment, soils infested with *R. solani* would reduce radish production. Even though seedling emergence was not reduced when grown in *R. solani* infected soil in a field trial, bulb yield was significantly reduced, most probably because *R. solani* infection prevented bulb formation. However, this explanation requires further investigation.

The second hypothesis that treatment of radish seeds with *Trichoderma* spp. would promote subsequent plant growth and provide control of *R. solani* was also partially supported because *Trichoderma* seed treatments reduced disease incidence (to a level comparable with the chemical fungicide thiram) and significantly increased bulb yield because of increased bulb size. All four isolates of the different *Trichoderma* species increased bulb yield, particularly LU785 (*T. hamatum*) which doubled the yield in the field. This isolate and species, applied as a seed treatment, shows promise for increasing radish crop yields.

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