Trichoderma species for biocontrol of soil-borne plant pathogens of pasture species

D.R.W. Kandula [a*], E.E. Jones[b], A. Stewart[a†], K.L. McLean[a] and J.G. Hampton[a]

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

Abstract Soil-borne plant pathogens such as *Rhizoctonia solani* (Kuhn), *Pythium ultimum* (Trow) and *Sclerotinia trifoliorum* (Eriks) can reduce grass and forage legume establishment. The potential for biocontrol of these pathogens by *Trichoderma* fungi was evaluated. Following dual culture assays, nine *Trichoderma* isolates (five of *T. atroviride* and one each of *T. hamatum*, *T. koningiopsis*, *T. viride* and *T. virens*) were chosen for assessment in pot experiments. In the presence of *R. solani*, perennial ryegrass (*Lolium perenne* L.) emergence was increased by 60-150% by two isolates of *T. atroviride* and by 35-212% by the isolate of *T. virens*, with the increase depending on growing medium and amount of pathogen inoculum. Red clover (*Trifolium pratense* L.) emergence in the presence of *S. trifoliorum* was significantly increased by two *T. atroviride* isolates and the *T. hamatum* isolate. In the presence of *P. ultimum*, white clover (*Trifolium repens* L.) emergence was increased by 25-42% by one isolate of *T. atroviride* and the *T. hamatum* isolate. However, for all three pasture species, some *Trichoderma* isolates reduced seedling emergence. Seedling growth (shoot and root fresh weight/plant) of the three pasture species was significantly increased by one or more *T. atroviride* isolates. On the basis of these results for both disease reduction and growth promotion, four *T. atroviride* isolates were selected for field assessment as biocontrol agents of soil-borne pathogens of pasture species.

Keywords: *Rhizoctonia solani*; *Pythium ultimum*; *Sclerotinia trifoliorum* . Damping-off; seedling establishment

*Corresponding author. E-mail: diwakar.kandula@lincoln.ac.nz
Present Address:
[†]Marrone Bio Innovations Inc., 2121 Second St, Davis, CA 95618, USA

1. Introduction

Soil-borne plant pathogens can be major biological constraints to pasture establishment (Falloon, 1985; Skipp & Watson, 1996) and production (Falloon & Fletcher, 1983; O'Rourke *et al*., 2009). In New Zealand, soil-borne pathogens commonly-associated with diseases of grass and forage legume seedlings include *Rhizoctonia solani* (Kuhn), *Pythium ultimum* (Trow) (Skipp & Watson, 1996) and *Sclerotinia trifoliorum* Eriks (Skipp & Hampton, 1996). *R. solani* and *P. ultimum* can reduce establishment of grasses and legumes (Skipp & Christensen 1989a,b; Waipara *et al*.,1996; Sarathchandra *et al*., 2000). In perennial ryegrass (*Lolium perenne* L.) these pathogens usually infect the seed or seedling prior to emergence (pre-emergence damping off – Falloon, 1985). In white (*Trifolium repens* L.) and red (*T. pratense* L.) clovers, *S. trifoliorum* initially causes brown leaf lesions before infection spreads to the stems and shoots, eventually killing the plant (Harvey and Harvey 2009). However, data for seedling losses are not readily available, primarily because in the field they have been difficult to define and quantify as damage frequently results from complex interactions among several soil-borne pathogens (Skipp & Watson, 1996).

While pasture seeds can be fungicide treated to combat damping-off (Charlton & Stewart, 2000), the use of fungicides to control sclerotinia rot is not considered to be cost effective (Harvey and Harvey 2009), and while Falloon (1980, 1981) recommended fungicide seed treatment, Latch (1996) considered that it was uneconomic to use fungicides to control pathogens in New Zealand pastures. However, biocontrol may offer an alternative strategy for New Zealand's pastoral farmers, as Skipp and Hay (1993) and Waipara (2000) suggested that the soil-borne fungus *Trichoderma* may have potential for control of damping-off diseases in pasture. Commercial *Trichoderma*-based products are available for biological control of soil-borne diseases in vegetables (Stewart, 2001).

The development of new biocontrol products against plant diseases requires screening of a high number of candidate antagonists which have to fulfil many requirements to be commercially successful (Kohl *et al*., 2011). The objective of this research was to identify New Zealand *Trichoderma* isolates able to improve seedling establishment in the presence of soil-borne pathogens and/or enhance seedling growth of pasture species important to New Zealand, specifically perennial ryegrass and white and red clover.

2. Materials and methods

Screening of *Trichoderma* isolates.

One hundred *Trichoderma* isolates from the Bio-Protection Research Centre's collection were included in dual culture assays (Ghaffar, 1969) with pairings against three pathogens *viz R. solani, P. ultimum* and *S. trifoliorum*. Isolates of these

pathogens were sourced from the Lincoln University microbial culture collection. Nine of these isolates which produced an antagonist 'B' type interaction (growing margins of the two colonies meet, pathogen growth inhibited and/or overgrown by the *Trichoderma* isolate) or 'D' type interaction (pathogen inhibited at a distance leaving a zone of inhibition (Ghaffar, 1969)) were chosen for use in pot experiments. Five of these *Trichoderma* isolates (LU 132, LU 140, LU 584, LU 633, LU 634) were *T. atroviride*, and one each were *T. hamatum* (LU 740), *T. koningiopsis* (LU 713), *T. viride* (LU 644), and *T. virens* (LU 540).

Production and application of *Trichoderma* inoculum

Trichoderma isolates were grown in sterile wheat bran and peat. Seventy-five grams of wheat bran and 25 g of sieved peat were added to each of 18 (two flasks/isolate) 500 ml flasks. The flasks were autoclaved at 121°C and 15 psi for 20 min on two consecutive days. The sterile medium was inoculated with five colonised agar plugs/flask, taken from the actively growing edge of a 5 day-old *Trichoderma* colony. For all the pot experiments described next, 1 g of colonized wheat bran/peat for each *Trichoderma* isolate (approx. 2-3 x 10^8 cfu/g) was added to 400 g of plant growing medium for each pot (see below) just prior to seed sowing. This gave a concentration of 1 x 10^6 cfu/g plant growing medium.

Preparation of pathogen inoculum

Rhizoctonia solani

Fifty grams of wetted wheat bran (2 parts bran: 1 part water) in each of six 250 ml flasks was autoclaved at 121°C and 15 psi for 20 min on two consecutive days. Sterile wheat bran was inoculated with five colonised agar plugs taken from the actively growing edge of a 3-day-old *R. solani* colony grown on PDA in the dark. Each flask was incubated at 20°C in the dark for 12 d.

Sclerotinia trifoliorum

S. trifoliorum was grown on sterile wheat grains. One hundred grams of wheat grain moistened with 240 ml deionised water was added to each of three 500 ml flasks which were then autoclaved at 121°C at 15 psi for 20 min on two consecutive days. Once cool, the sterile wheat grains were inoculated with five colonised agar plugs taken from the actively growing edge of a 4-day-old *S. trifoliorum* culture grown on PDA at 20°C in the dark. The flasks were incubated at 20°C for 14 d in the dark.

Pythium ultimum

P. ultimum was grown on sterile white clover roots. One hundred grams of roots were added to each of two 250 ml flasks. The flasks were autoclaved at 121°C and 15 psi for 20 min. Once cool, the sterile roots in each flask were inoculated with five colonised agar plugs taken from the actively growing edge of a 3 day old culture grown on PDA. Flasks were then incubated at 20°C for 14 d in the dark. The *P.*

ultimum oospore colonised roots were homogenised in a blender by adding an equal volume of deionised water.

Pot experiments

Rhizoctonia solani/perennial ryegrass

The first pot experiment used plant growth~~ing~~ medium (composted pine bark (80%); pumice 1-4 mm grade (20%); 5 kg m^{-3} slow release fertiliser comprising 1 kg m^{-3} horticultural lime, 1 kg m^{-3} hydraflo wetting agent, and 3 kg m^{-3} osmocote extract [16N-35P-10K]), with 400 g of this medium being placed in each pot. A preliminary experiment had determined that 2 g *R. solani* colonised wheat bran added to each pot resulted in approximately 50% of the emerged perennial ryegrass seedlings damping off (Table 1), and this was the inoculum rate used in the subsequent experiments. There were 11 treatments, the nine *Trichoderma* isolates (Table 2~~1~~) challenged by *R. solani*, a pathogen control and an uninoculated control. All treatments except the two controls were replicated four times, while each control treatment was replicated eight times. Forty perennial ryegrass seeds were sown in each pot at a depth of 5-6 mm. Pots were arranged in a completely randomized block design on tables in a glasshouse which was maintained at an average temperature of 20°C for the duration of the experiment. The pots were watered using overhead irrigation as required.

Fifteen days after sowing (DAS) seedling emergence was recorded. For each pot, at least one non-emerged seed or seedling was retrieved, surface sterilised in 1% NaOCl for 2 min followed by rinsing twice in sterile distilled water, and then plated onto PDA. In all cases *R. solani* grew from these non-emerged seeds or seedlings. At 8 weeks after sowing, all plants from each pot were carefully removed, washed to remove potting mix, dried between paper towels and fresh weight of roots and shoots was recorded.

For the second pot experiment, a Wakanui silt loam soil naturally infested with *R. solani* was sourced from Lincoln University. A preliminary experiment using three soil: pumice dilutions (3:1, 2:2, 1:3) showed the 1:3 dilution produced approximately 50% damping off of emerged seedlings, and this rate was used for the experiment. For each pot, 500 g soil (sieved to 2 mm) and pumice mixture were used. Three *Trichoderma* isolates (LU 132, LU 140, LU 540, chosen based on the results in plant growth medium; Table 2~~1~~) were assessed after having been prepared and added to the soil as previously described. There were nine treatments (Table 3~~2~~), as the three *Trichoderma* isolates were challenged in naturally infected soil, and also in the same soil inoculated with *R. solani* at the same rate as for the plant growth medium experiment described above. Each treatment was replicated ten times. Seed sowing, glasshouse environment and experimental design were also as previously described. Seedling emergence was recorded 21 DAS, and 7 weeks after sowing, all plants per pot were cut at ground level and shoot fresh weights were recorded.

Sclerotinia trifoliorum / red clover

The first pot experiment for this pathogen/host system used the plant growth medium mix described previously. Twelve grams of *S. trifoliorum* colonised wheat

grains were thoroughly mixed with 400 g plant growth medium in each of 48 pots. A preliminary experiment had determined that this rate provided approximately 50% of plants with sclerotinia rot in the disease control treatment (Table 1). There were eleven treatments (Table 43); the nine *Trichoderma* isolates challenged by *S. trifoliorum*, a pathogen control and an uninoculated control, all of which were replicated eight times. Forty red clover seeds were sown in each pot at a depth of 5-6 mm. Treatment randomisation and glasshouse conditions were as described previously for the perennial ryegrass experiments.

Twenty one DAS, seedling emergence was recorded and 7 weeks after sowing shoot weights and disease scores (where 1 = no symptoms evident, to 5 = seedling severely diseased) were recorded for each pot.

The second pot experiment used a Wakanui silt loam soil naturally infested with *S. trifoliorum* sourced from Lincoln University. A 3:1 soil-pumice dilution was used. There were two sets of treatments (Table 54) involving four *Trichoderma* isolates (LU 140, LU 634, LU 740, LU 644) chosen based on the results in plant growth medium (Table 43). The four isolates were challenged in naturally infected soil and also in field soil inoculated with *S. trifoliorum* at the same rate as for the plant growth medium experiment (described above). Each treatment was replicated eight times. Seed sowing, glasshouse environment and experimental design were also as previously described. Seedling emergence was recorded 21 DAS, and shoot weight and disease score recorded at 7 weeks after sowing.

Pythium ultimum / white clover

The first experiment used 400 g plant growth medium/pot to which 0.8 ml inoculum was added and mixed thoroughly. This level was based on a preliminary experiment which determined that this rate of inoculum resulted in approximately 50% diseased seedlings (Table 1). The 11 treatments were the same as for the red clover experiment, except that the pathogen challenge was *P. ultimum* (Table 65). Each treatment was replicated ten times. Seed sowing, glasshouse environment, experimental design and assessments were as described for red clover (above).

Growth promotion by *Trichoderma*

For perennial ryegrass, red clover and white clover, the nine *Trichoderma* isolates were added to pathogen-free plant growth medium (Table 76) as previously described. The inoculum rate, seed sowing, glasshouse environment, experimental design and assessments were as described for the pathogen challenge experiments.

A second growth promotion experiment using only perennial ryegrass and three formulations of one *Trichoderma* isolate (LU 584) was also conducted in plant growth medium (Table 87). The three *Trichoderma* treatments were colonised wheat bran plus peat (2.3 x 10^7 cfu/g), a seed coating (1 x 10^7 cfu/g seed) and a proprietary granule supplied by AgResearch Ltd (2.5 x 10^7 cfu/g granule) applied to the plant growth medium at a rate equivalent to 30 kg/ha. Each treatment was replicated ten times. Seed sowing, glasshouse environment and experimental design were as

previously described. Seedling emergence was recorded 15 DAS and shoot weights from each pot determined at 8 weeks.

Statistical analysis

All data were subjected to a one way ANOVA with a probability level of $P<0.05$ used for mean separation using Fisher's unprotected LSD test.

3. Results

Disease Control

Rhizoctonia solani / perennial ryegrass

In the plant growth medium, emergence in the no pathogen control was 86% but the presence of the pathogen reduced emergence to 38% (Table 2~~1~~). Three of the *Trichoderma* isolates (LU 132, LU 140 and LU 540) significantly increased emergence over that of the pathogen control (Table 2~~1~~), but their emergence was lower than from that of the uninoculated control. However emergence for LU 584, LU 633, LU 740 and LU 713 was significantly lower than that of the pathogen control (Table 2~~1~~). Shoot and root fresh weight per pot did not differ between the two controls, but LU 584 and LU 633 produced greater shoot fresh weights than the pathogen control (Table 2~~1~~). Treatment with LU 584 and LU 633 also significantly increased shoot dry weight per pot compared with the control, but only LU 633 increased root weight per plant. LU 740 and LU 713 had lower root fresh weights per pot than the pathogen control. No post-emergence damping off of seedlings in any of the treatments was recorded.

In soil naturally infested by *R. solani*, seedling emergence was significantly lower (50%) than that of the potting mix control or LU140 (both 80%, Table 3~~2~~A). The other two isolates did not significantly increase emergence. When this field soil was inoculated with the pathogen, both LU 140 and LU 540 increased seedling emergence over that of the *R. solani* control (Table 3~~2~~B). Shoot fresh weight per pot was increased by LU 140 and LU 540 incorporated into naturally infested field soil (Table 3~~2~~A), and all three isolates increased shoot fresh weight when the naturally infested soil was inoculated with *R. solani* (Table 3~~2~~B). Shoot fresh weight per plant was not increased by any of the isolates in either soil type (Table 3~~2~~A, 3~~2~~B). All seedlings were healthy in all the treatments.

Sclerotinia trifoliorum / red clover

In plant growth medium, seedling emergence was low for all treatments (range 42-61%) and did not differ between the two controls (Table 4~~3~~). One isolate, LU584 significantly increased emergence over that of the pathogen control. Five isolates (LU 140, LU 634, LU 740, LU 713, LU 644) increased shoot fresh weight per pot over that of the pathogen control, and one (LU 740) had a higher shoot weight per pot and per plant than the no pathogen control (Table 4~~3~~). LU 140, LU 634, LU 740 and LU 644 all had higher shoot weights per plant than the pathogen control. All the isolates significantly reduced the disease score compared with the pathogen control,

with LU 140, LU 634, LU 740 and LU 644 not differing significantly from the pathogen-free control.

In soil naturally infested with *S. trifoliorum,* seedling emergence was only 10%, and while LU 140 and LU 634 had a significantly greater emergence, it was still only around 20% (Table 54A). *S. trifoliorum* significantly reduced shoot fresh weight per pot compared with that of the plant growth medium control but none of the isolates increased shoot weight (per pot or per plant) over that of the field soil control. Disease score for all the isolates was significantly greater than that for the plant growth medium, but apart from LU 644, was significantly lower than that for the field soil control (Table 54A). When *S. trifoliorum* was inoculated into the naturally infested field soil, very few seedlings emerged (range 4 to 9 per pot, Table 54B). LU 634 and LU 740 gave a small, but significant, increase in emergence, and LU 740 also increased shoot fresh weight per pot but not per plant (Table 54B). Disease score was significantly reduced by all the isolates except LU 140 (Table 54B).

Pythium ultimum / white clover

In plant growth medium, emergence was 88% for the no pathogen control but only 59% in the presence of the pathogen (Table 65). Only two isolates, LU 633 and LU 740 gave increased emergence in the presence of the pathogen. LU 634 increased shoot fresh weight per pot and per plant over that of the pathogen control, but this was still substantially less than that of the no pathogen control (Table 65). Two isolates (LU 634 and LU 740) significantly reduced the disease score compared with that of the pathogen control, but disease scores for the other seven isolates were not reduced (Table 65).

Growth Promotion

Four isolates (LU 634, LU 740, LU 713 and LU 644) significantly reduced perennial ryegrass seedling emergence from that of the control (Table 76), but the mean number of seedlings per pot only fell from 34 (control) to 30 or 31 (data not presented). LU 584 and LU 633 produced large increases (178 and 172%) in shoot fresh weight per plant and also in root fresh weight per plant (100 and 86%; Table 76).

Emergence of red and white clover seedlings was not affected by the presence of the *Trichoderma* isolates with the exception of LU 140 which slightly reduced white clover emergence (Table 76, from 36 down to 32 seedlings per pot). Similarly, shoot fresh weight per plant was not significantly affected by the isolates for both species, with the one exception of LU 584 which gave an increase in red clover shoot fresh weight per plant (Table 76).

Perennial ryegrass seedling emergence for the three *T. atroviride* LU584 application methods did not differ from that in the plant growth medium control, but only the wheat bran treatment increased shoot weight per plant (Table 87).

4. Discussion

Fungal pathogens present in New Zealand soils can kill seedlings of forage plants, thus causing poor establishment (Falloon, 1981, Skipp & Watson, 1987). Initially this was demonstrated through experiments with fungicide seed treatments which improved establishment of ryegrasses (*Lolium* spp.) and red clover (*T. pratense* L.) (Falloon, 1980; Skipp *et al*., 1986). Skipp and Watson (1996) listed fungal pathogens of grass and forage legume species in New Zealand including *R. solani*, *P. ultimum* and *S. trifoliorum*, but noted that it was often difficult to define and quantify losses caused by these pathogens because more than one may be involved. Skipp *et al*. (1986) did record a 30% reduction in red clover emergence in a field soil naturally infested with *Pythium* spp. (primarily *P. ultimum*). In the current plant growth medium experiments, *R. solani* reduced perennial ryegrass seedling emergence by 57%, *S. trifoliorum* reduced red clover seedling emergence by 17%, and *P. ultimum* reduced white clover seedling emergence by 31%. As the pathogen inoculum levels added to the potting mix were chosen to produce approx. 50% seedling death, these results were as expected for perennial ryegrass and white clover, but lower than expected for red clover, although the pathogen did increase the disease score of emerged seedlings. However, in comparisons between seedling emergence of seeds sown into field soil known to contain the pathogens and those sown into sterile plant growth medium, inoculation with *R. solani* reduced perennial ryegrass emergence by 38% and inoculation with *S. trifoliorum* reduced red clover emergence by 78%.

These results, and those of Skipp *et al*. (1986), were obtained from a glasshouse maintained at around 20^{o}C where pots were regularly watered to avoid moisture stress. They are therefore somewhat removed from a field situation, where average 10 cm soil temperature at sowing in New Zealand in autumn and spring is from 8-14^{o}C depending upon location (Hampton *et al*., 1987), and soil moisture contents may vary considerably. Such conditions exert more stress on seeds as germination may decrease and time to emergence increase as soil temperature decreases (Charlton *et al*., 1986), thus potentially allowing more time for pathogen attack. Pathogen activity will also be affected by soil temperature, but "cold preferring isolates" of *R. solani* are active at 13^{o}C or less (Doornik, 1981) and *S. trifoliorum* can sporulate at 5-10^{o}C (Raynal, 1990). Damping-off caused by *P. ultimum* can occur at 10^{o}C or less (Martin & Loper, 1999) and Falloon (1985) reported that *Pythium* spp. were more virulent at 7.5^{o}C than at higher temperatures.

The success of seedling emergence of pasture species depends on many factors including seed lot quality, method and time of sowing, depth of sowing, soil type, previous cropping history, water availability, the presence of soil pests and the presence of soil-borne pathogens. Fungal soil-borne pathogens have the potential to cause significant establishment problems. The present study has demonstrated that in the presence of *R. solani*, perennial ryegrass seedling emergence was increased by 60-150% by two isolates of *T. atroviride* (LU 132, LU 140) and by 35-212% by the isolate of *T. virens* (LU 540). These increases depended on growing medium and inoculum level. The isolates of *T. hamatum* and *T. viride*, for which species Lewis *et al*. (1996), Bailey and Gilligan (1997) and Naeimi *et al.* (2010) had reported strains with activity against *R. solani*, did not increase seedling emergence. Lumsden and

Locke (1989) increased *Zinnia elegans* plant stand by reducing damping off caused by *R. solani* following the addition of *Gliocladium* (*Trichoderma*) *virens* to the growing medium, while *T. atroviride* (formerly *T. harzianum*) biocontrol of *R. solani* has been reported by a number of authors (Lewis *et al*.,1996; Ha, 2010; Naeimi *et al*., 2010; U-Rehman *et al*., 2012). U-Rehman *et al*. (2012) for example reported a 14% increase in cauliflower emergence and a 64% decrease in post-emergence damping off caused by *R. solani* for an isolate of *T. atroviride*. Isolates of *T. atroviride* have been shown to parasitise fungi such as *R. solani* (Grinyer *et al*., 2005) through the ability to release cell wall-degrading enzymes and antibiotics (Lorito *et al*., 1994, 1996).

The red clover emergence data recorded in the present study were poor, even for the plant growth medium control, primarily because a low vigour seed lot was used. Despite this, when challenged by *S. trifoliorium*, two isolates of *T. atroviride* (LU 584, 634) and the *T. hamatum* isolate (LU 740) gave a small but statistically significant increase in emergence. No reports are available for the use of *Trichoderma* spp. for biocontrol of *S. trifoliorum*, but all the isolates used here reduced seedling disease score, indicating bioactivity. Most research and commercial production has focussed on biocontrol of *S. sclerotiorum* (Verma *et al.,* 2009). The mechanism by which *T. atroviride* isolates control this pathogen is likely to be through mycoparasitism of hyphae or sclerotia (Inbar *et al.*, 1994; Jones & Stewart, 2000). An isolate of *T. hamatum* has also been shown to reduce *S. minor* and *S. sclerotiorum* infection in lettuce and cabbage, respectively (Rabeendran *et al*., 2006; Jones *et al*., 2014) with the mechanism of action likely to be either through protection of the host rhizosphere from myceliogenic infection or induced resistance to protect from ascospore infection of above ground host tissues.

When challenged by *P. ultimum*, one isolate of *T. atroviride* (LU 633) and one of *T. hamatum* (LU 740) increased white clover seedling emergence by 25-42%. A reduction in pre-and post-emergence damping off caused by *P. ultimum* has been previously reported for isolates of *T. atroviride*/*harzianum* (Lumsden *et al*., 1990; Chen *et al*., 2005; Siameto *et al.,* 2010), and *T. hamatum*, *T. viride* and *T. virens* (Harman *et al.*, 1981; Ha, 2010). *Trichoderma* spp. can aggressively attack and kill *P. ultimum* mycelia (Harman, 2000; Yang *et al*., 2004), and also produce antibiotic compounds which inhibit *Pythium* growth (Abdollahi *et al.,* 2012) as was noted in the preliminary dual culture assays of the present study (Kandula, unpublished data). *Trichoderma* may also compete with this pathogen on the host surfaces or induce host resistance (Harman, 2000).

For perennial ryegrass, seedling emergence in the presence of *R. solani* was reduced by the *T. hamatum* (LU 740) and *T. koningiopsis* (LU 713) isolates, and in the absence of the pathogen by these two isolates plus the *T. viride* (LU 644) and one of the *T. atroviride* (LU 634) isolates. *Trichoderma* spp. produce numerous secondary metabolites with biological activity which can enhance plant growth (Harman *et al*., 2004). However, some strains can produce phytotoxins which can reduce plant growth. For example, strains of *T. virens* produce the phytotoxin viridiol, which when placed in close proximity to the seeds, can reduce emergence (Howell & Stipanovic, 1994). This compound causes severe necrosis of the radicle and subsequent seedling

death (Jones *et al*.,1988). The *T. virens* isolate used in the present study did not reduce seedling emergence. The reason(s) for the recorded reductions in emergence by the four isolates were not determined in this study.

In the presence of *R. solani*, two *T. atroviride* isolates (LU 584, LU 633) increased perennial ryegrass shoot fresh weight. In the absence of the pathogen, *T. atroviride* (LU 633) increased shoot fresh weight. Care should be exercised when interpreting pot experiment results for plant growth promotion by *Trichoderma* spp. Results expressed per pot may not reflect any growth promotion effects if the increase recorded can be explained by fewer seedlings and decreased competition for resources, thereby allowing increased individual seedling growth. For *T. atroviride* isolates LU 584 and LU 633, the number of emerged seedlings was lower than that of the *R. solani* control, indicating a growth promotion response. This was verified by the data for shoot and root fresh weight per seedling which were increased by these isolates in both the presence and absence of the pathogen. *Trichoderma* isolates producing increases in shoot fresh weight per plant in the presence of the pathogen for red and white clover differed from those which produced a similar response in perennial ryegrass viz *T. atroviride* (LU 140, LU 634), *T. hamatum* (LU 740), *T. koningiopsis* (LU 713) and *T. viride* (LU 644). In the absence of *S. trifoliorum*, only LU 584 increased shoot fresh weight per plant in red clover, and only *T. hamatum* (LU 740) increased white clover growth in the absence of the pathogen. Mechanisms for growth promotion are probably multiple (Harman *et al*., 2004) and may include reduced activity of deleterious root microflora, inactivation of toxic compounds in the root zone, solubilisation of nutrients in the soil, increased nutrient uptake, improved nutrient use efficiency, increased resistance to abiotic stresses and the production of auxin-like compounds (Harman *et al.,* 2004). Although specific mechanisms for plant growth promotion were not assessed in the present study, it is likely that one or a combination of these mechanisms were responsible. As is the case in many studies, this effect was restricted to observations of improved plant growth with no indication of the possible mechanisms involved (Whipps, 2001).

The most useful *Trichoderma* strains for disease control and growth promotion are those that are rhizosphere-competent which have the ability to colonise and grow in association with plant roots (Harman, 2000). This requires delivery mechanisms which allow development of this close association between biocontrol agents and host plants. For the nursery industry, incorporation of *Trichoderma* into plant growing media before sowing achieves this result (Harman *et al*., 2004), but for field use, *Trichoderma* must be introduced at the time of seed sowing. Possible methods for this include seed coating or granules (Kandula *et al.* 2008), neither of which negatively affected perennial ryegrass seedling emergence or shoot fresh weight in the plant growth medium experiment.

As noted by Howell (2003), the production and activities of the enzymes and antibiotics associated with biocontrol and growth promotion by *Trichoderma* spp. are profoundly affected by soil temperature, the presence and activities of other soil microflora, the pathogen challenge, and soil types. While screening putative antagonists in pots containing test plants and pathogens probably increases the

chances of selecting active agents (Knudsen *et al.*, 1997), field studies are essential to confirm their efficacy. Using the *Trichoderma* activity summary data presented in Table 9~~8~~, four *T. atroviride* isolates (LU 132, LU 140, LU 584, LU 633) were selected for field assessment as a mixture prototype product (Chohan *et al*., 2010) on the assumption that multiple biocontrol agents as mixtures will provide a greater chance of success in a field environment (Stewart, 2001).

Acknowledgements

This research was funded by New Zealand Ministry of Business, Innovation and Employment (Project LINX 0804). We thank Dr Jayanthi Swaminathan of AgResearch Ltd, Lincoln for provision of the proprietary granule, and Professor Richard Falloon, Bio-Protection Research Centre, for reviewing the manuscript.

References

Abdollahi, M., Ommati, F., & Zaker, M. (2012). The *in vitro* efficacy of *Trichoderma* isolates against *Pythium aphanidermatum*, the causal agent of sugar beet root rot. *J Research Agr Sci*, *8*, 79-87.

Bailey, D. J., & Gilligan, C. A. (1997). Biological control of pathozone behaviour and disease dynamics of *Rhizoctonia solani* by *Trichoderma viride*. *New Phytologist*, *136*, 359-367.

Charlton, D., & Stewart, A. (2000). *Pasture and Forage Plants for New Zealand.* Grassland Research and Practice Series No. 8, New Zealand Grassland Association, Palmerston North, 74pp.

Charlton, J. F. L., Hampton, J. G., & Scott, D. J. (1986) Temperature effects on germination of New Zealand herbage grasses. *Proceedings of New Zealand Grassland Association*, *47*, 165-172.

Chen, J., Harman, G. E., Comis, A., & Cheng G. W. (2005). Proteins related to the biocontrol of *Pythium* damping-off in maize with *Trichoderma harzianum* Rifai. *Journal of Integrative Plant Biology*, *47*, 988-997.

Chohan, P., Kandula, D. R. W., Stewart, A., & Hampton, J.G. (2010). Biological control of *Rhizoctonia solani* in perennial ryegrass using *Trichoderma atroviride* isolates. *Proceedings of the 6th Australasian Soilborne Diseases Symposium*, 35.

Doornik, A. W. (1981). Temperature dependence of the pathogenicity of several isolates of *Rhizoctonia solani* in some bulb crops as an intrinsic property of the isolate. *Netherlands Journal of Plant Pathology*, *87*, 139-147.

Falloon, R. E. (1980). Seedling emergence responses in ryegrass (*Lolium* spp.) to fungicide seed treatments. *New Zealand Journal of Agricultural Research*, *23*, 385-391.

Falloon, R. E. (1981). Fungicide seed treatment to improve establishment of ryegrasses and other forage plants. *Proceedings of the 34th New Zealand Weed and Pest Control Conference*, 43-47.

Falloon, R. E. (1985). Fungi pathogenic to ryegrass seedlings. *Plant and Soil*, *86*, 79-86.

Falloon, R. E., & Fletcher, R. H. (1983). Increased herbage production from perennial ryegrass following fungicide seed treatment. *New Zealand Journal of Agricultural Research*, *26*, 1-5.

Ghaffar, A. (1969). Biological control of white rot of onion. I. Interactions of soil micro-organisms with *Sclerotinia cepivorum* Berk. *Mycopathologia et Mycologia Applicata,* 38, 101-111.

Grinyer, J., Hunt, S., McKay, M., Herbert, B. R., & Nevalainen, H. (2005). Proteomic response of the biological control fungus *Trichoderma atroviride* to growth on the cell walls of *Rhizoctonia solani*. *Current Genetics*, *47*, 381-388.

Ha, T. N. (2010). Using *Trichoderma* species for biological control of plant pathogens in Vietnam. *J ISSAAS*, *16*, 17-21.

Hampton, J. G., Charlton, J. F. L., Bell, D.D., & Scott, D.J. (1987). Temperature effects on the germination of herbage legumes in New Zealand. *Proceedings of the New Zealand Grasslands Association*, 48,177-183.

Harman, G. E. (2000). Myths and dogmas of biocontrol: changes in perception derived from research on *Trichoderma harzianum* T-22. *Plant Disease*, *84*, 377-393.

Harman, G. E., Chet, I., & Baker, R. (1981). Factors affecting *Trichoderma hamatum* applied to seeds as a biocontrol agent. *Phytopathology*, *71*, 569-572.

Harman, G. E., Howell, C.V., Viterbo, A., Chet, I., & Lorito, M. (2004). *Trichoderma* species – opportunistic, avirulent plant symbionts. *Nature Review Microbiol 2*, 43-56.

Harvey, I. C., & Harvey, B. M. (2009). *Pasture Diseases in New Zealand*. Bio-Protection Research Centre, Lincoln University, Lincoln, 144p.

Howell, C. R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts *Plant Disease*, *87*, 4-10.

561 Howell, C. R., & Stipanovic, R. D. (1994). Effect of sterol biosynthesis inhibitors on
562 phytotoxin (viridiol) production by *Glioclaldium virens* in culture.
563 *Phytopathology*, *84*, 969-972.
564
565 Inbar, J., Abramsky, M., Cohen, D., & Chet, I. (1994). Plant growth enhancement and
566 disease control by *Trichoderma harzianum* in vegetable seedlings grown
567 under commercial conditions. *European Journal of Plant Pathology*, *100*,
568 337-346.
569
570 Jones, E. E., Rabeendran, N., & Stewart, A. (2014). Biocontrol of *Sclerotinia*
571 *sclerotiorum* infection of cabbage by *Coniothyrium minitans* and
572 *Trichoderma* spp. *Biocontrol Science and Technology, 24, 1363-1382*.
573
574 Jones, E. E., & Stewart, A. (2000). Selection of mycoparasites of sclerotia of
575 *Sclerotinia sclerotiorum* isolated from New Zealand soils. *New Zealand*
576 *Journal of Crop and Horticultural Science*, *28*, 105-114.
577
578 Jones, R.W., Lanini, W. T., & Hancock, J. G. (1988). Plant growth response to the
579 phytotoxin viridiol produced by the fungus *Gliocladium virens. Weed*
580 *Science*, *36*, 683-687.
581
582 Kandula, D. R. W., Stewart, A., McDermid, J., Gale, D., Swaminathan, J. (2008). Bio-
583 inoculant formulations for enhanced seedling emergence and pasture
584 growth. In: McGill, R & Rowarth, J. S. (eds). *Seeds for Futures*, Agronomy
585 Society of New Zealand Special Publication No. 13; Grassland Research and
586 Practice Series No. 14, Palmerston North, New Zealand, pp 147-148
587
588 Knudsen, I. M. B., Hockenhull, J., Jensen, D. F., Gerhardson, B., Hokeberg, M.,
589 Tahvonen, R., Teperi, E., Sundheim, L., & Henriksen, B. (1997). Selection of
590 biological control agents for controlling soil and seed-borne diseases in the
591 field. *European Journal of Plant Pathology*, *103*, 775-784.
592
593 Kohl, J., Postma, J., Nicot, P., Ruocco, M., & Blum, B. (2011). Stepwise screening of
594 micro-organisms for commercial use in biological control of plant-pathogenic
595 fungi and bacteria. *Biological Control*, 57, 1-12.
596
597 Latch, G. C. M. (1996). Overview of New Zealand agriculture and pasture pathology.
598 In: Chakraborty S *et al*. (eds), *Pasture and Forage Crop Pathology*, ASA, CSSA,
599 SSSA, Madison, Wisconsin, pp 22-32
600
601 Lewis, J. A., Lumsden, R. D., & Locke, J. C. (1996). Biocontrol of damping-off diseases
602 caused by *Rhizoctonia solani* and *Pythium ultimum* with alginate prills of
603 *Gliocladium virens*, *Trichoderma hamatum* and various food bases.
604 *Biocontrol Science and Technology*, *6*, 163-174.
605
606 Lorito, M., Hayes, C. K., di Petro, A., Woo, S. L., & Harman, G. E.(1994). Purification,
607 characterization and synergistic activity of a glucan 1,3-β-glucosidase and an

608 *N*-acetyl-β-D-glucosaminidase from *Trichoderma harzianum*. *Phytopathology*,
609 *84*, 398-405.
610
611 Lorito, M., Farkas, V., Rebuffat, S., Bodo, B., & Kubicek, C. P. (1996). Cell wall
612 synthesis is a major target of mycoparasitic antagonism by *Trichoderma*
613 *harzianum. Journal of Bacteriology*, *178*, 6382-6385.
614
615 Lumsden, R. D., & Locke, J.C. (1989). Biological control of damping-off caused by
616 *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in soilless
617 mix. *Phytopathology*, *79*, 361-366.
618
619 Lumsden, R. D., Carter, J. P., Whipps, J. M., & Lynch, J. M. (1990). Comparison of
620 biomass and viable propagule measurements in the antagonism of
621 *Trichoderma harzianum* against *Pythium ultimum. Soil Biology and*
622 *Biochemistry*, *22*, 187-194.
623
624 Martin, F. N., & Loper, J. E. (1999). Soilborne diseases caused by *Pythium* spp.:
625 ecology, epidemiology and prospects for biological control. *Critical Review of*
626 *Plant Sciences*, *18*, 111-181.
627
628 Naeimi, S., Okhovvat, S. M., Javan-Nikkhah, M., Vagvolgyi, C., Khosravi, V., & Kredics,
629 L. (2010). Biological control of *Rhizoctonia solani* AG1-1A, the causal agent of
630 rice sheath blight with *Trichoderma* strains. *Phytopathologia Mediterranea*,
631 *49*, 287-300.
632
633 O'Rourke, T. A., Scanlon, T. T., Ryan, M. H., Wade, L. J., McKay, A. C., Riley, I.T., Li, H.,
634 Sivasithamparam, K., & Barbetti, M. J. (2009) Severity of root rot in mature
635 subterranean clover and associated fungal pathogens in the wheatbelt of
636 Western Australia. *Crop and Pasture Science*, *60*, 43-50.
637
638 Rabeendran, N., Jones, E. E., Moot, D., & Stewart, A. (2006). Biocontrol of *Sclerotinia*
639 lettuce drop by *Coniothyrium minitans* and *Trichoderma* species. *Biological*
640 *Control*, 39, 352-362.
641
642 Raynal, G. (1990). Kinetics of the ascospore production of *Sclerotinia trifoliorum* Eriks
643 in growth chamber and under natural climatic conditions. Practical and
644 epidemiological incidence. *Agronomie*, *10*, 561-572.
645
646 Rehman, S., Lawrence, R., Kumar, E. J., & Badri, Z. A. (2012). Comparative efficacy of
647 *Trichoderma viride*, *T. harzianum* and carbendazim against damping-off
648 disease of cauliflower caused by *Rhizoctonia solani* Kuehn. *Journal of*
649 *Biopesticides*, *5*, 23-27.
650
651 Sarathchandra, S. U., Watson, R. N., Skipp, R. A., Burch, G., Brown, J. A., & Cox, N. R.
652 (2000). Microbial pathogens and plant parasitic nematodes in pastures with
653 declining vigour. *New Zealand Journal of Agricultural Research*, *43*, 549-558.
654

655 Siameto, E. N., Okoth, S., Amugune, N. O., & Chege, N. C. (2010). Antagonism of
656 *Trichoderma harzianum* isolates on soilborne plant pathogenic fungi from
657 Embu District, Kenya. *Journal of Yeast and Fungal Research*, *1*, 47-54.
658
659 Skipp, R. A., & Christensen, M. J. (1989a) Fungi invading roots of perennial ryegrass
660 (*Lolium perenne* L.) in pasture. *New Zealand Journal of Agricultural Research,*
661 *32*, 423-431.
662
663 Skipp, R. A., & Christensen, M. J. (1989b) Host specificity of root-invading fungi from
664 New Zealand pasture soils. *Australasian Plant Pathology*, *18*, 101-103.
665
666 Skipp, R. A., Christensen, M. J., & Biao, N. Z. (1986). Invasion of red clover (*Trifolium*
667 *pratense*) roots by soilborne fungi. *New Zealand Journal of Agricultural*
668 *Research, 29*, 305-313.
669
670 Skipp, R. A., & Hampton, J. G. (1996). Fungal and bacterial diseases of pasture plants
671 in New Zealand. In: Chakraborty S *et al*. (eds). *Pasture and Forage Crop*
672 *Pathology*, ASA, CSSA, SSSA, Madison, Wisconsin, pp 213-236.
673
674 Skipp, R. A., & Hay, F. S. (1993). Screening for biocontrol agents of root pathogens of
675 pasture legumes. *Proceedings of the XVII International Grassland Congress*,
676 pp 936-937.
677
678 Skipp, R. A., & Watson, R. N. (1987). Pot experiments with pasture soils to detect
679 soilborne pathogens of white clover and lucerne, and effects of field
680 application of fungicides. *New Zealand Journal of Agricultural Research, 30*,
681 85-93.
682
683 Skipp, R. A., & Watson, R. N. (1996). Disease complexes in New Zealand pastures. In:
684 Chakraborty S *et al.* (eds). *Pasture and Forage Crop Pathology,* ASA, CSSA,
685 SSSA, Madison, Wisconsin, pp 429-451.
686
687 Stewart, A. (2001). Commercial biocontrol – reality or fantasy? Australasian Plant
688 Pathology, *30*, 127-131.
689
690 Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R. Y., & Valero, J. R. (2007).
691 Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. *Journal of*
692 *Biochemical Engineering*, *37*, 1-20.
693
694 Waipara, N. W. (2000). Biodiversity of *Trichoderma* species in agricultural soils and
695 their potential use as seed coat amendments for the biocontrol of soilborne
696 plant pathogens (Abstract). *Proceedings of the 53rd New Zealand Plant*
697 *Protection Conference,* 450.
698
699 Waipara, N. W., Di Menna, M. E., Cole, A. L. J., & Skipp, R. A. (1996) Potential
700 pathogenicity of pasture plant root-colonising fungi to seedlings of legumes

and grasses. *Proceedings of the 49th New Zealand Plant Protection Conference*, *49*, 212-215.

Whipps, J. M. (2001). Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*, *52*, 487-511.

Yang, Y., Chang, K.F., Hwang, S. F., Callan, N. W., Howard, R. J., & Blade, S.F. (2004). Biological control of *Pythium* damping-off in *Echinacea angustifolia* with *Trichoderma* species. *Journal of Plant Disease Protection* III, 126-136.

Table 1. Effect of different inoculum concentrations (g or mL/pot in 400g plant growth medium) of three different root pathogens on seedling emergence of three different pasture species in experiments to determine inoculum thresholds.

Rhizoctonia solani [a] / Perennial ryegrass		*Sclerotinia trifoliorum* [b] / Red clover		*Pythium ultimum* [c] / White clover	
Inoculum concentration g/pot	Seedling emergence [d] (%)	Inoculum concentration g/pot	Seedling emergence [d] (%)	Inoculum concentration ml/pot	Seedling emergence [d] (%)
0	90 (5.4)	0	79 (6.9)	0	91 (4.8)
1	73 (5.5)	3	70 (8.9)	0.4	77 (14.8)
2	52 (11.1)	6	62 (8.9)	0.8	51 (7.5)
4	28 (4.3)	12	51 (5.9)	1.6	23 (10.6)
8	11 (5.5)	24	20 (7.4)	3.2	7 (4.3)

[a] *R. solani* colonised wheat-bran; [b] *S. trifoliorum* colonised wheat grains; [c]homogenised roots colonised with *P. ultimum* oospores; [d]40 seeds sown/pot. Mean values of four replicates are shown with standard deviation in parenthesis.

Table 2. Effect of nine *Trichoderma* isolates on perennial ryegrass seedling emergence and shoot fresh weight and root dry weight when grown in plant growth medium inoculated with *Rhizoctonia solani*.

Treatment	Number of seedlings emerged[a]	Shoot weight		Root weight	
		g/pot	g/plant	g/pot	g/plant
Control (no pathogen)	35	8.0	0.23	3.9	0.11
Control (+ *R. solani*)	15	5.7	0.38	2.6	0.17
LU 132	29	9.2	0.32	4.2	0.14
LU 140	28	8.1	0.29	3.2	0.11
LU 584	6	11.3	2.06	1.8	0.27
LU 633	6	15.8	2.84	3.0	0.48
LU 634	10	4.5	0.44	1.8	0.18
LU 740	3	2.1	0.50	0.6	0.13
LU 713	4	2.0	0.32	0.8	0.10
LU 540	27	7.4	0.27	3.7	0.14
LU 644	12	6.5	0.56	2.8	0.23
LSD (P<0.05)					
Between isolates	3.3	2.7	0.35	1.19	0.09
Isolates vs control	4.5	3.7	0.47	1.61	0.12
Between controls	4.7	3.8	0.49	1.69	0.13

[a]out of 40 seeds sown per pot

Table 3. Effect of three *Trichoderma* isolates on perennial ryegrass seedling emergence and shoot fresh weight when grown in field soil containing (A) natural *Rhizoctonia solani* infestation and (B) natural infestation plus inoculated infestation.

Treatment	Number of seedlings emerged[a]	Shoot weight g/pot	Shoot weight g/plant
A.			
Control (plant growth medium)	32 c	12.2 c	0.38 b
Control (field soil)	20 ab	3.5 a	0.18 a
LU 132	18 a	4.1 ab	0.23 ab
LU 140	32 c	5.3 b	0.17 a
LU 540	27 bc	4.9 b	0.22 a
LSD (*P*<0.05)	7.1	1.3	0.13
B.			
Control (field soil + *R. solani*)	8 a	1.8 a	0.20 a
LU 132	13 ab	3.8 b	0.30 a
LU 140	20 bc	4.9 bc	0.32 a
LU 540	25 c	5.8 c	0.24 a
LSD (*P*<0.05)	7.3	1.6	0.17

[a]out of 40 seeds sown per pot

Mean values followed by a different letter in the columns indicate significant differences according to Fisher's unprotected LSD test.

Table 4. Effect of nine *Trichoderma* isolates on red clover seedling emergence, shoot fresh weight and disease score when grown in plant growth medium inoculated with *Sclerotinia trifoliorum*.

Treatment	Number of seedlings emerged[a]	Shoot weight g/pot	Shoot weight g/plant	Disease Score[b]
Control (no pathogen)	23 bc	8.8 de	0.38 bc	1.0 a
Control (+ *S. trifoliorum*)	19 ab	3.6 a	0.19 a	3.0 d
LU 132	21 abc	4.5 ab	0.23 a	1.8 bc
LU 140	22 bc	7.9 cd	0.38 bc	1.1 a
LU 584	25 c	5.0 ab	0.20 a	2.1 c
LU 633	17 a	5.0 ab	0.30 ab	2.0 c
LU 634	21 abc	10.6 ef	0.51 cd	1.1 a
LU 740	23 bc	11.6 f	0.53 d	1.1 a
LU 713	21 abc	6.3 bc	0.30 ab	2.3 c
LU 540	21 abc	4.5 ab	0.22 a	2.1 c
LU 644	20 ab	8.3 cd	0.43 bcd	1.3 ab
LSD (*P*<0.05)	4.5	2.2	0.14	0.6

[a]out of 40 seeds sown per pot

[b]where 1 = no evidence of disease and 5 = severely diseased

Mean values followed by a different letter in the columns indicate significant differences according to Fisher's unprotected LSD test.

Table 5. Effect of four *Trichoderma* isolates on red clover seedling emergence, shoot fresh weight and disease score when grown in field soil containing (A) natural *Sclerotinia trifoliorum* infestation and (B) natural infestation plus inoculated infestation.

Treatment	Number of seedlings emerged[a]	Shoot weight g/pot	Shoot weight g/plant	Disease Score[b]
A.				
Control (plant growth medium)	19 c	17.1 c	0.90 ab	1.0 a
Control (field soil)	4 a	4.3 ab	1.08 ab	3.8 d
LU 140	9 b	7.1 b	0.78 ab	2.5 bc
LU 634	8 b	6.4 ab	0.80 ab	2.6 bc
LU 740	7 ab	7.5 b	1.07 b	2.3 b
LU 644	6 ab	3.8 a	0.63 a	3.3 cd
LSD (P<0.05)	3.5	3.2	0.39	0.8
B.				
Control (field soil + *S. trifoliorum*)	4 a	2.8 a	0.70 a	4.1 c
LU 140	4 a	3.9 ab	0.97 a	3.2 bc
LU 634	9 b	6.1 ab	0.67 a	2.6 ab
LU 740	9 b	6.7 b	0.74 a	2.0 a
LU 644	8 ab	5.5 ab	0.69 a	2.6 ab
LSD (P<0.05)	4.4	3.4	0.49	0.9

[a]out of 40 seeds sown per pot

[b]where 1 = no evidence of disease and 5 = severely diseased

Mean values followed by a different letter in the columns indicate significant differences according to Fisher's unprotected LSD test.

Table 6. Effect of nine *Trichoderma* isolates on white clover seedling emergence, shoot fresh weight and disease score when grown in plant growth medium inoculated with *Pythium ultimum*.

Treatment	Number of seedlings emerged[a]	Shoot Weight g/pot	g/plant	Disease Score[b]
Control (no pathogen)	35 e	11.9 e	0.34 d	1.0 a
Control (*P. ultimum*)	24 ab	3.2 abc	0.13 ab	3.3 de
LU 132	25 abc	3.7 abcd	0.13 ab	3.0 cd
LU 140	21 a	1.4 a	0.07 a	4.0 e
LU 584	29 bcd	3.1 ab	0.10 ab	3.2 de
LU 633	30 cde	4.1 abcd	0.14 ab	2.8 bcd
LU 634	26 abc	6.4 d	0.25 c	2.1 bc
LU 740	34 de	5.9 cd	0.18 bc	1.9 ab
LU 713	27 bc	3.2 abc	0.11 ab	3.3 de
LU 540	24 abc	3.9 abcd	0.15 ab	2.9 cd
LU 644	28 bc	4.4 bcd	0.16 ab	2.8 bcd
LSD (P<0.05)	5.9	2.8	0.09	0.9

[a]out of 40 seeds sown per pot

[b]where 1 = no evidence of disease and 5 = severely diseased (illustrated in Figure 1)

Mean values followed by a different letter in the columns indicate significant differences according to Fisher's unprotected LSD test.

Formatted: Font color: Red

Table 7. Effect of nine *Trichoderma* isolates on seedling emergence (seed em.), and growth promotion in pathogen-free plant growth medium (data expressed as percentage of the control).

Isolate	Perennial ryegrass			Red clover		White clover	
	Seed Em.	Shoot weight	Root weight	Seed Em.	Shoot weight	Seed Em.	Shoot weight
LU 132	-9	12	41	-6	5	1	15
LU 140	-2	10	24	0	10	-11*	-13
LU 584	2	178*	100*	1	33*	-3	34
LU 633	-8	172*	86*	0	2	-2	-24
LU 634	-15*	33	78	3	-6	-1	-22
LU 740	-13*	10	2	-5	12	2	-12
LU 713	-13*	36	23	-8	-8	-1	-8
LU 540	-9	4	18	-6	19	0	-1
LU 644	-11*	25	28	-1	15	0	11

*indicates a significant difference from the control at P<0.05

Table 8. Effect of application method of *Trichoderma atroviride* LU 584 on perennial ryegrass seedling emergence and fresh shoot weight in plant growth medium.

Treatment	Number of seedlings emerged[a]	Shoot weight g/pot	g/plant
Control (no *Trichoderma*)	34.3 ab	10.3 a	0.30 a
Wheat bran	34.8 ab	14.3 b	0.42 b
Seed coat	33.6 a	10.6 a	0.31 a
Granules	36.2 b	13.3 b	0.37 ab
LSD *P*<0.05	2.093	2.0	0.07

[a]out of 40 seeds sown per pot

Mean values followed by a different letter in the columns indicate significant differences according to Fisher's unprotected LSD test.

Table 9. Summary of seedling disease control activity against specific pathogens and growth promotion of three pasture species from nine *Trichoderma* isolates in a range of pot experiments.

Isolate	Disease Control			Growth Promotion		
	R.s.[a]	S.t.[b]	P.u.[c]	P.r.[d]	R.c.[e]	W.c.[f]
LU 132 (*T. atroviride*)	++[g]	+	[h]	+		
LU 140 (*T. atroviride*)	+++					
LU 584 (*T. atroviride*)				+++	++	++
LU 633 (*T. atroviride*)		+	+	+++		
LU 634 (*T. atroviride*)		+++		+		
LU 740 (*T. hamatum*)		+++	++			
LU 713 (*T. koningiopsis*)						
LU 540 (*T. virens*)	+++	+				
LU 644 (*T. viride*)		+				

[a]*Rhizoctonia solani*, [b]*Sclerotinia trifoliorum*, [c]*Pythium ultimum,* [d]perennial ryegrass, [e]red clover, [f]white clover, [g]activity rating: +++ = high, ++ = medium, + = low, [h]empty cells indicate a nil response

Formatted: Tab stops: Not at 1.5 cm

Formatted: Centered

Formatted: Centered

Formatted: Centered

Figure 1 (A) *Pythium ultimum* disease score where 1=no symptoms evident and 5=seedlings severely diseased; (B) five representative white clover plants with a disease score of 1; (C) five representative white clover plants with a disease score of 4. Scale = 50mm

Formatted: Font: Italic, Font color: Dark Red