***Trichoderma* bio-inoculant improves seedling emergence, plant growth and seed yield of *Camelina sativa* (L.) Crantz**

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ABSTRACT *Camelina sativa* seedling emergence and plant growth can be reduced by the soil-borne pathogen *Rhizoctonia solani*. The effect of a mixture of *Trichoderma atroviride* isolates applied in granule and prill formulations at a rate equivalent to 15 kg/ha on two varieties of *C. sativa* (Suneson and 4164) was evaluated in glasshouse experiments using *R. solani* infested soil. The bio-inoculant treatments significantly increased seedling emergence by 30-60%, and increased total shoot and root dry weight by 4-8 fold over that of the untreated control. In a field experiment in 2012-2013, using *C. sativa* variety 4164, the prill formulation of the bio-inoculant was applied at sowing. Seedling emergence was not significantly increased but shoot dry weight was increased by 20% and seed yield was increased by 25% by the bio-inoculant treatment over that of the control. *Trichoderma* bio-inoculants may have a role in the integrated crop management of this second generation bio-fuel crop.

Keywords *Camelina sativa*, *Trichoderma* bio-inoculant, biodiesel, false flax, plant growth enhancement, *Rhizoctonia solani*.

INTRODUCTION

Camelina sativa (L.) Crantz (Siberian oilseed or false flax) is currently attracting interest as an alternative oilseed crop in many regions of the world (Yuan et al. 2008; Dale et al. 2010; Pikul et al. 2014) because of its potential value for food, feed and industrial applications. The crop can grow in harsh environments and has a low requirement for water and nutrients (Moser 2012). In New Zealand its potential as a renewable biodiesel feedstock is being investigated (McKenzie et al. 2011; Fasi et al. 2012), particularly its ability to grow on marginal lands unsuited to production of other crops (Cai et al. 2011).

Camelina sativa has generally been considered to be a disease-resistant crop (Seguin-Swartz et al. 2009), but can be attacked by the soil-borne pathogen *Rhizoctonia solani* Kuhn (Conn et al. 1994). This pathogen causes sore shin and damping-off characterised by a brown discoloration on the root and hypocotyl and rotting of the crown (Seguin-Swartz et al. 2009). *Rhizoctonia solani* occurs commonly in New Zealand soils (Harvey & Harvey 2010), and caused damping-off in a *C. sativa* field experiment at Lincoln University where a plant loss of 49% between seedling establishment and final harvest was recorded (Fasi et al. 2012).

Trichoderma spp. are ubiquitous fungi inhabiting soil and roots of numerous plant species and have been widely used to control plant pathogens and promote plant growth (Harman et al. 2004). Kandula et al. (2015) demonstrated that a *Trichoderma* bio-inoculant improved seedling emergence and dry matter production of herbage species in *R. solani* infected soils. This study sought to determine the ability of a *Trichoderma* bio-inoculant, applied in either a granule or prill formulation, to reduce the deleterious effects of *R. solani* on *C. sativa* seedling emergence and plant growth in glasshouse and field experiments.

MATERIALS AND METHODS

Glasshouse experiments

Soil from a Lincoln University field known to contain *R. solani* (Kandula et al. 2015) was collected, passed through a 3 mm sieve and mixed with pumice in a 3:1 soil: pumice ratio. This mix was then used to fill 45×30 cm growing trays. Glasshouse temperature was maintained at an average of 20°C for the duration of the experiments and the trays were watered using overhead irrigation as required.

Experiment One

Two varieties of *C. sativa* (Suneson and 4164) were used. Four treatments were used for each variety, viz. bare seed, seed hand-treated with carboxin+thiram (Vitaflo 200) at the label rate and bare seed with the *Trichoderma* bio-inoculant (a mixture of four strains) applied in a prill or granule equivalent to a field rate of 15 kg/ha. The prills were manufactured by Agrimm Technologies Ltd, Lincoln, and the granules by AgResearch, Lincoln. Both the formulations had a bioactive loading of 2×10^7 cfu /g product.

A complete randomised block design with six replicates per treatment was used. For each tray (replicate), 40 *C. sativa* seeds were sown at a depth of 1 cm on 17 April 2012. Trays were watered daily and seedling emergence was recorded at 7, 14 and 21 days after sowing (DAS). At 33 DAS, seedlings were hand removed from the trays, washed under running tap water to

remove adhering soil from the roots, the roots and shoots separated and oven dried at 60°C for 3 days, following which dry weight was recorded.

Experiment Two

The same basic methodology as in Experiment One was used but there were four changes: (1) because the emergence of bare seed treatment in Experiment One was less than 10%, this treatment was removed, (2) the prills and granules were mixed into soil containing carboxin+thiram treated seeds, (3) the number of replicates for the three remaining treatments was increased to eight and (4) the sowing date was 23 May 2012. Both the formulations had a bioactive loading of 2×10^7 cfu/g product.

Field experiment

The experiment was conducted at Lincoln University on an irrigated Wakanui silt loam soil known to contain *R. solani* (Fasi et al. 2012). The previous crop was ryegrass/white clover pasture, and the site was prepared by conventional cultivation. Diammonium phosphate at 250 kg/ha was applied prior to sowing, and the herbicide trifluralin (Treflan™) at 3 litres/ha (480 g ai/litre) was incorporated into the soil prior to sowing. *Camelina sativa* variety 4164 treated with carboxin+thiram was sown on 30 October 2012 at 4 kg/ha using an Oyjord cone seeder with sowing depth of 1.0-1.5 cm. Treatments were seed only and seed plus *Trichoderma* prills (2×10^7 cfu/g product) applied to the soil at 15 kg/ha between the drill rows. Plot size was 12 m long × 4.2 m wide, and there were eight replicates of each treatment arranged in a randomised complete block design.

Seedling emergence was assessed at 28 DAS. Nitrogen (50 kg N/ha as urea) was applied at 50% flowering, after which the plots were covered with bird netting to avoid seed loss. At 98 DAS (7 February 2013) all plants from a 1.8 × 1.8 m area in each plot were hand-cut at ground level and hung to dry in ambient conditions for 2 weeks before the seed was threshed using a Kurt Pelz thresher and hand cleaned. The non-seed material was oven-dried at 60°C for 24 h

following which dry weight was recorded. Seed yield was recorded at 10% seed moisture content.

Data analysis

Data were subjected to analysis of variance (ANOVA) using GenStat 14 software (VSN International Ltd) followed by comparison of mean values of the treatments using Fisher's unprotected least significance difference.

RESULTS

Experiment One

For both varieties, the emergence of untreated seeds was less than 10% (Figure 1). Fungicide

Figure 1 Seedling emergence in *R. solani*-infested soil of two varieties of *Camelina sativa* in the presence and absence of *Trichoderma* bio-inoculants (prill and granule) and fungicide (Experiment One). Values with the same letter do not differ significantly according to Fisher's unprotected LSD test.

Figure 2 Seedling root and shoot dry weight of two varieties of *Camelina sativa* grown in *R. solani*-infested soil in the presence and absence of *Trichoderma* bio-inoculants (prill and granule) and fungicide (Experiment One). Values with the same letter do not differ significantly according to Fisher's unprotected LSD test.

seed treatment significantly increased ($P < 0.05$) the emergence of both varieties, to 35% for Suneson and 70% for 4164. Both the granule and prill formulations significantly increased emergence of both varieties over the untreated control, to levels similar to those resulting from the fungicide treatment (Figure 1).

All three treatments significantly increased ($P < 0.05$) both root and shoot total dry matter (Figure 2) with the granule formulation increases being significantly greater than those of the fungicide for both varieties. These dry matter totals were a reflection of plant numbers per tray, as dry matter per seedling did not differ between the treatments (data not presented).

Experiment Two

Seedling emergence was greater for 4164 than for Suneson for all three treatments (Figure 3). For Suneson, the addition of *Trichoderma* (both formulations) increased emergence significantly over that of the fungicide treatment alone, but emergence of 4164 did not differ between the treatments (Figure 3).

Total root and shoot dry matter were significantly increased ($P < 0.05$) by the granule in Suneson but not by the prill, while in 4164, application of granules and prills produced more total root and shoot dry matter than the fungicide only treatment (Figure 4). For root dry matter there were no differences between the

varieties for these two treatments, but for shoot dry matter, 4164 was significantly greater than Suneson (Figure 4). Root and shoot dry matter per seedling were increased by both the granule and prill for 4164, but other differences were not significant (data not presented).

Figure 3 Seedling emergence in *R. solani*-infested soil of two varieties of fungicide treated *Camelina sativa* in the presence and absence of *Trichoderma* bio-inoculants (prill and granule) (Experiment Two). Values with the same letter do not differ significantly according to Fisher's unprotected LSD test.

Figure 4 Root and shoot dry weight of two varieties of fungicide treated *Camelina sativa* grown in *R. solani*-infested soil in the presence and absence of *Trichoderma* bio-inoculants (prill and granule) (Experiment Two). Values with the same letter do not differ significantly according to Fisher's unprotected LSD test.

Table 1 Seedling emergence (plants/m²), shoot biomass (kg DM/ha) and seed yield (kg DM/ha) of *Camelina sativa* plants grown with or without the *Trichoderma* bio-inoculant prill.

Treatment	Seedling emergence	Shoot biomass	Seed yield
Seed only	232	2475	1285
Seed + bio-inoculant	237	2937	1605
LSD (P<0.05)	NS	205	160

Field experiment

Seedling emergence did not differ between the two treatments (Table 1). Plant death as a result of *R. solani* crown rotting was subsequently observed in the trial but this was not quantified. At harvest, the *Trichoderma* bio-inoculant significantly ($P<0.05$) increased shoot dry weight (+20%) and seed yield (+25%) (Table 1) compared to the control.

DISCUSSION

Camelina sativa is characterised as a low-input oilseed crop with remarkable resistance against common and widespread diseases of the Brassicaceae (Vollmann & Eynck 2015). However, it is known to be susceptible to *R. solani* (Fleenor 2011), and Fasi et al. (2012) reported *R. solani* infected plants in a field trial at Lincoln University. *Rhizoctonia solani* is a pathogen of arable, vegetable and forage crops, and Kandula et al. (2015) reported a 38% reduction in perennial ryegrass seedling emergence in soil naturally infested by *R. solani*. *Camelina sativa* seedling

emergence in the current study was reduced by nearly 80% by the pathogen in the glasshouse experiments. Similarly to that reported for perennial ryegrass, this loss occurred as a result of pre-emergence damping-off (Falloon 1985), as no post-emergence damping-off was recorded.

Fungicide seed treatment allowed significantly improved emergence in both varieties, with the emergence of Suneson being lower than that of 4164. This varietal response also occurred for the two *Trichoderma* treatments in both glasshouse experiments suggesting that Suneson is more susceptible to *R. solani* than 4164. Both the granule and prill significantly increased emergence over that of bare seed, but not that of fungicide-treated seed. Kandula et al. (2015) reported that a number of individual *Trichoderma* strains increased perennial ryegrass seedling emergence by between 35 to 212% in the presence of *R. solani*, while Chohan et al. (2010) reported a similar response in perennial ryegrass for the four strain mix used in the present study.

Interestingly, seedling emergence in the field experiment did not differ between the bare seed and bare seed plus *Trichoderma*. The mean of 235 plants/m² recorded was lower than the 290 plants/m² reported by Fasi et al. (2012) for an October sowing at the same site, but these authors reported plant losses during the growing season ranging from 20 to 88% (greater for autumn than spring sowings), which they attributed to damage caused by *R. solani*. While plant population at harvest was not assessed, plants in plots treated with *Trichoderma* had a significantly ($P < 0.05$) higher shoot dry weight than the control. The reason for this cannot be determined from the data collected, but two possibilities exist. The first is that plant death/damage caused by *R. solani* was reduced by the presence of the *Trichoderma*. The second is that the *Trichoderma* promoted plant growth (Harman 2011; Kandula et al. 2015).

Camelina sativa seed yields in North America range from <1000 kg/ha to >3000 kg/ha depending on variety, location, water availability and fertiliser inputs (Gugel & Falk 2006; Fleenor 2011). However, the yields reported by Fasi et al. (2012) at

Lincoln University ranged from 664 to 1412 kg/ha depending on sowing date. Their October sowing produced a seed yield of only 865 kg/ha, one-third to one-half lower than the yields recorded a year later in the present experiment from a similar sowing date. Unfortunately seed yield component data were not recorded, and the reason why the *Trichoderma* treatment significantly increased seed yield cannot be explained. It is possible that either better plant survival or greater production per plant for the reasons provided above allowed the positive response, but this will need to be determined in a further set of experiments.

ACKNOWLEDGMENTS

Thanks to the Ministry of Business, Innovation and Employment (Project LINX0802) for funding this work; Voni Fasi for supplying the seed; Alan Marshall, Dave Jack and Daniel Dash (Lincoln University) for their technical assistance; Dylan Coleman, Agrimm Technologies Ltd, Lincoln, for supplying the bioactive and prill formulation and assistance with the field experiment; and Mrs Jayanthi Swaminathan, AgResearch, Lincoln, for supplying the granule formulation.

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