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Testing *Trichoderma* species as biological agents for control of *Dothistroma septosporum* in *Pinus radiata*

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Abstract Biological control of pathogens can be an important tool for long-term management of diseases. Species in the fungal genus *Trichoderma* have been used for a broad range of agricultural functions including biological control. Here we tested if isolates and mixes of *Trichoderma* spp. could be effective in reducing symptoms of dothistroma needle blight, caused by the foliar pathogen *Dothistroma septosporum*, on *Pinus radiata*. Pot trials with natural and artificial *D. septosporum* infection and field trials were undertaken. The majority of *Trichoderma* treatments were not significantly different from the control. However, in one experiment, the *Trichoderma* treatments significantly reduced disease symptoms in one seedlot but not in the other seedlot tested. Conversely, in the field trial, one *Trichoderma* treatment significantly increased symptoms in comparison to the control. PCR analysis indicated that *Trichoderma* can persist over time, 16 months after inoculation, in the soil *P. radiata* seedlings were grown in, however, further investigation is required. Overall, the results from our trials showed that the *Trichoderma* isolates and mixes tested were unable to control dothistroma needle blight in *P. radiata* and are not suitable as biological control agents.

Keywords *Dothistroma septosporum,* dothistroma needle blight, *Trichoderma atroviride, T. hamatum,* biological control, field trials, artificial inoculation

INTRODUCTION

Trichoderma spp. have been used for a broad range of agricultural functions, from growth promotion through to biological control agents (Zin et al. 2020). As biological control agents they can purportedly function through suppressing pathogen growth, based on their antagonistic behaviours *in vitro* (El Komy et al. 2015), or through the induction of resistance responses (Ruocco et al. 2015, Yoshioka et al. 2012). In New Zealand, numerous *Trichoderma* products are sold to promote plant growth and protection. Research into their role as biological control agents has shown *Trichoderma atroviride* is able to reduce dieback caused by *Diplodia pinea* in *Pinus radiata* seedlings by enhancement of systemic resistance responses (Reglinski et al. 2011) and some *Trichoderma* spp. have shown promise as biocontrol agents against *Dothistroma septosporum* based on *in vitro* antagonism (McDougal et al. 2011).

Dothistroma septosporum is a devastating foliar pathogen that causes a disease known as dothistroma needle blight (DNB) and has caused damage to pine species throughout the world (Bulman et al. 2016). Combinations of chemical management and silviculture are currently used to control this disease on susceptible pines. *Pinus radiata*, the most widely planted commercial forestry species in New Zealand, is known to be susceptible to *D. septosporum* and the predominant method of control in New Zealand is aerial spraying of copper oxides in high-risk areas (Watt et al. 2011). A potential alternative way of controlling DNB is through biological control agents that work to reduce disease symptoms. Such agents may be able to control this disease by either directly interacting with *D. septosporum* or stimulating induced resistance mechanisms within the host, ultimately providing enhanced resistance against the pathogen.

In this manuscript we present results from several trials undertaken to determine if isolates or mixtures of isolates of *Trichoderma* spp. could provide control against *D. septosporum* in *P. radiata.* Trials included: (1) pot trials with natural *D. septosporum* infection; (2) pot trials with artificial *D. septosporum* inoculation; and (3) field trials with natural *D. septosporum* infection. In all trials, plants were pre-inoculated with *Trichoderma* spp. The *Trichoderma*

isolates used were selections that showed the greatest promise as successful biological agents, based on previous research undertaken in the Forest Owners Association's 'Bioprotection – enchancing growth and health' research programme. In addition, a PCR-based detection method was used for one of the pot trials with natural *D. septosporum* infection to determine if the *Trichoderma* isolates used could persist in the seedlings and surrounding soil over time.

MATERIALS AND METHODS

Pot trials 1 and 2, with natural *D. septosporum* infection

Pinus radiata plants were obtained from the Scion nursery, Rotorua. All plants had been grown in potting mix and were in their second year of growth and were maintained in Scion's shade house and watered regularly. Two pot trials were undertaken; in each trial 100 seedlings from five randomly chosen seedlots were used; seedlots PR1A, PR2A, PR3A, PR4A and PR5A for pot trial 1, and 5PA, 5PB, 5PC, 5PD and 5PE for pot trial 2. None of the seedlots were known to be resistant to *D. septosporum*.

Four *Trichoderma* isolates were used in pot trial 1, each as a separate treatment: *T. atroviride* isolates LU132, LU525 and LU519, and *T. hamatum* LU592. In pot trial 2 treatments were all *T. atroviride:* isolates LU132, LU525, LU519, each as a separate treatment, and a mixture of *T. atroviride* isolates, LU584 and LU633, for the fourth treatment. The isolates were sub-cultured onto fresh 2 % (w/v) malt extract agar (MEA) and carrot agar (CA; Ganley et al. 2015) plates. The plates were left unsealed but placed into ziplock plastic bags and incubated under ultraviolet light at 20°C to induce sporulation. When the colonies had reached the edge of the plates, each colony was scored with a scalpel to further induce sporulation. Once the strains had sporulated (approximately two weeks later), spore suspensions were prepared for each of the *Trichoderma* isolates.

Spore suspensions were prepared by flooding each plate with 5 mL of sterile water and the resulting spore suspension was collected. A spore suspension, 200 mL, was prepared for each *Trichoderma* isolate with a final spore concentration of 10^6 – 10^7 spores per mL water; 50 µL of the surfactant AGPRO organosilicone (AGPRO NZ Ltd) was added to the suspension. The control solution contained 200 mL of sterile water and 50 µL of AGPRO organosilicone.

The *P. radiata* seedlings, 20 seedlings per treatment, were pre-inoculated with the *Trichoderma* isolates, as described above, or the control solution. Pot trial 1 was inoculated in October 2009 and pot trial 2 in October 2010. The needles of each seedling were sprayed twice and the soil was soaked with the relevant spore suspension or control solution. After the needles were sprayed, the remaining spore solution was sprayed equally between the seedlings into the soil, so each seedling/pot received approximately 12 mL of spore suspension in total. The seedlings were allowed to dry for 1 hour, then the needles were sprayed with a fine mist of water (enough to allow total leaf wetness but not dripping) and the seedlings were sealed in a humid plastic chamber and left for 48 hours. The seedlings were checked regularly to ensure they were not overheating and that constant leaf wetness was maintained. After 48 hours the plants were removed and returned to the Scion shade house. The seedlings were maintained in the shade house at for at least one month to allow sufficient time for colonisation of the seedlings by the *Trichoderma* isolates and for any induced resistance mechanisms to develop.

The *Trichoderma* pre-inoculated and control plants were challenged with *D. septosporum* under natural conditions. The pots were placed under mature *P. radiata* trees that had visible symptoms of DNB in Kaingaroa Forest, Central North Island. The seedlings were randomly divided into four blocks. Each block contained 25 plants, one from each seedlot (five seedlots) for each treatment (five treatments). The seedlings were regularly monitored and watered.

For pot trial 1, plants were placed in the field in November 2009 and collected in March 2010 for assessment, once symptoms of DNB were visible on the seedlings. For pot trial 2, the plants were placed in the field in December 2010, and collected in August 2011 for assessment, once symptoms of DNB were visible on the seedlings. The pot-trial 2 plants were maintained in the Scion shade house until October 2011 when they were returned to the field and collected again in January 2012 for a second assessment. Three scorers visually assessed the plants for the percentage of DNB infection (0% to 100%) over the entire seedling. The disease symptoms were confirmed to be caused by *D. septosporum* through isolation of the pathogen from lesions (data not shown).

The DNB scores were averaged, then transformed with an arc-sine transformation. Homogeneity of variance across treatments was checked. A mixed model was used to analyse data with Seedlot and Block considered as random factors; interactions between random effects and the interaction of random effects with treatment were not included as this markedly reduced the error degrees of freedom. Data were analysed with PROC MIXED using SAS software.

PCR-based detection method for pot trial samples

Three types of samples (pine needles, roots and surrounding soil) were obtained for this experiment in February 2012, from pot trial 2, 16 months after inoculation with *T. atroviride* isolates LU584 and LU633 in order to determine if these isolates were still present. The samples were plated separately on to 4 % (w/v) potato dextrose agar (PDA) containing antibiotics and allowed to grow microorganisms for four to five days at 22°C. For the needles and roots, between 5-10 needles and 5-10 roots fragments (1-2 cm in length) were plated. For soil, 50-100 g soil was sampled per pot, the soil was mixed in 100-200 mL sterile water to make a solution that could be pipetted and a 50 µL aliquot was plated for microorganism identification. For each sample type, there were five replicates from each of the five different seedlots used in the pot trial.

Fungal colonies with different morphologies were isolated and purified. DNA was extracted from four cultures whose morphology (colour, mycelial form, concentric rings and spores) on PDA resembled *Trichoderma* spp. DNA was also extracted directly from mixed samples of pine needles and pine roots, using a cetyltrimethyl ammonium bromide (CTAB) method as described in Doyle and Doyle (1987). PCR was conducted with fungal-specific ITS primers, as a

positive control, and then with *T. atroviride*-specific primers (RM3/4), *T. atroviride* LU584-specific primers (RM1/2) and *T. atroviride* isolate LU633-specific primers (RM5/6) (Table 1).

PCR reactions were performed using a FIREPol® DNA Polymerase kit (Solis BioDyne), according to the manufacturer's instructions. Each 25 µL PCR reaction contained 10x Buffer BD (2.5 µL), 1.5 mM MgCl2, 0.4 µM of each primer, 1.25 U of FIREPol® DNA Polymerase, 1 µL of DNA (approx. 50 ng) and PCR-grade water (up to 25 µL total volume). The cycling conditions consisted of an initial denaturation step of 95°C for 4 min, then 30 cycles of 94°C (1 min), primer-specific annealing temp (Table 1) (1 min) and 72°C (1 min), and a final extension step of 72°C (10 min). Negative controls (no DNA) and positive controls (DNA from separate *T. atroviride* isolates LU584 and LU633) were included. Gels were run on 2.5% agarose in TBE and PCR products were visualised after staining with ethidium bromide.

Pot Trial 3 - with artificial *D. septosporum* inoculation

Pinus radiata seed was provided by PF Olsen Ltd and was sown and maintained in Scion's nursery. Two seedlots were used in this experiment: seedlot A, from *D. septosporum* susceptible, GF16; and seedlot B, from *D. septosporum* resistant, GF26. Eighty seeds from each seedlot were sown, 160 in total.

Three *Trichoderma* spp. mixes that showed the greatest promise as successful biological agents, based on previous research undertaken in the Forest Owners Association's 'Bioprotection – enchancing growth and health' research programme were used: mix C, D and E. Mix C was also known as *Trichoderma* mixture 'no.11' (*Trichoderma atrobrunneum* isolates FCC318, FCC319 and FCC320; *Trichoderma koningiopsis* isolate FCC322; and *Trichoderma harzianum* isolate FCC340). Mix D was also known as Trichoderma mixture 'no. 6 + 180' (*Trichoderma asperellum* isolate FCC13; *T. atroviride* isolates FCC14 and FCC15; *Trichoderma* sp. isolate FCC16, and *Trichoderma crissum* isolate FCC180). Mix E was also known as "*Trichoderma* mixture best 2012 trial" (*Trichoderma* spp. isolates FCC49 and FCC275; *T. harzianum* isolate FCC55; *Trichoderma crassum* isolate FCC362; and *T. atrobrunneum* isolate FCC368)). Inoculum (4.64×10^6 spores per mL for mix C, 4.08×10^6 spores per mL for mix D and 2.45×10^6 spores per mL for mix E) was prepared and provided by Robert Hill (Bio-Protection, Lincoln University). The *Trichoderma* spp. suspensions, 10 mL per pot, were applied directly to dampened potting mix when the *P. radiata* seeds were sown on the 26 September 2013.

The trial was set up as a split-plot design consisting of four replicate blocks with 'biological control treatment' as whole-plot factor and 'seedlot' as split-plot factor. Biological control treatment was a factor with four levels: Control, mix C, mix D, mix E, and seedlot was a factor with two levels: seedlot A and B. Eighty seedlings per seedlot were randomly allocated to each treatment.

Plants were inoculated with *D. septosporum* using the protocols described by Kabir et al. (2013) and using laboratory prepared *D. septosporum* inoculum. A freshly isolated culture of *D. septosporum* was grown and sub-cultured on a pine needle minimal media with glucose (PMMG; McDougal et al. 2011) for three generations to enhance sporulation of the culture. Inoculated plates were incubated at 22°C for seven days. Spores were harvested by flooding the plates with sterile distilled water and were diluted to 3.65×10^6 spores per mL. An aliquot of the spore suspension was plated onto PMMG to confirm spore germination.

The spore suspension was sprayed onto *P. radiata* plants on 22 April 2014, using a hand sprayer and ensuring that the inoculum was distributed over the surface of all the needles. After airdrying for 20-30 min, the plants were covered with a pre-moistened polythene bag for 4 days, then housed in a purpose-built inoculation chamber at Scion, at 20°C, with a 12/12 hour night day cycle), 95% humidity and misting for 10 sec every 15 min.

The plants were monitored regularly for signs of DNB symptoms. Once *D. septosporum* lesions and fruiting bodies were observed on needle lesions, nine weeks after inoculation, the seedlings were individually assessed and the percentage of DNB symptoms over the whole plant was

Table 1 Primers used to detect *Trichoderma atroviride*

Primer name	Primer sequence (5'- 3')	PCR product size (bp)	Remarks
ITS1F	CTTGGTCATTTAGAGGAAGTAA	800-1000	White et al. 1990. Fungal-specific, annealing temp 58°C
ITS4	TCCTCCGCTTATTGATATGC		
RM1	CACTTCCAACTTATTTTCTGCC	95	*T. atroviride* isolate LU584 specific, annealing temp 58°C
RM2	CCGTACATGATGCCTCACA		
RM3	TATTAACTGGTTCCATCGTTGG	154	Mellow et al. 2016.
RM4	TGTGTCCTGGTCGACGTTG		*T. atroviride*-specific, annealing temp 58°C
RM5	GCAAGTTGGATACAGTTGGCT	72	Mellow et al. 2016.
RM6	CCAGTAACTAGAATCCCATG		*T. atroviride* isolate LU633-specific, annealing temp 60°C

scored. The plant symptoms were categorised from 0 to 4 as follows: 0 = none = no lesions; 1 = low = 1% to 25% of the seedling infected; 2 = medium = 26% to 50% of the seedling infected; 3 = high = 51% to 75% of the seedling infected; 4 = very high = 76% to 100% of the seedling infected.

A cumulative link mixed model (CLMM) fitted by Laplace approximation and using a logit link function (R Core Team 2014) was used to analyse the scores for symptomatic needles caused by *D. septosporum*. The model comprised BCA treatment, seedlot, and their interaction as fixed effects and a random term containing BCA treatment nested within block. There was one missing score pertaining to a control seedling from seedlot A in one of the blocks, which died prior to inoculation with *D. septosporum*. Comparisons between models with different threshold structures showed that the model with equidistant threshold was best suited for this data set as judged by the lower value of the Akaike Information Criterion (AIC = 292.6 for the model with flexible thresholds vs. AIC = 288.9 for the model with equidistant thresholds). The significance of the fixed terms was assessed using a backwards selection procedure based on likelihood ratio testing (Zuur et al. 2009). A significant interaction term was followed up with separate models for each seedlot and the significance of the treatment levels was judged using the z-values of the model outputs (Christensen 2013).

Field trial with natural *D. septosporum* infection

A field trial established in an area of Kaingaroa Forest prone to DNB in 2013 by Robert Hill (Bio-Protection, Lincoln University) was assessed in October 2015. The plant material used for this trial was seed from a control pollinated seedlot that consisted of seed from 150 crosses produced from 70 different parents. The same seedlot was used for all treatments. The field trial had a randomised block layout with three blocks (R1- R3), and each block had six randomly allocated treatment plots: T1 *Trichoderma* mixture 'A' PBI (*T. atroviride* isolates LU132, LU140, LU584, LU633); T2 *Trichoderma* mixture 'no.11' (*T. atrobrunneum* isolates FCC318, FCC319 and FCC320; *T. koningiopsis* isolate FCC322; and *T. harzianum* isolate FCC340); T3 *Trichoderma* mixture 'no. 6 + 180' (*T. asperellum* isolate FCC13; *T. atroviride* isolates FCC14 and FCC15; *Trichoderma* sp. isolate FCC16, and *T. crissum* isolate FCC180); T4 *Trichoderma* mixture 'best 2012 trial' (*Trichoderma* sp. isolate FCC49; *T. harzianum* isolate FCC55; *T. crassum* isolate FCC362; and *T. atrobrunneum* isolate FCC368); T5 *Trichoderma* mixture '2nd best 2012 trial' (*T. harzianum* isolates FCC333 and FCC327, *Trichoderma* sp. isolates FCC410 and FCC424); and T6 untreated control (Ganley & Todoroki 2015).

Three scorers visually assessed the trees and gave each tree a score between 1% and 100% to indicate the percentage of DNB infection. Disease levels were scored in 5% increments, except if the level of disease was less than 5%, in which case they were scored between 1% - 4%. Thirty plants were scored per treatment per replicate; a total of 90 plants per treatment. The disease symptoms were confirmed to be caused by *D. septosporum* through isolation of the pathogen from lesions (data not shown).

All analyses were performed using R (R Core Team, 2013), with the "lattice" (Sarkar 2008) and "ggplot2" (Wickham 2009) libraries for graphical plots and data visualisation. The data were analysed using mixed effects modelling with post-hoc comparisons by Dunnett's test. Homogeneity of variance was observed across treatments.

RESULTS

Pot trials 1 and 2 with natural *D. septosporum* infection

For pot trial 1, all plants displayed symptoms of DNB. There were no significant differences in the percentage of DNB infection among any of the treatments (P = 0.1175); the *Trichoderma* isolates did not increase or decrease DNB symptoms in comparison to the control (Table 2).

In pot trial 2, all plants displayed symptoms of DNB in September 2011 but the level of infection was not sufficient to accurately score or analyse (data not shown). The plants were re-deployed to the field and DNB infection was high enough to be scored when re-assessed in February 2012. The results from the February 2012 assessment also showed there were no significant differences in the percentage of DNB infection (P = 0.3710) among any of the treatments and the control (Table 2).

Homogeneity of variance across treatments was observed for both trials, and neither the random seedlot nor block effects were significant.

PCR-based detection method

Nineteen different types of microorganisms were isolated from pine needle, root and surrounding soil samples taken to determine if the *Trichoderma* isolates had persisted over a 16-month period (data not shown). Most of the cultures were obtained from needles (ten) and the least from the root samples (two). Four cultures from the soil samples had morphologies similar to those of *Trichoderma* species and DNA was extracted from these cultures.

When the fungal-specific ITS primers were used, DNA was amplified from all four cultures as well as the root sample but no fungal DNA was detected in the pine needle sample.

Amplification with the *T. atroviride*-specific (RM3/ RM4) and LU584-specific primers (RM1/ RM2) gave PCR products of the expected size from two soil-sample cultures, suggesting these cultures are *T. atroviride* LU584. No PCR

Table 2 Average dothistroma needle blight (DNB) infection percentages for *Trichoderma* species and control treatments in *Pinus radiata* pot trials 1 and 2.

Treatment	DNB (%)	
	Pot trial 1	Pot trial 2
Control	45	28
T. atroviride LU132	39	24
T. atroviride LU519	51	25
T. atroviride LU525	52	26
T. hamatum LU592	44	-
T. atroviride LU584 and LU633	-	38
P-value	*0.1175*	*0.3710*

products were amplified from the two other cultures or from root and needle samples with either the *T. atroviride*-specific or LU584-specific primers suggesting that those samples provided did not contain *T. atroviride*. The LU633-specific primers did not amplify DNA in any of the samples tested apart from the positive controls.

Pot trial 3 with artificial *D. septosporum* inoculation

Infection levels were suitable for disease screening, with over 99% of the seedlings infected and severity ranging from low (1 to 25 % of needles infected) to very high disease levels (over 75% of needles infected).

Analyses showed there was a significant *Trichoderma* spp. mix x seedlot interaction (L = 8.57, df = 3, $P = 0.036$; Figure 1), with reduced DNB infection in seedlot A plants whereas individuals from seedlot B remained largely unresponsive to any of the treatments. Separate analysis for seedlot A showed that the largest and statistically significant effects were from *Trichoderma* mixes D and E (mix D: z = -1.995, $P = 0.046$; mix E: z = -2.731, $P < 0.01$), whereas mix C failed to produce a significant effect relative to the control (z = -1.114, $P = 0.256$). The most effective treatment for seedlot A was mix E, which halved the proportion of plants showing high disease levels (51 to 75 % symptomatic needles) compared

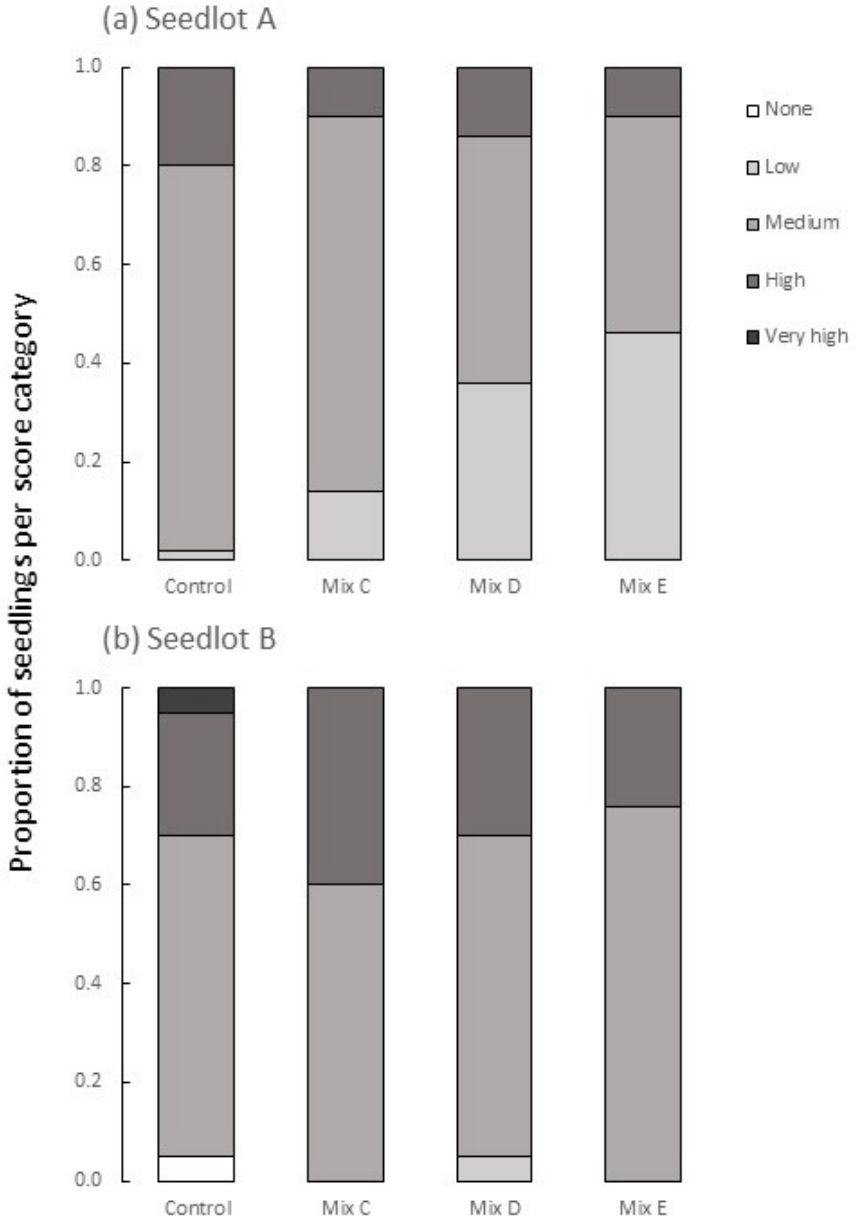

Figure 1 Proportions of *Pinus radiata* seedlings infected with the foliar pathogen *Dothistroma septosporum* per symptom score category. Seedlings were derived from two seedlots (panels a and b) and were either pre-inoculated with one of three mixes of biological control agents (mix C, D and E) or not inoculated (control). The percentage of symptomatic needles per seedling was assessed on a 5-point rating scale shown in simplified form in the figure legend (None = no lesions seen, Low = 1 to 25 %, Medium = 26 to 50 %, High = 51 to 75 %, Very High = 76 to 100 %).

to the control and reduced the proportion of seedlings with medium disease levels (26 to 50 %) from 0.75 in the control to 0.45 (Figure 1a).

Field trial with natural infection

Disease levels in trees in the trial ranged from 1% to 40% of the needles showing symptoms of DNB. Overall, there was no significant difference between treatment means (control 19%, T1 24%, T2 18%, T3 17%, T4 25% and T6 22%) at $\alpha = 0.05$, but at $\alpha = 0.10$ there was a significant difference between means of Treatment 4 and the control ($P = 0.079$), with higher levels of disease symptoms reported in Treatment 4 than in the control. This suggests that none of the treatments were able to reduce symptoms of DNB in comparison to the control (Ganley & Todoroki 2015).

DISCUSSION

Based on the results of these trials it is unlikely that the *Trichoderma* isolates tested suppressed *D. septosporum* or induced a resistance response in the *P. radiata* seedlings, making it unlikely they could function as biological control agents for control of DNB. Results from the pot trials with artificial *D. septosporum* inoculation showed two of the *Trichoderma* mixes were able to significantly reduce disease symptoms but this effect was seedlot specific and was not observed in the other seedlots tested. Conversely, the results from the field trial indicated one of the *Trichoderma* treatments significantly increased disease symptoms. The variation in response across seedlots highlights the complexity of fungal-host interactions and the need to test potential biological control agents against a range of genotypes. Similar testing of *Trichoderma* isolates in different *P. radiata* pine genotypes to infection by the stem canker pathogen *D. sapinea* has shown a wide variation in the response, with responses ranging from an increase in proportion of healthy trees, in comparison to the control, through to no difference or a decrease in the number of healthy trees (T. Reglinski, Plant & Food Research, pers. comm.).

Amaral et al. (2019) showed that timing of pre-inoculation of *P. radiata* with *Trichoderma viride* prior to pathogen (*Fusarium circinatum*) inoculation was important. Their results suggested that *T. viride* was likely recognised as an invading organism and that if plants were not given sufficient time to recover from the pre-inoculation that it could exacerbate subsequent pathogen infection. In our studies it is unlikely the timing between pre-inoculation to infection influenced results. Pot trial 1 was left for over a month before exposure, pot trial 2 for over two months and pot trial 3 for over a year. The timing between *Trichoderma* species inoculation and out-planting of the field trial is unknown, but as this trial was assessed two years later, pre-inoculation timing is unlikely to have influenced disease expression.

Although the *Trichoderma* isolates have been shown to direct inhibit growth of *D. septosporum* in culture (McDougal et al. 2011), there was no evidence of significant direct inhibition in this study. The PCR results also suggest the *Trichoderma* isolates did not colonise the needle tissue, but a lack of sensitivity of the PCR assay to detect fungal DNA in needle tissue cannot be ruled out. A nested primer assay may be more effective at detecting *T. atroviride* from environmental samples. *Trichoderma* species are typically soil/root associated fungi but some *Trichoderma* species are able to colonise leaf tissue, although the frequency of isolation has been low (Bailey et al. 2009). Alternatively, it is possible that the direct inhibition observed in culture is not functional *in planta* (Köhl et al. 2019). Viable *T. atroviride* isolates were obtained from some soil samples but not from any of the needles or roots tested. This result indicates there is some level of persistence, although the ability of *Trichoderma* species to persist over time in association with pine requires further investigation.

Some of the same *Trichoderma* mixes or isolates were used in multiple trials. Isolates LU132 and LU633 were used in the pot trials with natural *D. septosporum* infection and combined with other isolates in field trial T1. None of these treatments were effective in significantly reducing disease symptoms despite different application approaches. Similarly, there were crossovers between treatments in the artificial *D. septosporum* inoculations and the field trials. Mix C (artificial inoculation) and Treatment 2 (field trial) were the same, with mix C reducing symptoms whereas in the field T2 had no effect. Different plant genotypes were used within and between the trials so a genotype influence cannot be ruled out. Conversely, artificial inoculations done under optimal conditions do not always reflect results under field conditions where a variety of environment factors can influence efficacy and broad-spectrum activity (Köhl et al. 2019, Velivelli et al. 2014). Mix E (artificial trial) and T2 (field trial) were very similar, the only difference was the absence of isolate FCC275 in T2. Comparison of the results from these trials showed a difference in response, with mix E significantly reducing symptoms whereas in the field trial it significantly increased symptoms in comparison to the control. Again, variation in plant genotype, isolate difference between the treatments and field trial environment could have influenced the different response.

CONCLUSIONS

Overall, the *Trichoderma* isolates and mixtures tested in *P. radiata* against *D. septosporum* in a variety of pot and field trials were not effective at significantly reducing DNB disease symptoms. Findings also indicated genotype-specific plant responses in some trials and persistence of *Trichoderma* species over time in inoculated soil. Further investigation of both is recommended.

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