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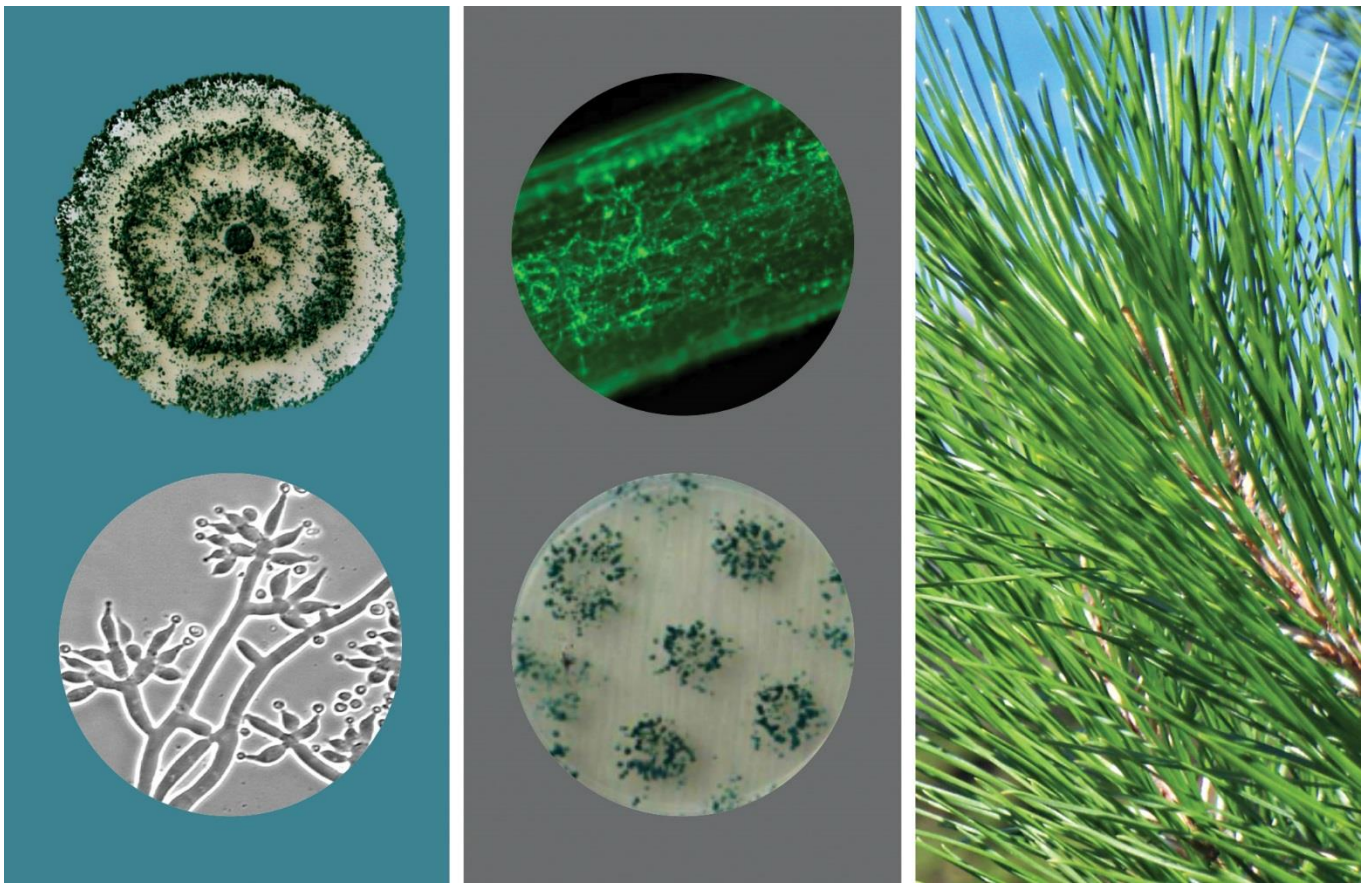

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Report Title	Scoring dothistroma needle blight symptoms in a *Trichoderma* biocontrol field trial
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Scoring dothistroma needle blight symptoms in a *Trichoderma* biocontrol field trial

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Background

Field trials using seed treated with *Trichoderma* species and strains have been set up on different sites across New Zealand. The *Trichoderma* treatments used in these trials are selections that show the greatest promise as successful biological agents, based on previous research undertaken in the 'Bioprotection – enchancing growth and health' research programme.

One of the field trails established, XPKANG 2013, is located in Kaingaroa Forest in an area prone to dothistroma needle blight (DNB) caused by the pathogen *Dothistroma septosporum.* The purpose of this project was to determine if the *Trichoderma* treatments could reduce the symptoms of DNB in comparison to the control treatment.

This project

Field trial XPKANG 2013 had a randomised block layout with three blocks (R1- R3). Each block had six randomly allocated treatment plots (Table 1).

Table 1. Treatments used in this study

Treatment number	Treatment name	Trichoderma isolates
T1	Trichoderma mixture 'A'	PBI (LU132, LU140, LU584, LU633)
T2	Trichoderma mixture 'no. 11'	FCC318, FCC319, FCC320, FCC322, FCC340
T3	Trichoderma mixture 'no. 6 + 180'	FCC13, FCC14, FCC15, FCC16, FCC180
T4	Trichoderma mixture best 2012 trial	FCC49, FCC55, FCC362, FCC368
T5	Trichoderma mixture 2nd best 2012 trial	FCC333, FCC327, FCC410, FCC424
Control (T6)	Untreated	n/a

The genetic material used for this trial was seed from 12/207, a control pollinated seedlot that consists of seed from 150 crosses produced from 70 different parents. The same seedlot was used for all treatments.

Three scorers visually assessed the trees and gave each tree a score between 1 and 100 to indicate the percentage of dothistroma 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. Disease levels in trees in the trial ranged from 1% to 40% of the crown showing symptoms of DNB (Figure 1). The disease symptoms were confirmed to be caused by *D. septosporum* through isolation of the pathogen from lesions (data not shown).

Figure 1. Trees in the field trial displaying symptoms of dothistroma needle blight: (A) approximately 1% infection in the crown and (B) approximately 35% of the crown showing symptoms.

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.

Key Results

There was no significant difference between treatment means at α = 0.05, but at α = 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 (Figure 2). This suggests that none of the treatments were able to reduce symptoms of DNB in comparison to the control.

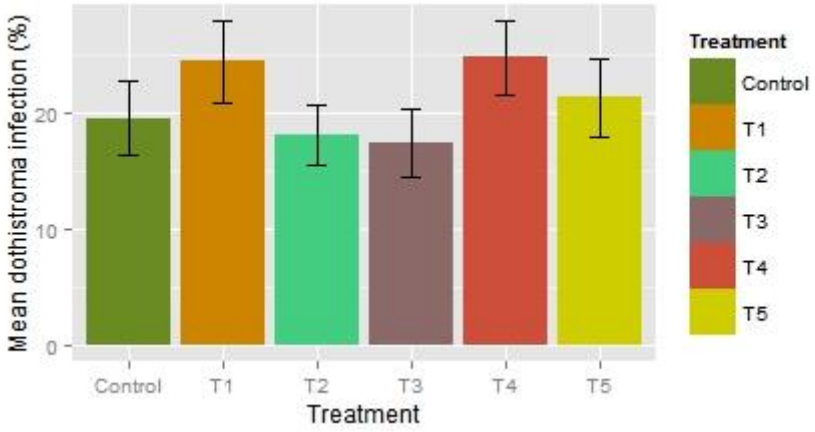

Figure 2. Mean dothistroma infection levels for *Trichoderma* treatments (Treatment 1-5 and Control) with 95% confidence intervals.

Differences between the mean disease scores of replicates were observed, particularly for the control and Treatment 1 (Figure 3). This level of variation could have been due to variation in disease levels across the trial or could have been a result of genetic variation between replicates. The seedlot used for this trial has a high level of genetic diversity (150 crosses produced from 70 different parents) and as DNB resistance is heritable, this could have influenced disease levels across the trial. The variation was not due to the scorers who were relatively consistent with scoring (data not shown).

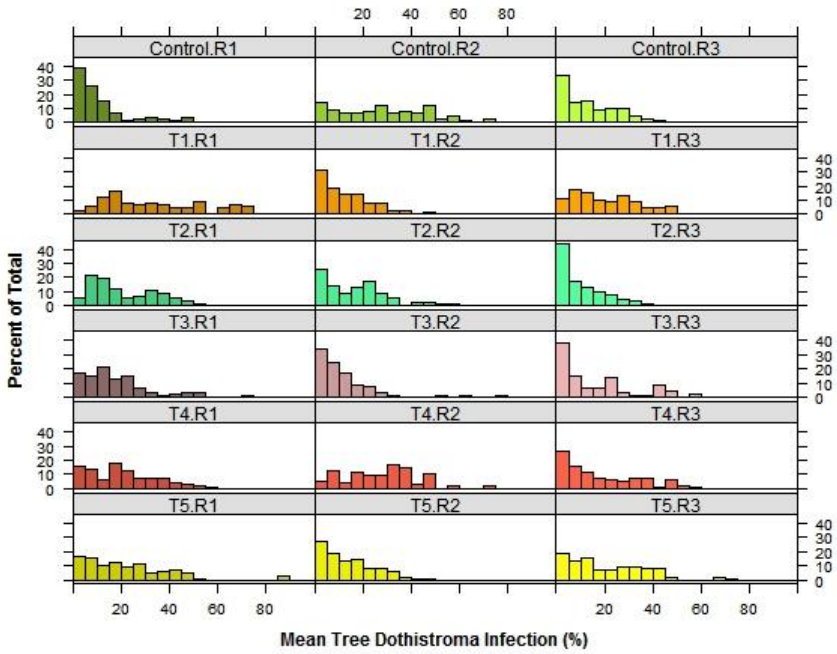

Figure 3. Distribution of mean dothistroma infection scores by treatment (Control, Treatment 1-5, shown by row) and replicate (R1, R2, R3, shown by column).

Comparison of results with controlled environment trials

Of the five treatments assessed in this field trial, the same *Trichoderma* mixes or some of the same species/strains have been previously tested against *D. septosporum* in potted plants exposed to inoculum in the field or in controlled environment trials. These include LU132[1]; LU633[2]; Treatment 2 (Trichoderma mixture 'no. 11', FCC318, FCC319, FCC320, FCC322, FCC340)[3], and Treatment 4 (Trichoderma mixture best 2012 trial, FCC49, FCC55, FCC362, FCC368)[3]. The plant material used in the field trial is not the same as that used in the controlled environment trials. In Ganley et al (2010)[1] seedlings from five, randomly chosen, different seed lots were used; in Ganley (2012)[2] seedlings were from a mixed seedlot; and in Ganley and Bader (2014)[3] seedlings were from control pollinated seedlots that were known to be *D. septosporum* susceptible and resistant.

[1] Ganley R, Bulman L, Gardner J, McDougal R, and R Bradshaw. 2010. Testing strains of *Trichoderma* against *Dothistroma septosporum*. Field Trial 2009/10. Scion report output 46215.

[2] Ganley R. 2012. Testing biological control agents against *Dothistroma septosporum*. Field trial 2010/11. Scion report output 48938.

[3] Ganley R and M Bader. 2014. Task 4. Challenge BCA pre-inoculated material against *Dothistroma septosporum* under greenhouse conditions. Scion report output 53973.

In the previous studies LU132, LU633 and the Treatment 2 *Trichoderma* mixture were not able to reduce disease symptoms of DNB in comparison to the control. These findings are in keeping with the results from this field trial. In contrast, Treatment 4 in a controlled environment trial was able to significantly reduce disease symptoms in comparison to the control in one seedlot tested, but in the other seedlot tested there was no significant difference in disease symptoms. When Treatment 4 was tested in the controlled environment trial, the *Trichoderma* mixture had also included *Trichoderma* isolate FCC275. This was not included in the mixture used for the field trial as it did not grow well in large scale production.

The results from the field trial for the Treatment 4 mixture were different from those from the controlled environment trial. The variation in response to genotype observed in the controlled environment trial suggests the effects on disease levels of this *Trichoderm*a mix could be host specific, and based on the results from both experiments the effects could range from increased resistance through to increased susceptibility, although the increased susceptibility observed in the field trial was only mildly significant at $\alpha = 0.10$. The genotypes used in the field trial were not the same as those used in the controlled environment trial, as field trial Treatment 4 did not include isolate FCC275 it is difficult to make definitive comparisons between the studies.

Implications of Results for Client

The *Trichoderma* mixtures tested in this field trial were not able to reduce the symptoms of dothistroma needle blight in radiata pine planted under field conditions.

Results from both the field and controlled environment trials suggest there might be a complex interaction between *Trichoderma* spp. and radiata genotypes, with effects from the *Trichoderma* treatments ranging from increased resistance through to increased susceptibility.

References

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Acknowledgements

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