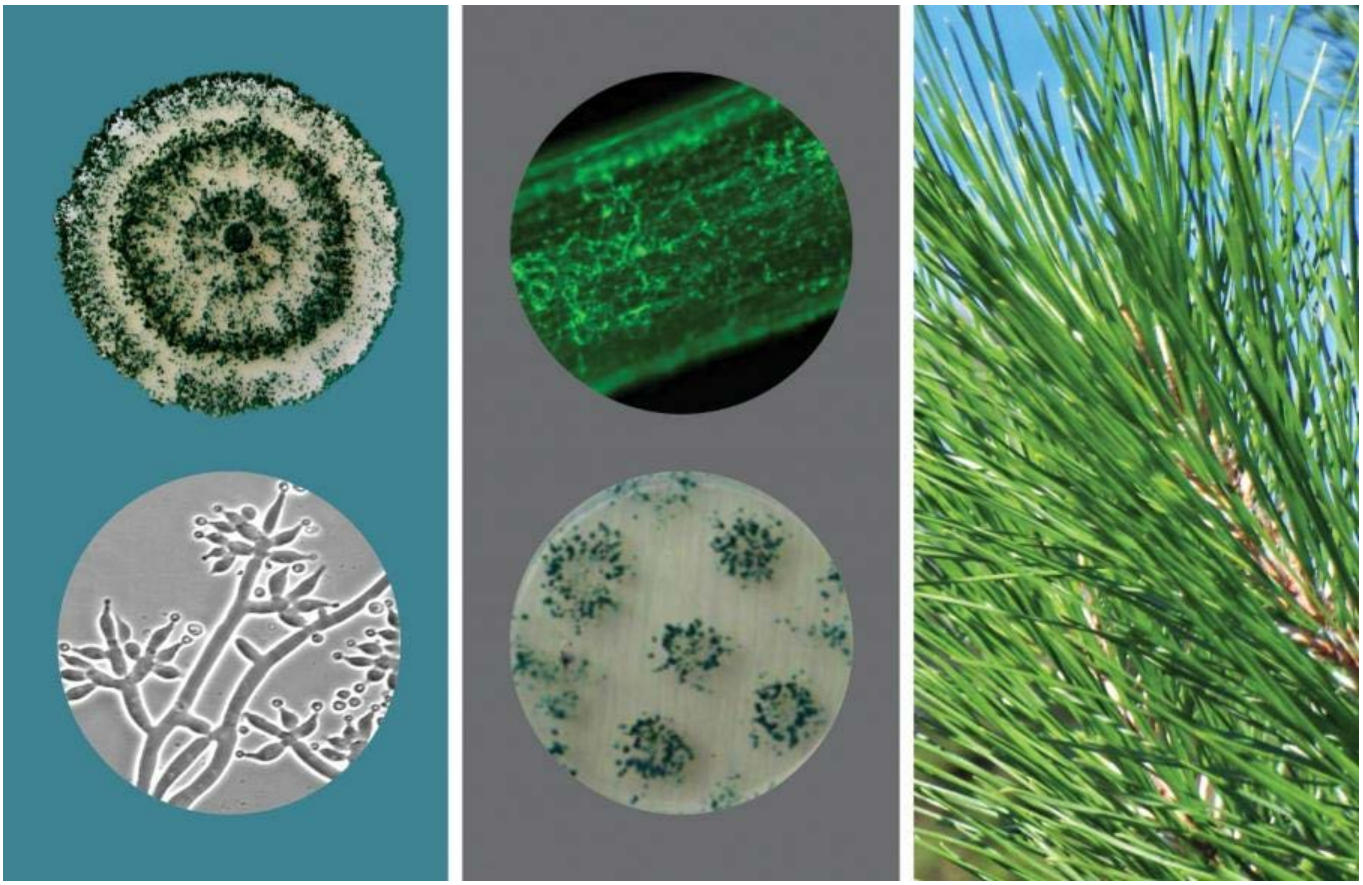
Bioprotection - enhancing growth and health Research Programme

Bio-Protection
Bioprotection science for New Zealand

Task 1.4 Testing biological control agent (BCA) inoculated material against *Phytophthora pluvialis in planta*

Rebecca Ganley and Martin Bader



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Rebecca Ganley and Martin Bader
Scion

June 2015

Table of Contents

EXECUTIVE SUMMARY

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Authors: Rebecca Ganley and Martin Bader

The problem

The "Bioprotection for foliar diseases and disorders of radiata pine" project is investigating organisms that have the potential to be used as biological controls. The objective of this task was to test the ability of biological control agents (BCAs), inoculated in *Pinus radiata*, to reduce symptoms of red needle cast (RNC).

This project

Three different seedlots of *Pinus radiata* (radiata pine) seed were pre-inoculated with *Trichoderma* spp. mixtures (mixes A and B) and later were inoculated with foliar endophytes. Four treatments were applied: *Trichoderma* mix A; endophytes; *Trichoderma* spp. mix A + endophytes; *Trichoderma* spp. mix B. A control treatment, not inoculated, was also included. Branches from the pre-inoculated plants were then challenged with *Phytophthora pluvialis* or water control treatments *in planta* and the needles were later scored for red needle cast symptoms. Using a linear mixed effects model (LME) the data were analysed to determine if there were significant differences in lesion length between the treatments and also analysed as proportion data, for number of lesions per fascicle, using a generalised linear mixed model (GLMM).

Key Results

In the *in planta* experiment, the endophytes alone treatment was able to significantly reduce the number of lesions in comparison to the control, but did not significantly reduce lesion lengths. There were no significant differences in red needle cast symptoms, in comparison to the control, for the rest of the treatments tested.

Significant differences in seedling height varied between the seed lots and there was no treatment that gave heights significantly greater than the control for all three seedlots. The *Trichoderma* treatments all had significantly greater steam diameter than the control and endophytes alone treatment.

Comparison of the detached needle and in planta assays

For the majority of treatments x seedlot combinations, there were no significant differences in comparison to the control for both the detached needle and *in planta* assays. The exceptions to this were some of the endophyte treatments, which showed significantly greater disease symptoms in comparison to the control in the detached needle assay but no difference in the *in planta* assay. Only one opposite result was observed; in one seedlot the endophyte treatment applied alone significantly increased lesion frequency in the detached needle assay but significantly decreased it in the *in planta* assay.

Implications of Results for Client

The *Trichoderma* mixes tested in this study were not effective in reducing red needle cast symptoms and would not be recommended for controlling this disease. The endophyte treatment showed promise as an effective biological control agent and needs further investigation. Detached needle assays are not recommended for testing foliar endophytes but could potentially be used for screening *Trichoderma* treatments.

Further Work

It is recommended that further testing of the endophyte treatment is undertaken to determine whether these could be effective biological control agents and to investigate whether the reduction in symptoms is an induced resistance response. Further testing of the detached needle assay versus *in planta* assay for *Trichoderma* treatments, and the relationship between plant growth and pathogen resistance, is also recommended.
Soil and root samples collected will be tested for the persistence of some of the *Trichoderma* strains applied to the plants using strain-specific primers.

Introduction

Red needle cast (RNC) was first reported in New Zealand forests in 2008 causing needle cast in *Pinus radiata*. The disease is characterised by olive green lesions with black resinous bands that turn yellow and then red, and are easily shaken from the trees. A new-to-science pathogen, *Phytophthora pluvialis,* was found to cause the disease (Dick et al. 2014). The disease can adversely impact productivity, in one site affected by RNC there was a 38% reduction in increment during the growth year immediately following severe infection (Beets et al*.* unpublished data[1]).

Artificial inoculation methods for *P. pluvialis* have recently been developed which include both *in planta* inoculations and detached needle assays (Dick et al. 2014; Williams unpublished data[2]). The ability of biological control agents (BCAs) to reduce symptoms of RNC have been tested using a detached needle assays (Ganley and Bader unpublished data[3]), but are yet to be tested *in planta*. The aim of this study was to determine whether BCAs, pre-inoculated in *Pinus radiata*, could reduce symptoms of RNC *in planta*.

This project is part of the "Bioprotection for foliar diseases and disorders of radiata pine" research programme designed to identify and develop biological control methods targeted at foliar diseases and disorders of *Pinus radiata.* This report summarizes the results from Milestone 1: Controlled environment trials, 1.4 Evaluate the most effective treatments for potential to control foliar pathogens (*Phytophthora pluvialis*) in at least two pine seedlots.

[1] Beets, P., McKinley, R., Oliver, G., Pearce, S., Bulman, L., & Graham, D. (2013). *Impact of red needle cast defoliation of radiata pine on stand growth at Wharerata Forest*. Scion internal report (SIDNEY output 50788).

[2] Williams, N. (2013*). Red needle cast SOP*. Scion internal report (SIDNEY output 51194).

[3] Ganley R and M Bader. (2014).Task 3: *Testing biological control agent (BCA) inoculated material against* Phytophthora pluvialis *using a detached needle assay.* Scion internal report (SIDNEY output 53018).

Materials and Methods

Plant material and biological control agents

Pinus radiata: Three seedlots were used in this study: Seedlot A, *Dothistroma septosporum* susceptible, GF16; Seedlot B, mid-range *Dothistroma septosporum* resistance, GF20; and Seedlot C, *Dothistroma septosporum* resistant, GF26. Forty-five seeds from each seedlot were sown, 135 in total. All seed was provided by Wei-Young Wang (PF Olsen Ltd) and was sown, treated and maintained at Scion under standard nursery conditions.

Biological control agents: Two *Trichoderma* spp. mixes were used, mix A and mix B. Inoculum was prepared by filtering spores from pre-inoculated peat/wheatbran/water mixtures provided by Robert Hill (Bio-Protection, Lincoln University). Spore suspensions of 9×10^6 spores per ml for mix A and 6.5×10^6 spores per ml for mix B were prepared.

One endophytes combination was used which contained isolates NZFS 3305 (E5), 3259 (E14), 3306 (E16), 3311 (E31), 3226 (E46) and 3289 (E55). The isolates were obtained from healthy trees in an RNC-affected stand[4] and have previously been tested against *Diplodia pinea* by Tony Reglinski at Plant and Food Research[5]. The fungal endophytes were grown on potato dextrose agar (PDA) for seven days, and then a spore and mycelial suspension was prepared by scraping the surface mycelium from all cultures and blending them in sterile water using a IKA®ULTRA TURRAX®Tube Drive. Endophyte suspensions contained a final concentration of 0.5 mg wet weight mycelium per mL for each endophyte isolate.

Experimental design

The trial was set up as a split-plot design using 4 BCA treatments (*Trichoderma* spp. mix A, endophytes, *Trichoderma* spp. mix A + endophytes, *Trichoderma* mix B) plus control as whole-plot factor, *Pinus radiata* seedlot (A, B and C) as split-plot factor and pathogen (uninoculated control, inoculated with *Phytophthora pluvialis*) as split-split-plot factor.

Biological control agent inoculations

Trichoderma spore suspensions were applied directly to dampened potting mix when the seed were sown. A total of 54 seeds were pre-inoculated with *Trichoderma* spp. mix A and 27 seeds with *Trichoderma* spp. mix B.

When the seedlings were one year old, half the untreated plants and half the *Trichoderma* spp. mix A treated plants were inoculated with a selection of foliar fungal endophytes.
The suspension of endophytes was applied immediately to *Pinus radiata* needles using hand-held sprayers. Plants were maintained in a growth chamber at 22 ^{o}C,100% relative humidity, with a 16-h day and 8-h night lighting regime for 48-h.

On plant inoculations

Inoculation: the *in planta* assays were undertaken when the plant material was 24 months old. Zoospore suspensions of *Phytophthora pluvialis* were prepared using the red needle cast Standard Operating Procedure (SOP)[2]. Suspensions were cultured and prepared in Scion's Forest Protection laboratory to produce sufficient quantities of inoculum containing zoospores at a final concentration of 4×10^3 per ml. Autoclaved pond water was used for controls.

The plants were inoculated with zoospore suspensions within two hours of inoculum being produced. For each tree, one branch was inoculated, using plastic bags, with 30 ml of zoospores suspension and another with 30 ml of pond water. The needles were left in the inoculum for 20 hours outside. The

[4] Ganley, R. (2009). *Selection of fungal endophytes for future disease resistance trials.* Scion internal report (SIDNEY output 45271).

[5] Reglinski, T, Taylor J.T., Spiers T.M. and Ah Chee A. (2011) *Effects of fungal endophytes on* Pinus radiata *resistance to* Diplodia pinea. Plant and Food Research internal report (SPTS No. 5625).

following day, the plastic bags were removed and the plants were left outside for a further 11 days to allow lesion development.

Lesion assessment: Ten fascicles, with the bases intact, were harvested from each inoculated branch, both *P. pluvialis* and control treatments, 11 days after inoculation. The number of needles present, the length of each lesion on each individual needle, and the total number of lesions per needle was recorded. Due to the inherent subjectivity when counting lesions, the number of independent scorers for *P. pluvialis*-infected needles was limited to ensure consistent lesion scoring.

Any needles displaying RNC-like symptoms from the controls and a selection of *P. pluvialis*-inoculated needles were tested for the presence of *P. pluvialis*. Lesions were sectioned from the needles, surface sterilised with 70% ethanol for 30 sec, and rinsed twice with sterile distilled water. After blotting dry, the needle sections were plated onto PARP agar and incubated at 17°C for 5-7 days before assessment.

In addition to the lesion scoring, plant height and stem diameter were measured and recorded for each plant. Soil and root material was also taken from several plants from the control and treatments. These samples will be sent to Massey University to determine persistence of select *Trichoderma* strains using strain-specific primers.

Data preparation

The total lesion length per fascicle was calculated followed by the computation of the average per 10 fascicles per tree. In order to analyse the data as a proportion of fascicles with lesions, a binary response variable had to be created first by calculating the number of fascicles with lesions per tree. This information was used to construct a two-column matrix holding the number of 'successes' (fascicles with lesions) and the number of 'failures' (fascicles without lesions) as response variable for a binomial regression model. For an analysis of the proportion of affected fascicles, any fascicle with at least one needle showing lesion development was considered to have lesions.

Statistical analysis

A linear mixed effects model (LME) fitted by restricted maximum likelihood was applied to analyse the cumulative lesion length per fascicle using R version 3.1.2 (R Development Core Team 2014, Pinheiro et al. 2014, R-package nlme). The model contained 'BCA', 'seed lot', 'pathogen' and their interaction as fixed effects. The random term of the model contained 'seed lot' nested in 'BCA' nested in 'tree' (block). Multiple comparison testing was performed using Tukey contrasts and the Benjamini & Hochberg (1995) method to adjust P-values for multiple testing (R-package multcomp). Graphical model validation tools were used to check the model assumptions of variance homogeneity and normality (plots of standardised residuals vs. fitted and explanatory variables and quantile-quantile plots). Variance heterogeneity was detected and modelled applying a combination of an exponential variance structure using the fitted values as variance covariate and a constant variance function using 'pathogen' as grouping variable (varExp and varIdent functions in R-package nlme).

The data set was also analysed as proportion data using a generalised linear mixed model (GLMM) with binomial errors and logit link fit by Laplace approximation (Bates et al. 2014, R-package lme4). The fixed and random terms were the same as described for the LME in the previous paragraph. A significant interaction term was followed up with a multiple comparison procedure using Tukey contrasts and the Benjamini & Hochberg (1995) method to adjust P-values for multiple testing (R-package multcomp). Deviance residuals were plotted against the fitted values and all explanatory variables for model validation.

The plant height and stem diameter data was analysed with similar linear mixed-effects models as described above. However, since an individual tree served as a block (individual branches served as control or were inoculated with *Phytophthora pluvialis*), the fixed term for these models only contained BCA, seedlot and their interaction. The same model validation and multiple comparison procedures as described above was applied to the final models.

Results

Lesion length
Lesion formation was greater in *Phytophthora pluvialis* inoculated material and there was a significant seedlot × pathogen interaction (F = 14.09, P = 0.019; Table 1A, Appendix). However, the BCA treatments had no significant effect compared to the *Phytophthora pluvialis* controls and did not differ significantly among each other (Fig. 1).

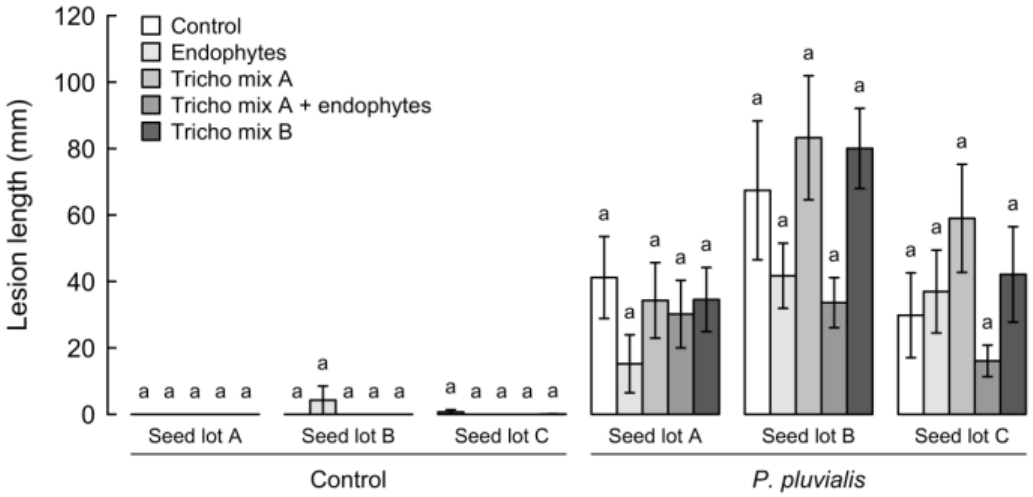


Figure 1 - Average lesion length per fascicle in *Pinus radiata* trees originating from three seedlots that were treated previously with BCA treatment and were then either inoculated with *Phytophthora pluvialis* or uninoculated (Control). Different lower-case letters indicate significant differences between BCAs within seedlot and pathogen treatment at α = 0.05, letters in brackets denote P = 0.05 – 0.1 (multiple comparison test using Tukey contrasts). Means ± SE, n = 9 trees.

Proportion of fascicles with lesions
Analysis of the proportion of fascicles with lesions showed there were significant seedlot and pathogen interactions (Table 2A, Appendix). In contrast to the lesion length results, there were also significant differences in the proportion of fascicles with lesions between the different treatments (χ^2 = 65.92, df = 13, P < 0.001; Fig. 1). Specifically, the endophyte treatment in seedlot A significantly reduced the number of fascicles with lesions and there was also a significant reduction with Trichoderma mix A + endophytes and Trichoderma mix B treatments for seedlot A (Fig. 2). For the seedlots B and C there were no significant differences in treatments applied.

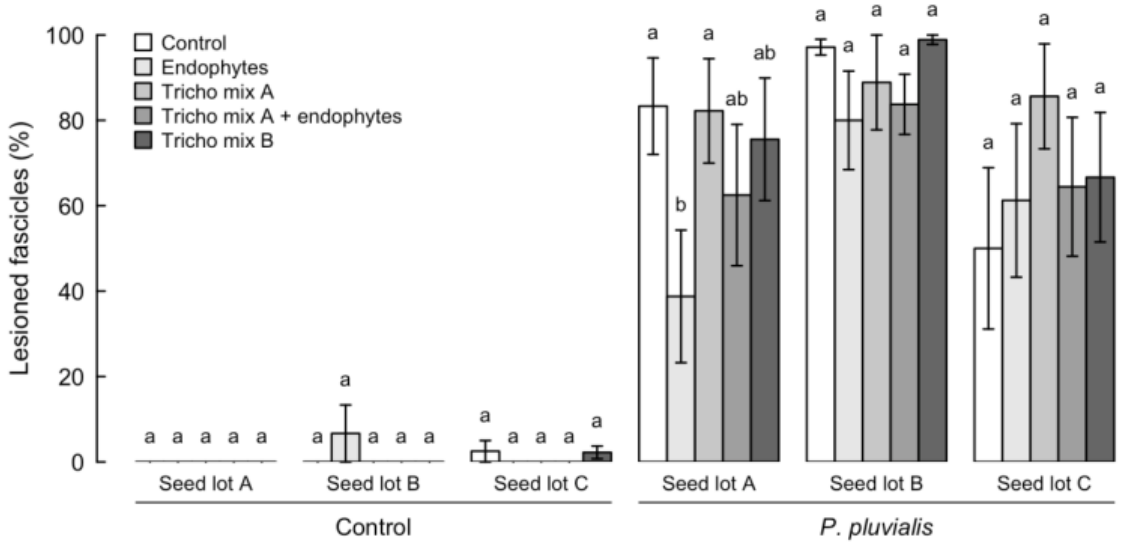


Figure 2 - Percentage of fascicles with lesions in *Pinus radiata* trees originating from three seedlots that were treated with BCAs and were either inoculated with *Phytophthora pluvialis* or uninoculated (Control). Different lower-case letters indicate significant differences between BCAs within seedlot and pathogen treatment at $\alpha = 0.05$ (multiple comparison test using Tukey contrasts). Means ± SE, $n = 9$ trees.

Phytophthora pluvialis was not re-isolated from any of the control needles tested, including those showing RNC-like symptoms, but was re-isolated from all of the inoculated material tested

Plant height

The presence of BCAs had a significant effect on plant height that varied with seedlot (BCA × seedlot interaction, $F = 3.69$, $P < 0.001$; Table 3). The endophyte treatment alone significantly reduced height in Seedlot B but did not significantly differ from many of the other treatments in seedlot A or C (Fig 3.). In seedlot A, Trichoderma mix A + endophytes significantly increased the height of the seedlings, but yet in seedlots B and C there was no significant difference between this treatment and the control or some of the other treatments (Fig 3.). Trichoderma mix A significantly increased plant height in seedlot A but there was no significant difference in comparison to the control for the remainder of seedlots (Fig 3.). Trichoderma mix B did not differ in height from the controls for any of the seedlots.

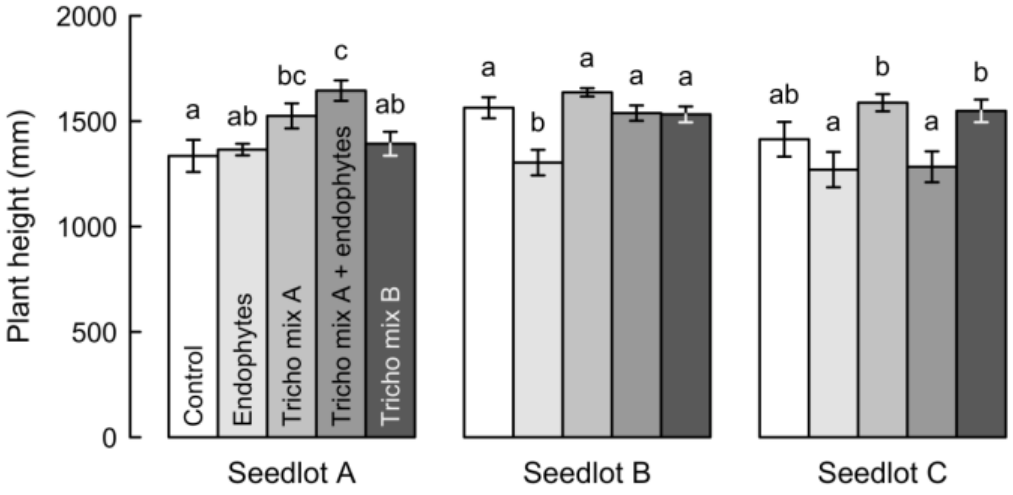


Figure 3 - Plant height of *Pinus radiata* from three seedlots that were treated with BCAs. Different lower-case letters indicate significant differences between BCAs within seedlot at $\alpha = 0.05$ (multiple comparison test using Tukey contrasts). Means ± SE, $n = 9$ trees.

Table 3 - Results from a linear mixed-effects model applied to the stem height and diameter linear data. DF_{num} = numerator degrees of freedom, DF_{den} = denumerator degrees of freedom, F = F-ratio, P = P-value.

Parameter	DF_{num}	DF_{den}	F	P
Stem height				
Intercept	1	91	13630.37	< 0.001 ***
Biocontrol agents	4	91	8.87	< 0.001 ***
Seedlot	2	91	2.43	0.094
Biocontrol agents × seedlot	8	91	3.69	< 0.001 ***
Stem diameter				
Intercept	1	91	8404.51	< 0.001 ***
Biocontrol agents	4	91	10.24	< 0.001 ***
Seedlot	2	91	0.78	0.463
Biocontrol agents × seedlot	8	91	0.60	0.777

Stem diameter

There was no significant BCA × seedlot interaction and no significant seedlot effect (Table 3). However, there was a significant increase in stem diameter with the treatments Trichoderma mix A, Trichoderma mix A + endophytes and Trichoderma mix B (Table 3, Fig. 4) Stem diameter in the endophyte alone treatment was not significantly different compared to the control (Table 3, Fig. 4).

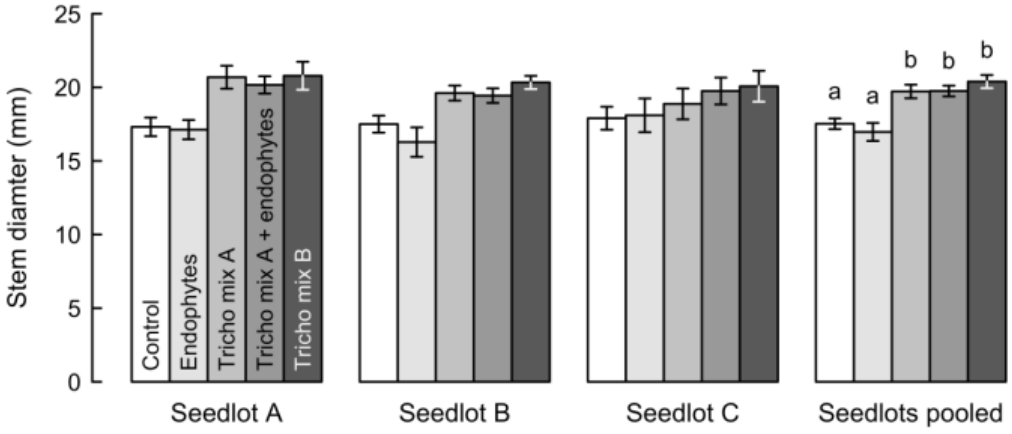


Figure 4 - Stem diameter of *Pinus radiata* from three seedlots that were treated with BCAs. Different lower-case letters indicate significant differences between BCAs at α = 0.05 (multiple comparison test using Tukey contrasts). Means ± SE, n = 9 trees.

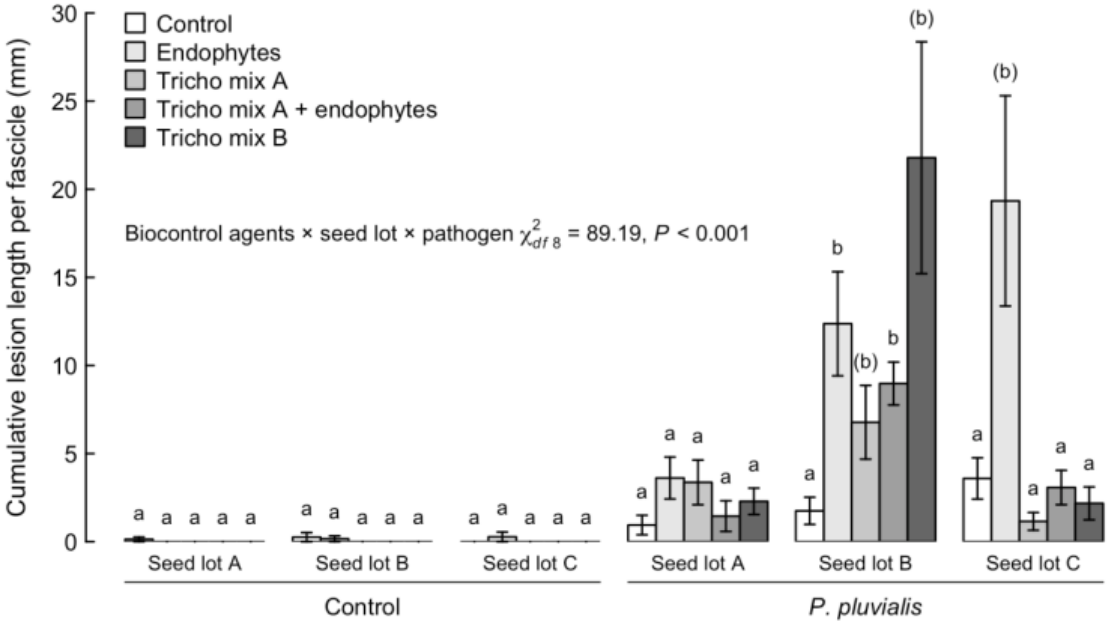
Comparison of detached needle and *in planta* results

Lesion length
There were no differences in results between the detached needle and the *in planta* assay for seedlot A; for all treatments and control there were no significant differences in lesion length (Fig 5). Similarly for seedlot C, there were no significant differences in treatments and control in both assays, with the exception of the endophyte treatment that significantly increased lesion length in the detached needle assay but not *in planta*. In contrast the results for seedlot B were very different between the detached needle assay and the *in planta* assay. In this seedlot all the treatments produced significantly longer lesions in the detached needle assay yet in the *in planta* assay there were no significant differences.

Percentage of fascicles with lesions
Similar to the lesion length comparison between the detached needle and the *in planta* assay, there was no a huge variation in results between the different types of inoculation. For seedlot A there was no significant difference in the number of lesions, with the exception of the endophyte treatment (Fig 6.). In the detached needle assay there was a significant increase in the number of lesions for the endophyte treatment, whereas in the in plant assay it was the opposite and the endophyte treatment significantly decreased the number of lesions. This was the only incident when an opposite effect was detected. In seedlot B the only difference in the two assays was a significant increase in the number of lesions in Trichoderma mix B, and no there were differences between the assay results for seedlot C.

Overall, the lesion lengths were smaller and number of lesions fewer in the detached needle assay than in the *in planta* assay.

A. Detached needle assay



B. *In planta* assay

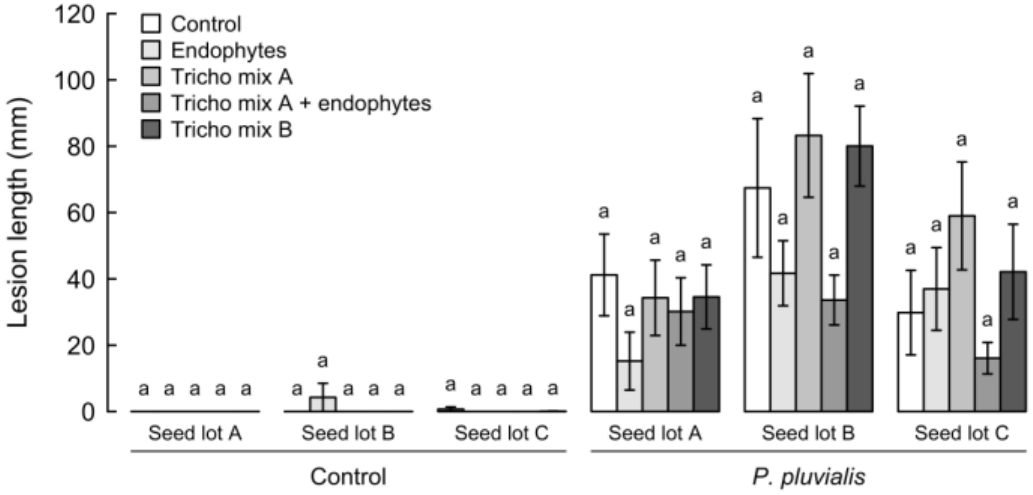
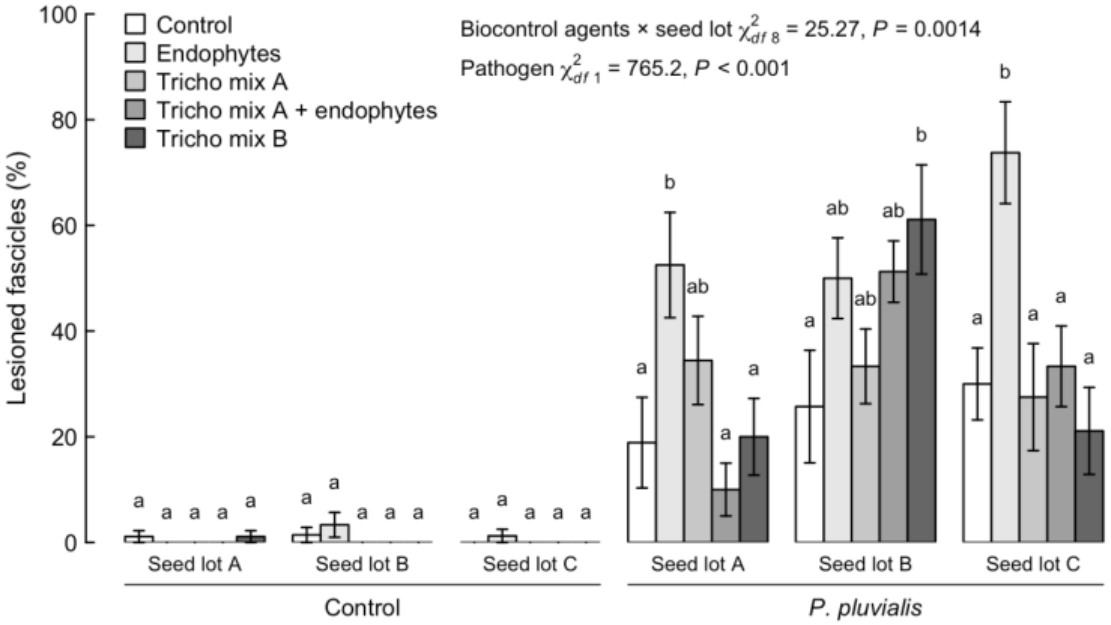


Figure 5 - Average lesion length per fascicle in *Pinus radiata* from three seedlots that were treated previously with BCA treatment and were then either inoculated with *Phytophthora pluvialis* or uninoculated (Control). Figure A are results from the detached needle assay and figure B from the *in planta* assay. Different lower-case letters indicate significant differences between BCAs within seedlot and pathogen treatment at $\alpha = 0.05$, letters in brackets denote $P = 0.05 – 0.1$ (multiple comparison test using Tukey contrasts). Means ± SE, $n = 9$ trees.

A. Detached needle assay



B. *In planta* assay

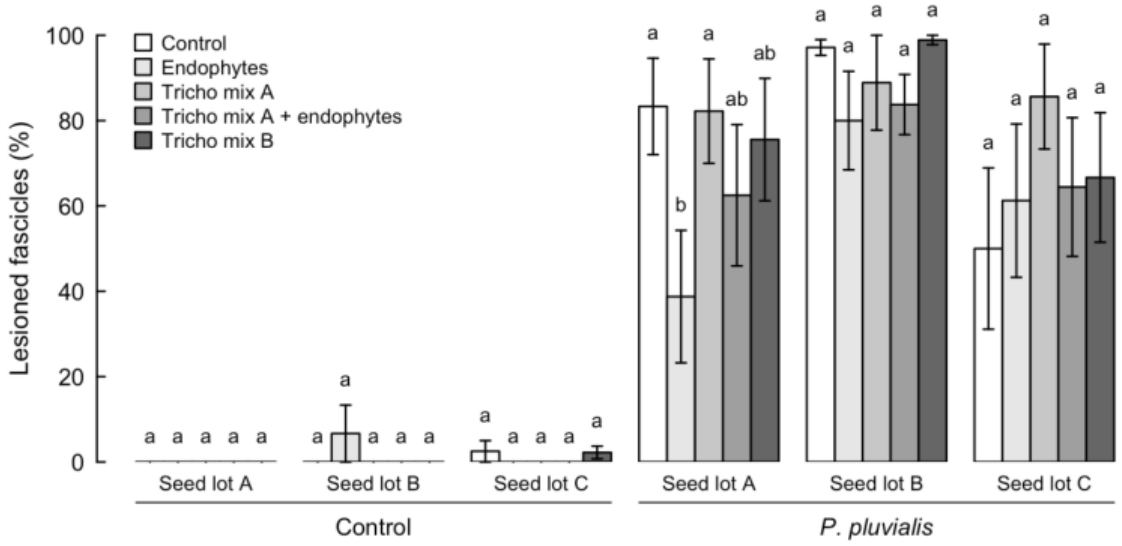


Figure 2 - Proportion of fascicles with lesions in *Pinus radiata* from three seedlots that were treated with BCAs and were either inoculated with *Phytophthora pluvialis* or uninoculated (Control). Figure A are results from the detached needle assay and figure B from the *in planta* assay. Different lower-case letters indicate significant differences between BCAs within seedlot and pathogen treatment at $\alpha = 0.05$ (multiple comparison test using Tukey contrasts). Means ± SE, $n = 9$ trees.

Discussion and Recommendations

The results from this *in planta* assay showed the *Trichoderma* spp. mixes tested did not differ significantly from the control. The only treatment that did significantly reduce lesions numbers was the endophytes applied alone. Based on these results we cannot recommend any of the *Trichoderma* mixes tested in this study as biological control agents for reducing symptoms of red needle cast on infected hosts.

The results from the endophyte treatment, where there was a significant reduction the number of lesions, does warrant further attention. The endophytes used in this study were the same as those previously tested against *Diplodia sapinea* and in one report were also shown to reduce symptoms in their hosts (Reglinski et al unpublished data[6]). Although these endophytes were able to reduce the number of red needle cast lesions in this study, there was no significant reduction in lesion length. Further testing of the effect of these endophytes on disease would be recommended. Of interest, the endophyte treatment was the only treatment in both the detached needle and *in planta* assays where an opposite effect was detected. In the detached needle assay this treatment was found to significantly increase lesion number in seedlot A, conversely in the *in planta* assay it significantly decreased the number of lesions. The endophytes also significantly increased lesion length in seedlots B and C in the detached needle assays, but had no significant effect in comparison to the control in the *in planta* assay. The reasons for this variation between the two assay types are unclear. It could be due to the detached needle assay process or could be an influence of the endophytes increasing the lesion death in the detached needles as a result of their natural senescence process (Unterseher, et al., 2013; Voriskova, et al., 2013).

In general the result from the detached needle assays were not hugely different from the *in planta* assays. The majority of treatments tested showed no significant difference, and any differences were not uniform across all seedlots. The biggest difference between the assays was with some of the endophyte treatments. As discussed previously, the increased lesion lengths in the treatments with endophytes could be due to needle senescence triggering the entophytes' natural decomposition role (Unterseher, et al., 2013; Voriskova, et al., 2013). Based on these results it is not recommended that detached needle assays are used for testing foliar endophytes. There were very little differences in the results between the

[6] Reglinski, T, Taylor J.T., Spiers T.M. and Ah Chee A. (2011) *Effects of fungal endophytes on* Pinus radiata *resistance to* Diplodia pinea. Plant and Food Research internal report (SPTS No. 5625).

Trichoderma treatments. Further testing is required to determine whether detached needle assays could be used in lieu of *in planta* assays. The detached leaf assays are routinely used for phytophthora disease testing worldwide and allow rapid and more screening of material than *in planta* assays (Jinek, et al., 2011; Widmer, 2014, 2015). Good correlation between detached needle assays and *in planta* assays have been observed when testing *Phytophthora pluvialis* pathogenicity (Ganley et al. unpublished data[7]).

Similar to previous red needle cast studies, differences in host susceptibility to *P. pluvialis* were observed. A seedlot x pathogen interaction was observed in both the lesion length and number of lesion analyses, indicating variation in susceptibility of the seedlots to *P. pluvialis*. These results highlight the important of using multiple seed genotypes in this type of screening assay. Lesions were smaller and fewer in the detached needle assay than they were in the *in planta* assay. The assays were not completed at the same time due to resource constraints to complete the experiment simultaneously and the plant material was not big enough to compete *in planta* inoculations when the detached needle assay was performed. The reason behind the observed differences in lesions is not known but could be due to a variety of factors such as climatic differences in the season or year when the assays were undertaken, physiological age differences in the plants, or differences in isolate virulence.

In the control for the *Phytophthora pluvialis* challenge, small bands were recorded infrequently for some of the treatments. These bands are not likely to be RNC lesions, but rather lesions that look similar and are probably due to water-related decay occurring at the fascicle in some needles. No *Phytophthora pluvialis* was isolated from any of the control lesions plated. The same portion of non-RNC lesions scored as RNC is expected to have occurred in the challenged material.

Plant height and stem diameter were measured at the end of experiment as the interaction between plant growth and resistance could influence the effect of biological control agents. In particular reports of growth reductions through resource allocation for defence, and conversely, increased susceptibility to pathogens when resources are diverted for growth (Heil, et al., 2002; Herms, et al., 1992). Although significant increases in plant height were observed with the *Trichoderma* treatments, these differences were not uniform across all seedlots. The endophytes alone treatment had the lowest plant height of all the treatments, and was either significantly smaller or no different from the control for all three seedlots. Interestingly, this was also the only treatment where a significant decrease in disease symptoms was observed. All the *Trichoderma* treatments had significantly greater stem diameters than the control and endophytes alone treatment for all seedlots. Whether the differences in plant growth are related to resource allocation is unknown but these results do highlight the need for further work to determine the influence of plant growth on host resistance.

Overall the results of this study showed the Trichoderma mixes were unable to reduce red needle cast symptoms but that the endophyte treatments showed promise as biological control agents. Further work is needed to investigate the effects of endophytes in reducing disease symptoms both against red needle cast and other foliar diseases.

Acknowledgements

The authors wish to thank Catherine Banham for her assistance with preparing the plant material used in this trial; Scion's Forest Protection red needle cast team for their assistance with the *Phytophthora pluvialis* challenge and Scion nursery staff for maintaining the plant material.

[7] Ganley, R., & Bader, M. (2013). *Milestone 3 (c), Tasks 3 & 4: Test best treatments in young plants against the foliar disease red needle cast.* Scion internal report (SIDNEY output 51311).

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Appendix

Table 1A - Results from the linear mixed-effects model for *Phytophthora pluvialis* lesions length (lesion length model).

DF_{num} = numerator degrees of freedom, DF_{den} = denumerator degrees of freedom, *F* = *F*-ratio, *P* = *P*-value.

Parameter	DF_{num}	DF_{den}	*F*	*P*
Intercept	1	112	5.14	0.025
BCAs	4	32	0.92	0.466
Seedlot	2	72	0.55	0.577
Pathogen	1	112	130.10	< 0.001 ***
BCAs × seedlot	8	72	1.083	0.385
BCAs × pathogen	4	112	2.32	0.061
Seedlot × pathogen	2	112	4.09	0.019 *
BCAs × seedlot × pathogen	8	112	1.08	0.380

Table 2A - Results from the generalised linear mixed-effects model for the proportion of fascicles with *Phytophthora pluvialis* lesions (fascicles with lesions model) based on Wald Chi-square values.
DF_{num} = numerator degrees of freedom, DF_{den} = denumerator degrees of freedom, *F* = *F*-ratio, *P* = *P*-value.

Parameter	χ^2	*DF*	*P*
Biocontrol agents	65.92	13	< 0.001 ***
Seedlot	106.18	10	< 0.001 ***
Pathogen	124.56	3	< 0.001 ***
Biocontrol agents × seedlot	56.90	8	< 0.001 ***
Biocontrol agents × pathogen	0.56	4	0.9671
Seedlot × pathogen	0	2	1
Biocontrol agents × seedlot × pathogen	0	8	1