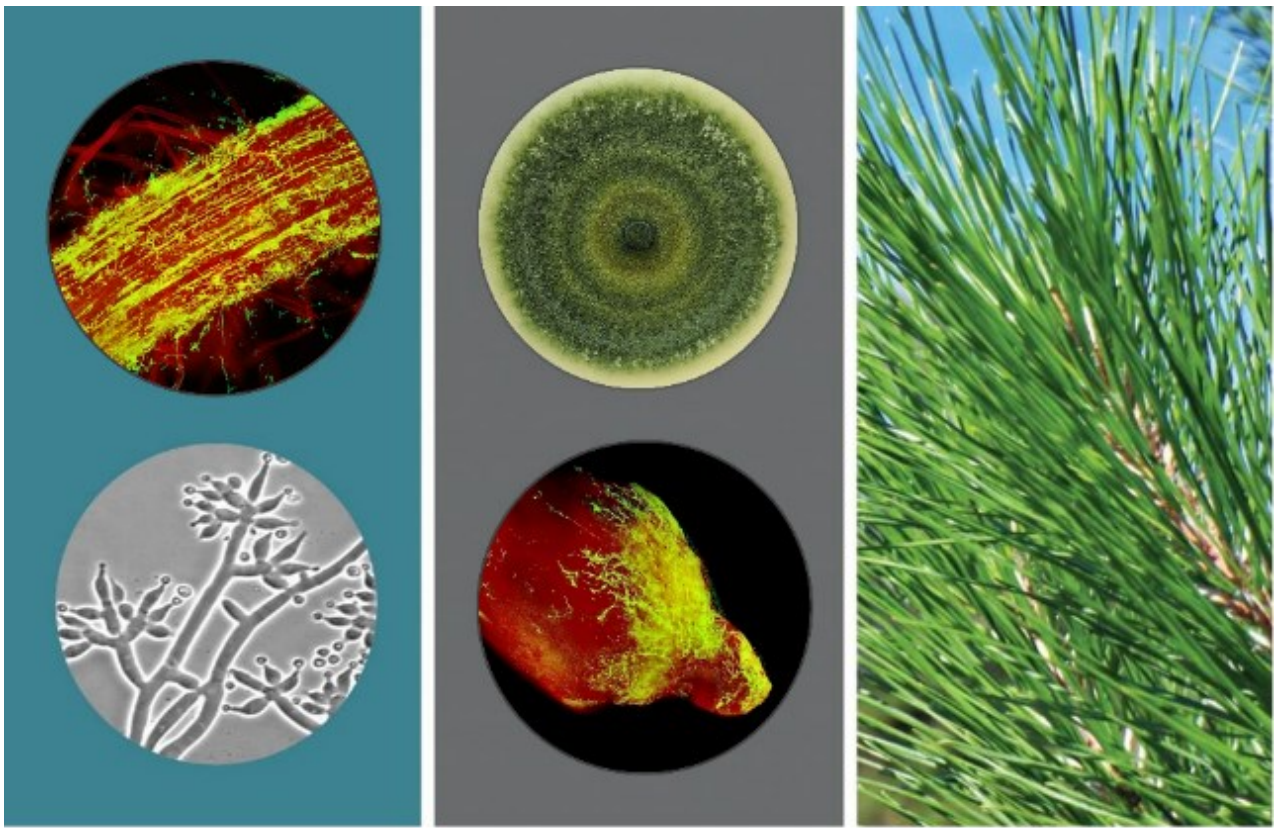
Bio-Protection
Bioprotection science for New Zealand

Bioprotection for foliar diseases and disorders of radiata pine

Project Overview
August 2019 to April 2020

Report prepared for New Zealand Forest Growers Research



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FOREST GROWERS RESEARCH

TABLE OF C0NTENTS

EXECUTIVE SUMMARY

Foliar diseases and disorders are the most significant cause of economic loss for the New Zealand forestry industry. In order to alleviate losses caused by existing diseases and to reduce potential impacts of biosecurity threats, ongoing work (initiated in 2012) is being conducted to establish a long-term symbiotic relationship between *Pinus radiata* (radiata pine), and other forestry tree species, and a beneficial microbe, *Trichoderma*. *Trichoderma* fungi can induce a broad-spectrum activity response from their host plants, enhancing the speed and strength of the plant's response to diseases. *Trichoderma* has also been shown to stimulate growth of plants. The products of this research project will be *Trichoderma* inoculated forestry tree species, with enhanced growth and improved foliar health, potential protection against biosecurity incursions, as well as reduced chemical application in nurseries and plantations.

This report summarises the results measured between August 2019 and April 2020:

- In 2019*, Trichoderma* inoculum was supplied for treatment of approximately 13 million radiata pine seeds in New Zealand forestry nurseries. Two nurseries now inoculate all of their radiata pine seed production with *Trichoderma*.

- Following laboratory, greenhouse and plantation screening programmes, two *Trichoderma* mixtures (PR6 and PR3a) were tested against an untreated Control in large plantation trials in four important forestry regions. One or both of the *Trichoderma* treatments had significantly ($P<0.05$) increased tree height by 7 to 15%, compared to the untreated control, 12 months after planting. When averaged over all trials, tree height was significantly ($P<0.05$) increased by 6% and 7% for PR3a and PR6 respectively, compared to the untreated Control. *Trichoderma* had no effect on tree survival in the majority of trials (average of 97% in all trials). *Dothistroma septosporum* infection was measured in one trial 15 months after plantation planting and *Trichoderma* treatments resulted in an approximately 39% (PR3a) and 50% (PR6) reduction (significant at $P<0.05$) in disease severity, compared to the untreated Control. Further measurements of tree growth and disease levels will be made to confirm the growth promotion and disease suppression effects as the trees mature.

- Application of five *Trichoderma* bioprotectants mixtures (by soil bed drench at setting) to two hard-to-root radiata pine clonal cuttings significantly ($P<0.05$) increased production values by up to 75% (average of 32%), compared to an untreated Control (Control minus fungicide; 20%). Production values were derived using two variables: plants with strongly growing roots in 3 or 4 quadrants, and plants with root collar diameters (RCD) > 6.9mm. *Trichoderma* significantly ($P<0.05$) increased the percentage of plants with strong roots in 3 or 4 quarters and with RCD > 6.9mm by 55% and 38% respectively, compared to the untreated Control. In addition, one or more *Trichoderma* treatments significantly ($P<0.05$) increased survival and root, shoot and plant dry weight by up to 12%, 10%, 11% and 10% respectively, compared to the Control minus fungicide treatment. *Trichoderma* increased survival, growth and production values by a similar amount when compared to another control treatment, Control plus fungicide, which represented nursery standard practice. Therefore, cutting survival, growth and production values of hard-to-root clones could be increased by reducing or eliminating fungicides from standard nursery management practice and replacing with one *Trichoderma* application at setting.

- Generally, *Trichoderma* treatments were not beneficial to the growth of trees measured four to 5.5 years after planting in six national trials, established between 2012 and 2015, compared to an untreated Control. These results were in contrast to results found in other trials established at the same time and measured between September 2017 and March 2019.

- The majority of New Zealand's plantation forests are un-inoculated with *Trichoderma* bioprotectants. Two pilot trials in 2017 and 2019, involving direct application of a *Trichoderma* mixture (PR6) to established plantation trees were set-up. *Trichoderma* was found to have colonised the roots and significantly ($P<0.05$) increased the increment trunk diameter at breast height (DBH) in one trial, and the increment height in the other trial, twelve months after application. Some application methods used in these trials (*eg.* root dowels, trunk spray and trunk injection) are unpractical for large-scale application of bioprotectants. An additional trial using foliar and ground sprays was established in 2019 to determine if *Trichoderma* could be inoculated into established trees in an efficient and effective manner to generate a growth and disease suppression effect.

- Containerised Douglas-fir *(Pseudotsuga menziesii)* seedling roots were highly compatible to six *Trichoderma* mixtures, applied as a potting mixture drench, with *Trichoderma* present in 30 to 97% of root pieces, sampled twelve months after application. Seedling survival of an Oregon provenance seedlot was significantly ($P<0.05$) increased by up to 10% (96%) in one *Trichoderma* treatment, compared to the untreated Control (87%). *Trichoderma* was also highly beneficial to Oregon seedling growth, with significantly ($P<0.05$) increased RCD, height, root, shoot and plant dry weight by up to 14, 25, 45, 38 and 42% respectively, compared to the untreated Control. These results and those found in a 2017 trial, suggest that *Trichoderma* could reduce costs in nurseries by increasing production values and improving establishment of seedlings due to larger initial plant size once planted in the field. The PR6 mixture (a treatment used in the radiata pine validation 2018 trials) had very high levels of root colonisation (>80%), survival and growth in the Oregon seedlings. This mixture may be suitable for containerised Douglas-fir nursery production but further research is required in other genotypes and production systems (*eg.* soil beds).

- In containerised cypress seedlings, *Trichoderma* (applied as a potting mixture drench) significantly ($P<0.05$) increased RCD and height by 7% and plant dry weight by 12% in one or more treatments, compared to an untreated Control. *Trichoderma* had the biggest impact on root dry weight (up to a 27% increase in the five applied treatments) and resulted in up to a 26% increased root/shoot ratio. Therefore, application of *Trichoderma* to cypress seedlings in nurseries may result in trees with improved establishment in the field, due to increased nutrient and water uptake and less socketing and windthrow, compared to untreated trees.

- The commercialisation of selected BPRC *Trichoderma* bioprotectants for nursery use is currently being investigated with a commercial partner.

1.0 INTRODUCTION

Foliar diseases cost the New Zealand forestry industry over NZ$150 million per annum in lost production (Hill, 2016). This research project was developed to find beneficial *Trichoderma* root endophytes that suppress foliar diseases and enhance tree growth in New Zealand's most important forest species, radiata pine.

This project used a novel approach, based on screening *Trichoderma* root endophytes isolated from exceptionally healthy, strongly growing radiata pine and non-forest plants, to streamline the selection of beneficial fungal isolates (Hill, 2016). Nursery and laboratory assays identified many *Trichoderma* isolates that promoted growth and reduced the incidence of Dothistroma, terminal crook and diplodia canker in radiata pine seedlings. The 24 most effective isolates were then tested, as individual or as mixture treatments, in 24 forestry plantation trials in seven forestry regions. Data indicated that many *Trichoderma* treatments significantly increased growth by up to 20%, and reduced disease severity by up to 60%, in trees less than six years of age (Hill, 2016, Whelan, 2019a). The two most effective mixtures, PR6 and PR3a were tested in eight large-scale validation trials in four important forestry regions. The ultimate goal will be the commercialisation of one or more of the mixtures for biocontrol of foliar diseases and growth promotion in forest nurseries and plantations, leading to increased productivity, economic gain and sustainability in the New Zealand forest industry.

NZ Forest Growers Research using recurring 12-month contracts funded this project.

2.0 BIOPROTECTION PROJECT MILESTONES

The project tasks completed for the period July 2012 to July 2019 were summarised in Hill (2016) and Whelan (2019a). Tasks completed between August 2019 and April 2020 are summarised in this report.

2.1 Milestone 1 – Production of *Trichoderma* inoculum

Trichoderma inoculum was supplied in 2019 to the following nurseries: PF Olsen Ltd Waiuku and Seddon, Southern Cypresses, Appletons Tree, Timberlands Te Ngae and Proseed, for seed-coat and/or drench application of seed (approximately 13 million), cuttings or ramets. Appletons Tree Nursery treated all of their radiata pine seeds in September 2019 after very positive results in a Nelson Forests *Trichoderma* plantation trial. In addition, PF Olsen Waiuku treated all of their radiata pine seed, as they have consistently done for the past twenty years. Timberlands Te Ngae Nursery applied *Trichoderma* to a large area of cuttings in late 2019.

2.2 Milestone 2 – Colonisation and Persistence of *Trichoderma* in *Pinus radiata*

A major question arising from the programme is whether the applied *Trichoderma* isolates can persist under nursery and field conditions to provide long-term growth benefits and protection from disease. Selected *Trichoderma* isolates were found to be fast, abundant and persistent

colonisers of containerised radiata pine seedlings and cuttings grown under greenhouse or nursery conditions (Whelan and Hill, 2017, Whelan, 2019b).

In six radiata pine plantation trials, molecular techniques using species- and strain-specific polymerase chain reaction (PCR) primers confirmed the persistence of an applied bioprotectant *T. atroviride* LU633, in the majority (94%) of root pieces tested, 3.5 years after tree establishment. In three of the trials measured with high concentrations of root LU633, one recorded significantly ($P<0.05$) increased tree height and trunk diameter 3.5 years after tree establishment. The persistence of this isolate in the field should be confirmed in future measurements. Molecular techniques for identification of isolates used in validation trials (see section 2.3.2) do not exist and should be investigated in the future (when funds allow) to determine the persistence of these isolates in the field.

The impact of nursery chemicals on colonisation and persistence of *Trichoderma* inoculants was investigated in late 2019 and continued into 2020. Results will be presented in late 2020.

2.3 Milestone 3 – Nursery and forest plantation trials in radiata pine

2.3.1 Effect of *Trichoderma* on rooting of hard-to-root clones

New Zealand forestry production is increasingly reliant on the use of high-quality clonal stock for improved crop quality and productivity, in preference to open and crossed pollinated material. However, clonal stock may have poor root initiation and be susceptible to early 'damping off' disease in the nursery, resulting in high production costs per cutting and low numbers of cuttings available for deployment.

The positive effects of *Trichoderma* bioprotectants on the growth of a hard-to-root radiata pine clone in 2017 (Whelan, 2018c) lead to the establishment of a large trial at Te Ngae Nursery, Rotorua in June 2018 (Figure 1). The 2018 trial had two clones, 48 and 57 (Forest Genetics Ltd, http://www.forest-genetics.com) arranged in a 2x7 factorial design with nine replicates.

Five *Trichoderma* treatments (no fungicide):

- GenMix (FCC320, FCC327, LU633)
- Mixture A (LU297, LU668, LU753, LU996 and LU1328; a mixture with root growth benefits, Hill *et al*., 2010)
- PBI (LU132, LU140, LU584, LU633)
- ModArb (LU655, LU659, LU660, LU661 and LU663)
- PR6 (FCC55, FCC318, FCC327, FCC340; mixture used in radiata pine 2018 validation trials – refer to section 2.3.2)

were compared to two controls:

- Control with no fungicide (Control - fungicide)
- Control and fungicide (Control + fungicide)

One Blue Shield® DF application (1kg copper hydroxide a.i./hectare) was applied in early December 2018; this was nursery fungicide practice in the 2018/2019 season.

Soil preparation in 2018 was similar to the 2017 trial (Whelan, 2018c) with planting-holes made three weeks before planting. Cuttings were hand-planted into three soil bed rows in plots of 1m wide and 3m length on 19 June 2018 (Figure 1). *Trichoderma* treatments were applied as a soil drench (2.1 litres @ 2.8 x 10^6 spores/ml per plot) one day after setting with a watering can. A relatively high rate was used due to the forecast of rain the following day. Cuttings were covered with frost cloth until removal in October 2018. Nursery practice in the trial included irrigation, herbicide applications (3 x Hurricane® (150g a.i haloxyfop-P/ha each spray) and 3 x Asset™ (1kg a.i terbuthylazinegens/ha each spray), lateral pruning, wrenching and topping of cuttings.

The central 32 plants (4 rows x 8 plants) were measured for survival, RCD and root, shoot and plant dry weight on 23 July 2019. Root initiation and development was assessed by scoring the presence of strongly growing roots in each quadrant (root scores 1, 2, 3 and 4 = roots in one, two, three and four quadrants respectively; Figure 2). The percentage of production cuttings was calculated as cuttings with a root score of 3 or 4 and also a RCD greater than 6.9mm. *Trichoderma* root colonisation (%) was determined for five random cuttings and 30 random root pieces, from each plot in replicates 1, 3, 4 and 6, on 20 February 2019 and 23 July 2019. Root pieces were sterilised and placed on malt yeast extract agar with rose bengal (MRB) plates for growth of *Trichoderma* colonies, using established protocols (Whelan, 2018c). Data were analysed for significance by analysis of variance (ANOVA) and unprotected least significant difference (LSD) tests at the 5% level (Genstat, v19).

Figure 1: Te Ngae Nursery 2018 trial at setting of cuttings in June 2018 (a), and harvest in July 2019 (b, c).

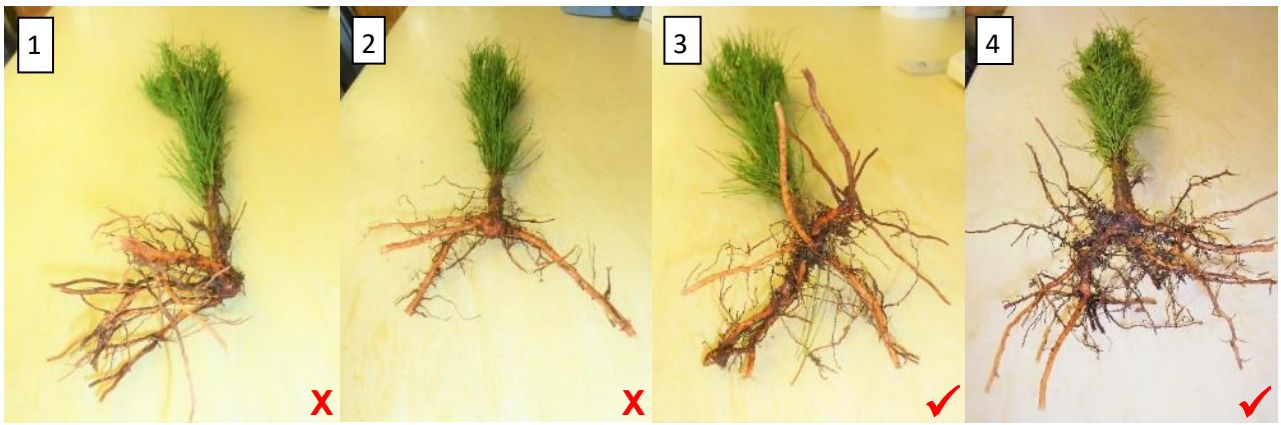

Figure 2: Root initiation and development were assessed by the presence of strongly growing roots in each quadrant. Images 1 and 2 had poor root scores of 1 and 2, whilst images 3 and 4 had good root scores.

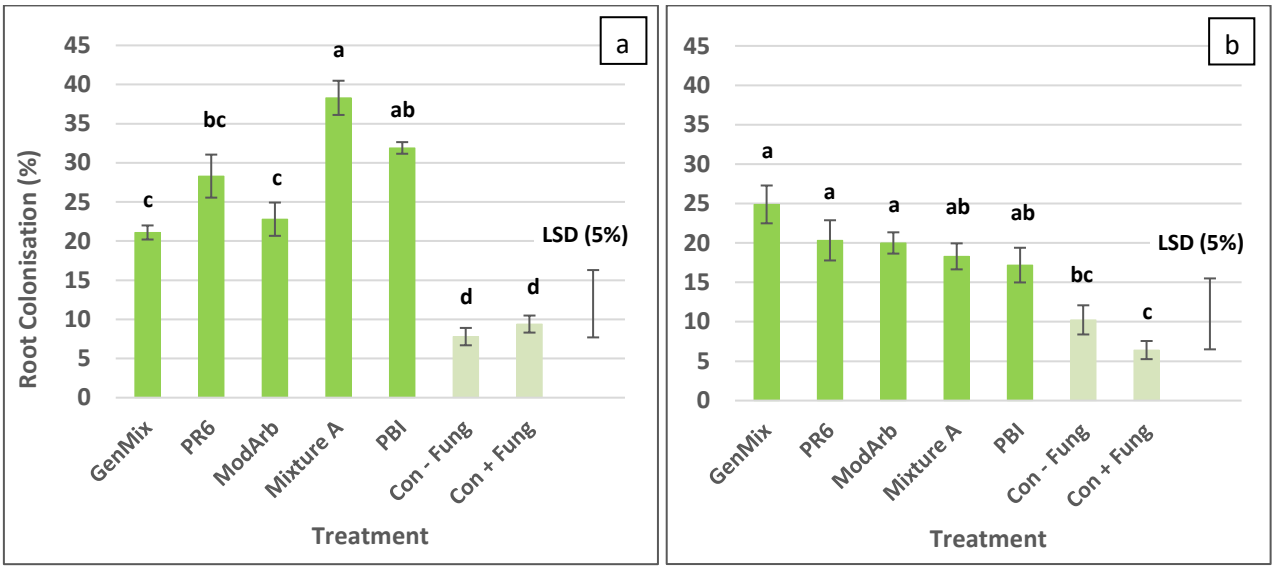

Figure 3: *Trichoderma* colonisation (%) of *P. radiata* roots in treatments, sampled eight (a) and twelve (b) months after setting of cuttings and treatment application, based on MRB plating data.

Soil drench application of *Trichoderma* inoculum resulted in relatively high (between 21% and 38%) colonisation and persistence of *Trichoderma* in cutting roots eight months after treatment (Figure 3a). Root colonisation in *Trichoderma* treatments were significantly ($P<0.05$) greater than both control treatments (<9%; Figure 3a). Although colonisation levels in *Trichoderma* treatments fell to between 17 to 25% at harvest, these were still significantly higher ($P<0.05$) compared to the Control + fungicide treatment (at 6%) but not to the Control - fungicide treatment (10.2%, Figure 3b). Overall, the control cuttings had relatively low levels (≤10%) of environmental *Trichoderma* (*eg*. from the soil and/or airborne spores in the nursery), therefore had only a minimal impact on the results.

No statistically significant interactions were observed between clone and *Trichoderma* treatment for any of the measured parameters (Table 1). Consequently, all results are discussed in terms of main effects. Larger cuttings were found at harvest in Clone 57, compared to Clone 48 (Table 1), as expected according to previous nursery production values (Nigel Heron *pers. comm.*). Clone 57 had significantly ($P<0.05$) increased RCD, root, shoot and plant dry weight and percentage of production cuttings, compared to Clone 48. However, no differences in survival, root/shoot ratio or number of plants with root score 3 or 4 or RCD > 6.9mm were measured between the two clones (Table 1).

Trichoderma treatment significantly improved cutting survival, growth and root initiation and development, compared to the standard nursery practice, the Control + fungicide treatment. All *Trichoderma* treatments had significantly (P<0.05) increased cutting survival at harvest, by 12 to 21%, compared to the Control + fungicide treatment (Table 1). In addition, one or more *Trichoderma* treatments significantly (P<0.05) increased RCD and root, shoot and plant dry weights by up to 6%, 10%, 10% and 9% respectively (Table 1), even though roots and shoots were trimmed during the year as part of standard nursery practice. The largest effect of *Trichoderma* treatment was a significantly (P<0.05) increased percentage of cuttings at the production standard of root score 3 or 4 (by 39.1 to 61.2%), compared to the Control + fungicide treatment (Table 1). *Trichoderma* also significantly (P<0.05) increased (by 13.9 to 30.3%) the number of plants with RCD above the production standard of 6.9mm, compared to the Control + fungicide treatment. This, when combined with cuttings with root scores 3 or 4, resulted in a significantly (P<.05) increased (by 35.4 to 64%) percentage of cuttings at production standard (Table 1).

Results for cutting survival, growth and root initiation and development were also similar when *Trichoderma* treatments were compared to the Control - fungicide treatment. One or more *Trichoderma* treatments significantly (P<0.05) increased survival, RCD, root, shoot and plant dry weight and percentage of plants with root scores 3 or 4, RDC >6.9mm and at production standard, by up to 12%, 7%, 10%, 11%, 10%, 55%, 38% and 75% respectively (Table 1). *Trichoderma* had no effect on the ratio of root and shoot dry weight (Table 1).

The positive effect of *Trichoderma* on cutting survival and size and initiation and development of roots is particularly relevant for nurseries producing clonal stock with low production values, when even small improvements in production values can lead to economic gains. In addition, one application of *Trichoderma* at setting, together with, or as a replacement of fungicide applications, could be a viable management tool for improved production rates. The effect of *Trichoderma* on final cutting size would also be economically important for forestry companies wanting large plants for improved plantation establishment, tree growth rates and ultimately wood production.

Of the five *Trichoderma* treatments, Mixture A and GenMix resulted in the highest survival, RCD, root score of 3 or 4 and the percentage of cuttings at production standard levels (Table 1). PR6, one of the mixtures under evaluation for improved growth and disease resistance in the validation plantation radiata pine trials (see Section 2.3.2), also performed well. PR6 significantly (P<0.05) increased the percentage of cuttings at production standard by 39 and 49%, compared to the Control + fungicide and Control - fungicide treatments respectively (Table 1). ModArb was the least beneficial treatment. In this trial, the number of cuttings at production standard levels in the Control + fungicide treatment (mean of 21.2% for the two clones) was similar to that found for these two clones in the nursery 2019 production figures (Nigel Heron, *pers. comm*.).

This study validated the results found in the 2017 pilot trial (Whelan, 2018c) thereby demonstrating consistent benefits of *Trichoderma* to seedling survival and growth in two hard-to-root cutting clones in seedbed production systems. In response to these results, Te Ngae Nursery applied *Trichoderma* soil drench to 13 hectares of cuttings in December 2019. Further research is required to determine if other plant genotypes and production systems (*eg.* containerised trays) modify the survival and growth effects of *Trichoderma*, in order to improve *Trichoderma* application decisions in radiata pine nursery production.

Table 1: Effect of *Trichoderma* and clone on survival, root collar diameter (RCD), root, shoot and plant dry weight, percentage of plants with root score 3 or 4 or RDC >6.9mm and production standard values of radiata pine seedlings twelve months after setting and inoculation on 23 July 2019.

	Survival of plants with roots (%)	Root Collar Diameter (mm) [a]	Mean Dry Weight (g)			Root / Shoot Ratio	No of plants with root score 3 or 4 (%)	No of plants with RCD > 6.9mm (%)	Production Standard Values (%) [d]
			Root [b]	Shoot [c]	Plant				
MAIN EFFECT MEANS: [e]									
a) Clone (main-plot treatment factor):									
57	77.6 a	7.39 a	1.62 a	13.3 a	14.9 a	0.123 a	41.4 a	43.4 a	32.2 a
48	82.1 a	6.79 b	1.43 b	11.4 b	12.8 b	0.126 a	34.5 a	37.0 a	24.7 b
LSD (5%)	10.1	0.18	0.15	0.9	1.0	0.011	8.7	7.3	7.3
Significance	NS	P<0.001	P<0.05	P<0.01	P<0.01	NS	NS	NS	P<0.05
b) *Trichoderma* (sub-plot treatment factor):									
Mixture A	83.5 ab	7.21 a	1.60 a	12.6 ab	14.2 ab	0.128 a	44.8 a	44.1 a	34.7 a
GenMix	85.9 a	7.15 a	1.54 ab	12.0 ab	13.6 abc	0.127 a	45.3 a	45.1 a	33.0 ab
PR6	81.2 ab	7.29 a	1.56 ab	12.7 ab	14.4 a	0.125 a	38.4 a	45.1 a	29.5 ab
PBI	81.6 ab	7.07 ab	1.59 ab	13.0 a	14.5 a	0.123 a	40.8 a	40.3 ab	32.1 ab
ModArb	79.2 ab	7.18 a	1.48 ab	12.4 ab	13.7 abc	0.121 a	39.1 a	39.4 ab	28.7 b
Control + fungicide	70.8 c	6.91 bc	1.45 b	11.8 b	13.3 bc	0.123 a	28.1 b	34.6 bc	21.2 c
Control - fungicide	76.9 bc	6.79 c	1.46 ab	11.7 b	13.2 c	0.124 a	29.3 b	32.8 c	19.8 c
LSD (5%)	7.5	0.24	0.14	1.0	1.1	0.008	7.5	6.4	5.8
CLONE X *TRICHODERMA* INTERACTION MEANS:									
Clone 57									
Mixture A	83.0	7.53	1.70	13.7	15.4	0.125	50.7	47.6	39.2
GenMix	83.3	7.43	1.64	12.7	14.3	0.130	51.0	49.0	38.9
PR6	79.9	7.63	1.60	13.5	15.3	0.120	41.0	49.3	32.6
PBI	78.8	7.39	1.72	14.0	15.6	0.123	49.0	43.4	39.6
ModArb	76.7	7.47	1.58	13.7	15.0	0.115	39.9	41.0	30.6
Control + fungicide	68.4	7.11	1.49	12.3	13.8	0.121	27.1	36.8	22.2
Control - fungicide	73.3	7.14	1.63	13.1	14.7	0.125	31.3	36.8	22.2
CLONE 48:									
Mixture A	84.0	6.88	1.50	11.6	13.1	0.130	38.9	40.6	30.2
GenMix	88.5	6.87	1.43	11.4	12.8	0.125	39.6	41.3	27.1
PR6	82.6	6.96	1.51	11.8	13.4	0.131	35.8	41.0	26.4
PBI	84.4	6.76	1.47	12.0	13.4	0.123	32.6	37.2	24.7
ModArb	81.6	6.89	1.38	11.1	12.4	0.126	38.2	37.9	26.7
Control + fungicide	73.3	6.71	1.42	11.4	12.8	0.125	29.2	32.3	20.1
Control - fungicide	80.6	6.44	1.29	10.3	11.6	0.124	27.4	28.8	17.4
LSD(5%) for comparing means for same level of Clone (main-plot treatment factor):									
	10.6	0.33	0.20	1.4	1.5	0.012	10.6	9.0	8.2
LSD(5%) for all other comparisons:									
	13.4	0.35	0.23	1.5	1.6	0.015	12.5	10.6	10.1
Significance of Clone x *Trichoderma* Interaction:									
	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] root collar diameter of plants with roots at harvest.

[b] mean dry weight of roots after lateral pruning and wrenching in autumn and winter respectively.

[c] mean dry weight of shoots after standard nursery topping at an average of 34cm height in autumn. Above- and below-ground shoot weights were combined.

[d] percentage of plants with both a root score of 3 or 4 and root collar diameter >6.9mm.

[e] letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. NS = not significant.

2.3.2 Forestry plantation 2018 validation trials for most-effective treatments

Following laboratory and screening programmes (Hill, 2016, Whelan 2019a), the two most effective *Trichoderma* mixtures, PR6 and PR3a were tested against an untreated control in eight large (two to three hectare) plantation trials in four important forestry regions (Table 2; Figure 4). PR6 comprised isolates FCC55 (*T. harzianum*), FCC318 (*T. atrobrunneum*), FCC327 (*T. harzianum*) and FCC340 (*T. harzianum*) whilst PR3a comprised isolates FCC13 (*T. asperellum)*, FCC14 (*T. atroviride*), FCC15 (*T. atroviride*) and FCC180 (*T. crassum*). PR3a was selected as a mixture that may be tolerant to cooler NZ forest locations, after high growth promotion in a cool Bay of Plenty/Waikato trial site, and two of the isolates were isolated from Canterbury plants.

The mixtures were applied as a seed-coating @ 1.0 $x10^6$ spores/seed and seedlings were grown in a commercial nursery for ten to twelve months before plantation planting in winter 2018. At planting, *Trichoderma* colonisation of seedling roots was 49%, 32% and 4% in PR6, PR3a and untreated Control treatments respectively.

The isolate mixtures were tested in two temperature regimes, with participating forestry companies selecting a high ("cold") and low ("warm") altitude site in each region. Trials were a randomised complete block design (RCBD) with large plots and seven to ten replicates. Plots contained 81 trees in a 9 x 9 grid pattern with plant density ranging from 800 to 1190 plants per hectare. Tree height and survival were measured in the central 5 x 5 = 25 plants approximately twelve months after planting, apart from the Nelson trials at ten months. Roots from two to three trees per plot (*ie:* 60 to 90 plants per treatment) were also sampled and bulked together in each trial. *Trichoderma* was then isolated from approximately 60 sterilised random pieces of roots per treatment, using MRB agar plates, according to established protocols (Whelan, 2018c).

For each trial, height and survival (%) were analysed for significance by analysis of variance (ANOVA) and unprotected LSD tests at the 5% level (Genstat, v19). The exception was in the Gisborne Tauwhareparae trial where wet soil conditions affected 9% of plants during establishment and early growth. Data for percentage of plants with yellow, stunted growth was used as a covariate in an analysis of covariance (ANCOVA) to reduce the variability of tree height data. Data were also analysed by ANOVA as eight blocks (= trials) with three treatments, with input data being treatment means for height and survival. In addition, differences between means for each of the eight trials (including the difference between PR6 and Control, PR3a and Control, PR6 and PR3a and mean of the two *Trichoderma* treatments and Control) were calculated, then analysed using an ANOVA that fitted the main effects of temperature and region, and used the temperature x region interaction as the residual (error) term.

Plant establishment was high with survival averaging 96.7% in all trials (Table 3). *Trichoderma* had no effect on tree survival in individual trials (Table 3), apart from in the Bay of Plenty and Nelson warm trials where survival was significantly ($P<0.05$) higher by 2.5% (in PR6) and 5% (in PR3a) respectively, compared to the untreated Control. When analysed over all trials, survival was not significantly ($P<0.05$) different between treatments (Table 3).

Trichoderma treatments had significantly ($P<0.05$) increased tree height by 7 to 15%, compared to the untreated Control in six of the eight trials (Figure 5). The largest increases in height due to *Trichoderma* treatment were in the Bay of Plenty/Waikato (11% and 15%) and Northland (11% and 12%) trials, followed by the Gisborne (7%) trials (Figure 5). *Trichoderma* had no effect on height in the Nelson trials, possibly because the Auckland-based plant stock was not suitable for the colder

Nelson sites (Craig Brown *pers. comm*.). When analysed over all trials, tree height was significantly (P<0.05) increased by 6% and 7% for PR3a (68.3cm) and PR6 (69.3cm) respectively, compared to the untreated Control (64.6cm; Figure 6).

Temperature, as selected by site altitude, did not significantly affect differences in tree height or survival between the two *Trichoderma* treatments or between either *Trichoderma* treatment and the control (Table 4). However, the difference in height between the average of the two *Trichoderma* treatments and the control was significantly (P<0.05) higher for colder than for warmer temperature sites (Table 4). This result suggested that neither *Trichoderma* mixture was better suited to the warmer or colder temperatures experienced in these trials, but that in general, the response to *Trichoderma* may be higher at colder sites. This result reinforces a recent study at BPRC (Whelan, 2019a) that isolated and characterised cold and warm tolerant *Trichoderma* isolates, including laboratory temperature testing of the eight isolates used in PR6 and PR3a mixtures. It concluded that deployment of PR6 and PR3a mixtures was appropriate in temperate or sub-tropical plantations (the majority of New Zealand's forestland). However, the mycelial growth rate of PR3a isolates was higher at low temperatures, compared to the PR6 isolates, and may be more suitable for colder temperate regions, south of Nelson/Marlborough.

There were issues with the use of altitude as a method for selecting nominal site temperature, due to other factors that affected plant growth (*eg.* methods for soil preparation, soil fertility, site exposure, disease levels). For example, the low altitude (nominally warm) site in Northland produced shorter trees, possibly due to the effect of the soil preparation method on early seedling growth, compared to the high altitude (nominally cold) Northland site. In the Bay of Plenty/Waikato warm trial, disease may have affected potential tree growth, resulting in similar tree heights between the warm and cold trials. In addition, the statistical combined analyses has an assumption that the difference between cold and warm temperature is the same in all four regions. However, this may not have been the case as the difference between cold and warm sites in Nelson may have been larger than that in the trials located in each region further north. Future work involving many sites with different soil temperatures (including Southland), but similar plant establishment conditions, may refine knowledge of the suitability of each *Trichoderma* mixture to sites with different temperatures.

Trichoderma was found in the tree roots of the three treatments, with some trial sites appearing to have high natural levels at the time of measurement, in particular, Gisborne cold and warm and Northland cold trials (Table 5). Generally in each trial, *Trichoderma* treatments that generated growth responses, had higher levels of root colonisation ten to twelve months after planting, compared to the controls. Therefore, the inoculated isolates appeared to have a more dominant role in the growth response compared to the natural *Trichoderma* strains. When analysed over all trials, root colonisation in the *Trichoderma* treatments was at least 70% higher compared to the Control treatments, but was not significantly (P<0.05) different due to high levels of variability between trials (Table 5).

Dothistroma septosporum was present at low levels (<4% mean disease severity) in the Bay of Plenty/Waikato trials and the Gisborne warm trial approximately twelve months after planting. *D. septosporum* was re-measured in the Bay of Plenty/Waikato warm trial 15 months after planting. Both *Trichoderma* treatments reduced disease severity from 9.2% in the Control, to 4.6% and 5.6%, a reduction of 50% and 39% in the PR6 and PR3a treatments respectively (Figure 7).

Table 2: Establishment details and twelve-month measurement comments for the 2018 plantation radiata pine trials.

Region	Nominal temperature within the region	Company	Forest / Trial Name	Location	Altitude (m)	Planting Date	No Replicates	Planting Density and spacing	Comments
Northland	warm	Rayonier	Topuni	-36.225173 174.413915	40	23/08/18	8	1000 (5m x 2m)	Wet site with high clay soil type. West 5° slope and herringbone ripping, therefore variable plant growth potential.
Northland	cold	Hancock	Whatoro	-35.708955 173.676602	330-350	08/08/18	10	830 (4m x 3m)	10km from coast, flat. Low weed burden apart from replicate 8 and 10. Socketing in 8% of plants. Poor growth in 7% of plants (probably due to soil compaction).
Bay of Plenty / Waikato	warm	Timberlands	Kaingaroa xPKANG 209/4	-38.559711 176.445696	180	24/07/18	10	925 (4m x 2.7m)	Spot-mounded, flat, free-draining soils; fleabane.
Bay of Plenty / Waikato	cold	Timberlands	Kaingaroa xPKANG 660/2	-38.868971 176.280092	240	23/07/18	10	925 (4m x 2.7m)	Spot-mounded, flat free-draining soils; fleabane.
Nelson	warm	Nelson Forests	Golden Downs / Sherry	-41.448233 172.651675	310-380	03/09/18	8	800 (5m x 2.5m)	West slope (7-10° bottom half and 17-32° top half of trial); replicates placed across the slope. Cold, snow-prone site but more sheltered from winds compared to Berrymans. The bottom 3 replicates had low survival (average 87%) due to frost damage. AGPRO Valzine 500 spot-spray in November 2018. Ex-Douglas-fir.
Nelson	cold	Nelson Forests	Golden Downs / Berrymans	-41.458333 172.90833	450	05/09/18	7	800 (5m x 2.5m)	South 8° slope with internal gullies; plots placed to avoid these. A range of slash levels but plots arranged to have similar levels in each replicate. Very cold, frosty, wind-exposed site. The bottom 3 replicates had low survival (average 83%) due to frost damage. Lower growth potential compared to Sherry.
Gisborne	warm	Juken	Patunamu	-38.90725 177.239278	200	18/07/18	9	1190 (2.9m x 2.9m)	East 15° slope, high amount of cut-over, potential high boxthorn/hawthorn burden in future.
Gisborne	cold	PFOlsen	Tauwhareparae	-38.198800 178.099317	400-425	11/08/18	10	1000 (3.3m x 3m)	East 15° slope, small gullies through trial. 9% of plants had yellow stunted growth or death due to wet soil.

Further measurement of tree growth and disease expression will be made in 2020 and 2022 to confirm the growth promotion and disease suppression effects as the trees mature.

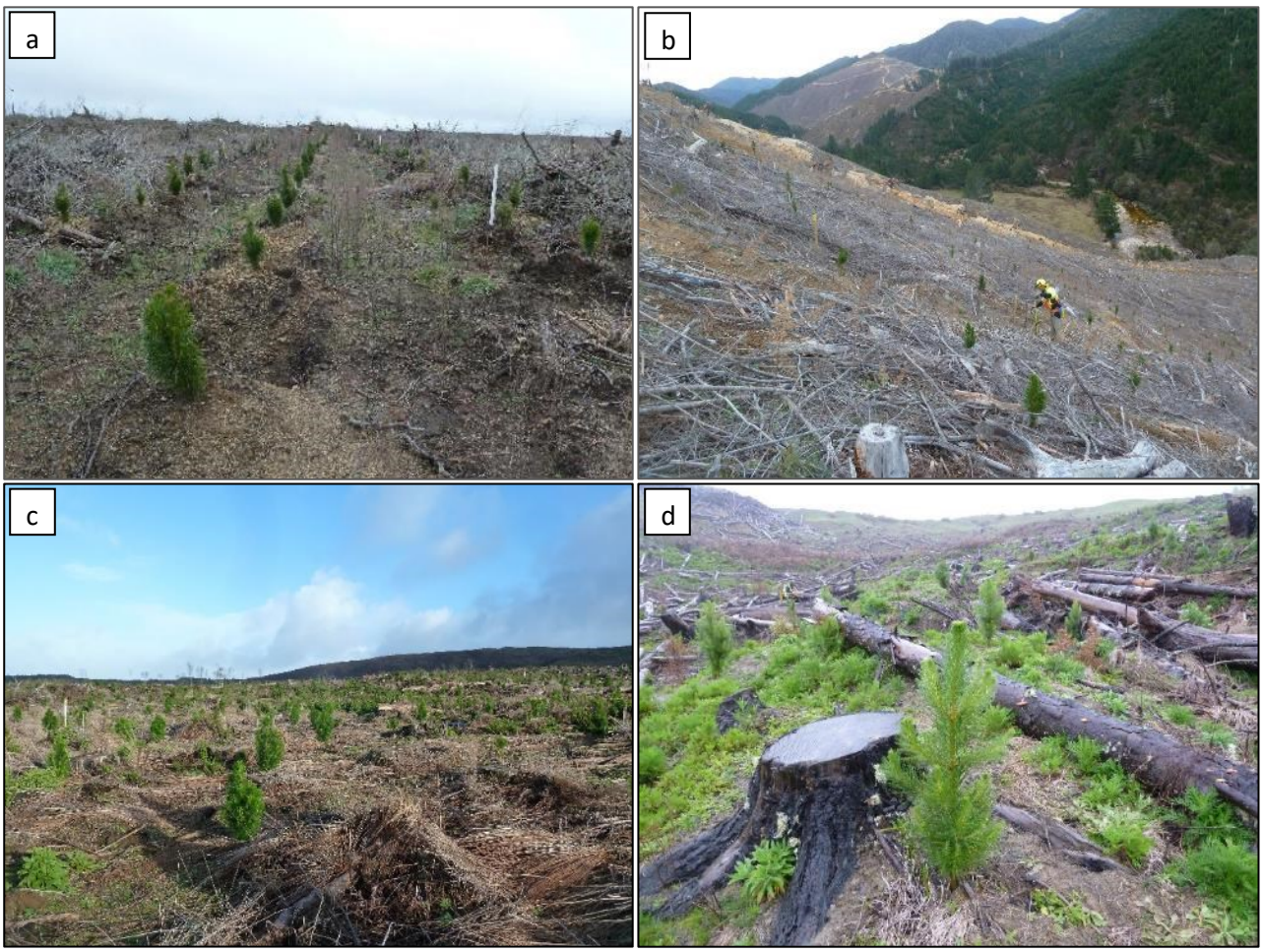

Figure 4: Trials in a) Kaingaroa Forest, Bay of Plenty/Waikato, b) Golden Downs Forest, Nelson, c) Whatoro Forest, Northland and d) Tauwhareparae Forest, Gisborne, approximately ten to twelve months after establishment.

Table 3: Effect of two *Trichoderma* mixtures on tree survival in the eight 2018 validation trials ten to twelve months after planting.

Trial	Site	Treatment			LSD (5%)	Significance
		PR6	PR3a	Control		
Gisborne	Warm	96.9 a[a]	98.7 a	99.1 a	3.2	NS
	Cold	98.0 a	95.2 a	96.8 a	4.9	NS
Bay of Plenty / Waikato	Warm	99.6 a	97.6 ab	97.2 b	2.3	P<0.05
	Cold	100.0 a	99.6 a	99.6 a	1.0	NS
Northland	Warm	96.5 a	99.0 a	97.5 a	3.5	NS
	Cold	98.8 a	98.4 a	98.4 a	2.2	NS
Nelson	Warm	93.5 ab	97.0 a	92.5 b	4.4	P<0.05
	Cold	91.9 a	93.1 a	86.9 a	8.6	NS
Mean (all trials)		96.9 a	97.3 a	96.0 a	1.8	NS

[a] In each trial, letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. NS = not significant.

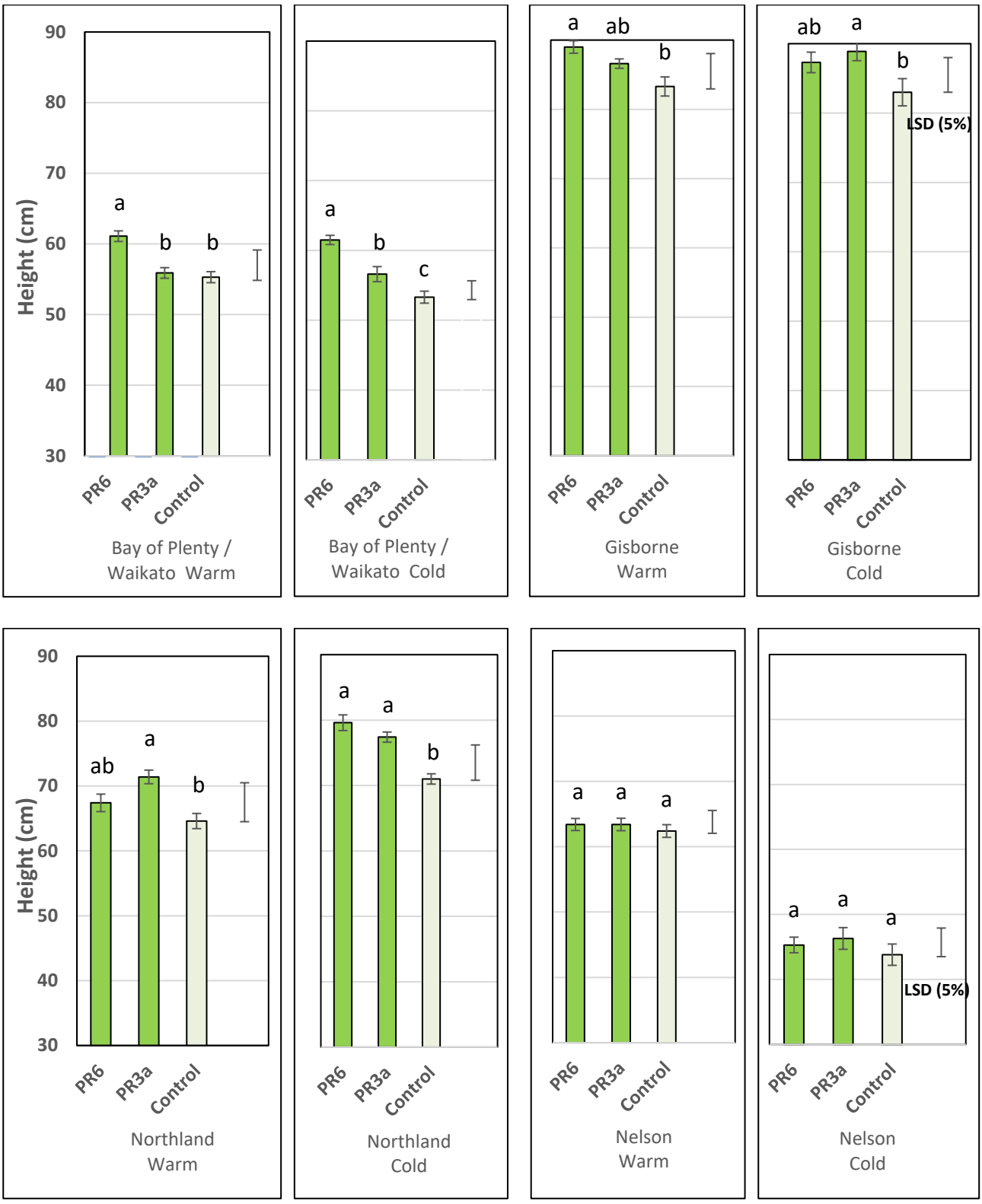

Figure 5: Effect of *Trichoderma* treatments on tree height approximately ten to twelve months after planting, in the eight validation plantation trials. In each trial, letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. Bars = SE of mean or the LSD at 5% level. Data for Gisborne cold site is adjusted means according to ANCOVA analysis, adjusted for % of plants with yellow, stunted growth.

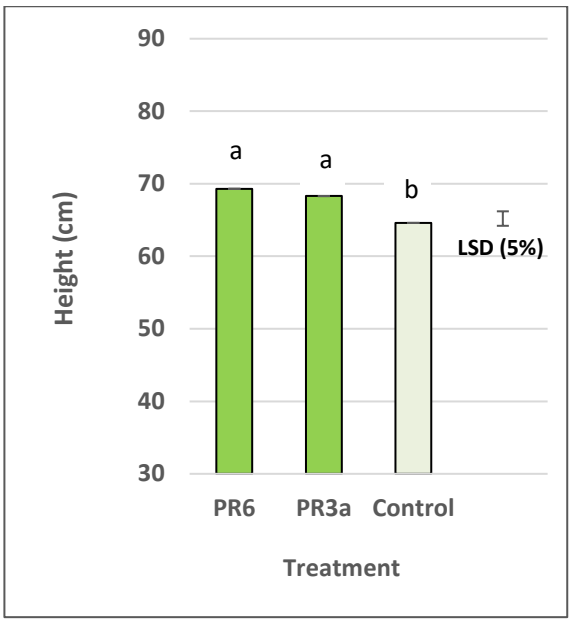

Figure 6: Effect of *Trichoderma* treatments on tree height approximately ten to twelve months after planting, averaged for the eight validation plantation trials. Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. Bar = LSD at 5% level.

Table 4: Effect of temperature, as defined by trial altitude, on the mean actual difference between treatments in tree survival and height ten to twelve months after planting, meaned over the eight 2018 validation trials.

Treatment Comparison	**Temperature**	**Mean Actual Difference**	
		Height (cm)	**Survival (%)**
PR6 - Control	Cold	5.7	1.8
	Warm	3.8	0.1
	LSD (5%)	4.9	4.3
	Significance	NS	NS
PR3a - Control	Cold	4.5	1.2
	Warm	2.9	1.5
	LSD (5%)	2.3	2.3
	Significance	NS	NS
PR6 - PR3a	Cold	1.1	0.6
	Warm	0.9	-1.5
	LSD (5%)	6.8	4.2
	Significance	NS	NS
Mean (PR6 and PR3a) - Control	Cold	5.1	1.5
	Warm	3.4	0.8
	LSD (5%)	1.7	2.7
	Significance	P=0.05	NS

Table 5: *Trichoderma* colonisation (%) of *P. radiata* roots in the 2018 validation trials, sampled ten to twelve months after planting, based on MRB plating data.

Region	Site	Treatment [a]			LSD (5%)	Significance
		Control	PR6	PR3a		
Gisborne	Warm	57.1	64.3	81.0	na	na
	Cold	72.2	59.5	92.9	na	na
Bay of Plenty / Waikato	Warm	22.2	88.6	36.7	na	na
	Cold	13.5	43.5	38.9	na	na
Northland	Warm	16.7	47.5	41.7	na	na
	Cold	32.3	34.6	70.1	na	na
Nelson	Warm	4.2	30.0	26.7	na	na
	Cold	6.7	14.0	20.0	na	na
Mean (all trials)[b]		28.1 a	47.8 a	51.0 a	26.5	NS

[a] Root pieces for each treatment plot were bulked together at each site.

[b] Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. NS = not significant. na = not applicable.

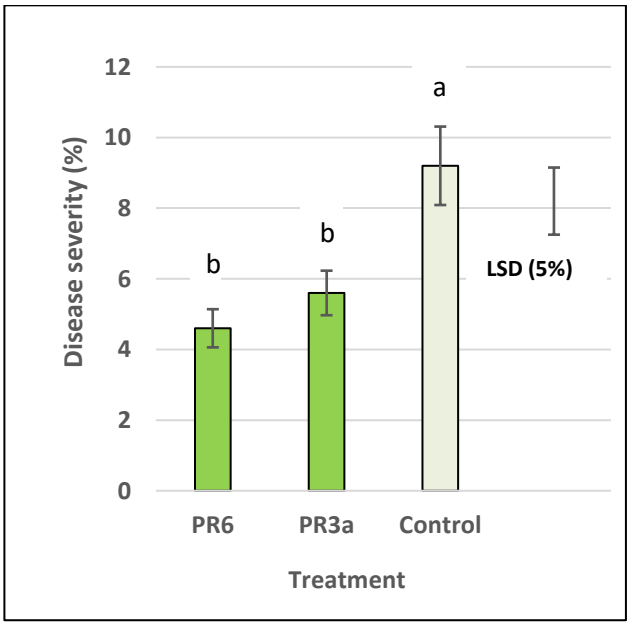

Figure 7: Disease severity (%) in a Bay of Plenty/Waikato trial fifteen months after planting. Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. Bars = SE of mean or the LSD at 5% level.

2.3.3 Forestry plantation trials 2012 to 2015

Between 2012 and 2015, 24 forestry plantation trials were established in radiata pine growing areas at locations throughout New Zealand (Table 6). The most effective 24 isolates identified in nursery screening trials and disease assays were tested individually or as mixtures. In 2019, ten of these trials were selected for continued measurement (Table 6) based on:

1. quality of trial at establishment and durability over time
2. regional spread
3. plants responding to expectation of the general environment, and
4. a lower priority to the 2014 trials due to the different control seedline (refer to section 5.6.3 of Hill, 2016, report).

Table 6: Establishment details for forestry plantation trials 2012 to 2015 and the trials selected for continuation after 2019.

Date established	Company	Locality	Forest plantation area	Trial continued
2012	Juken NZ Ltd	Gisborne	Wharerata	
2012	PF Olsen	Rotorua	Pinnacles	
2013	Timberlands	Kaingaroa	XP KANG	✓
2013	Juken NZ Ltd	Gisborne	Cricklewood	
2014	Timberlands	Kaingaroa	XP KANG	
2014	Hancock	Kinleith	Phoenix Horohoro	
2014	Hancock	Kinleith	Waituna_Kinleith	
2014	Hancock (now Tasman Pine)	Nelson	MTRN	✓
2014	Hancock (now Tasman Pine)	Nelson	Pearse	
2014	Hancock	Northland	Otaenga	✓
2014	Hancock	Northland	Pipiwai	
2014	Ernslaw One Ltd	Gisborne (Inland Tokomaru Bay)	Waiau	✓
2014	Ernslaw One Ltd	Gisborne (Inland Whatatutu)	Waipaoa	
2014	Ernslaw One Ltd	Ohakune	Karioi	
2014	Ernslaw One Ltd	Wanganui	Harakeke	✓
2014	Nelson Forests Ltd	Nelson	Ngaruru	
2014	Nelson Forests Ltd	Nelson	Kohatu	✓
2014	Nelson Forests Ltd	Nelson	Kings Ridge	
2014	Rayonier	Wanganui	Lismore	
2015	Timberlands	Kaingaroa	XP KANG	✓
2015	Rayonier	Northland	Glenbervie	✓
2015	Rayonier	Matariki	Maramarua Forest	
2015	Hancock	Kinleith	Phoenix Horohoro	✓
2015	Hancock	Kinleith	Giles Rd Kinleith	✓

Survival, height and health score measurements for many of the 2012 to 2014 trials, one year after establishment, were presented in Hill's (2016) report. Measurements taken in the trials between 2015 and 2019 were summarised in Whelan (2019a) and reports sent to the respective forestry companies (Hill and Whelan, 2016; Whelan, 2018a and b; Whelan, 2019c to f and Whelan 2020a to g). Recent measurements are summarised in the following section (2.3.3.1 to 2.3.3.6).

In Figures 9, 11, 13, 15 and 17, letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. Bars = SE of mean or the LSD at 5% level.

Isolate mixtures used in the trials are listed below:

2014 trials:

T1 = *Trichoderma* mixture = PBI (LU132, LU140, LU584, LU633)

T2 = *Trichoderma* mixture = PR1 (FCC318, FCC319, FCC320, FCC322, FCC340)

T3 = *Trichoderma* mixture = PR2 (FCC362, FCC368, FCC49, FCC55)

T4 = *Trichoderma* mixture = PR3 (FCC180, FCC327, FCC275, FCC161)

T5 = Control, untreated

2015 trials:

T1 = *Trichoderma* FCC320

T2 = *Trichoderma* FCC327

T3 = *Trichoderma* mixture = PR5 (FCC161, FCC180, FCC275, FCC327)

T4 = *Trichoderma* mixture = PR6 (FCC55, FCC318, FCC327, FCC340)

T5 = *Trichoderma* mixture = PBI (LU132, LU140, LU584, LU633)

T6 = Control, untreated

2.3.3.1 Ernslaw One Ltd. Waiau 2014 trial

Trial assessed at Year 5.5 (5 September 2019; Figure 8):

Figure 8: The Waiau trial on 5 September 2019.

- PBI treatment (7.6m) had a similar tree height to the Control (7.7m; Figure 9).
- PR1, PR2, and PR3 treatments had significantly (P<0.05) reduced tree heights, by up to 6% (0.5m), compared to the Control.
- All treatments had similar DBH measurements (between 155 to 158mm), apart from PR3 (143mm) that was significantly (P<0.05) reduced (Figure 9).
- Tree survival ranged from 82% (PR1) to 95% (PR3) but was not significantly different (P<0.05) between treatments, due to the large amount of variability (Figure 9).
- Dothistroma needle blight (*D. septosporum*) was present in the trial in September 2019 but only at low levels (2% or less in each treatment).
- Disease severity (%) was significantly (P<0.05) reduced in the PR2 treatment, compared to the Control (Figure 9), however, this difference may not be of practical importance.

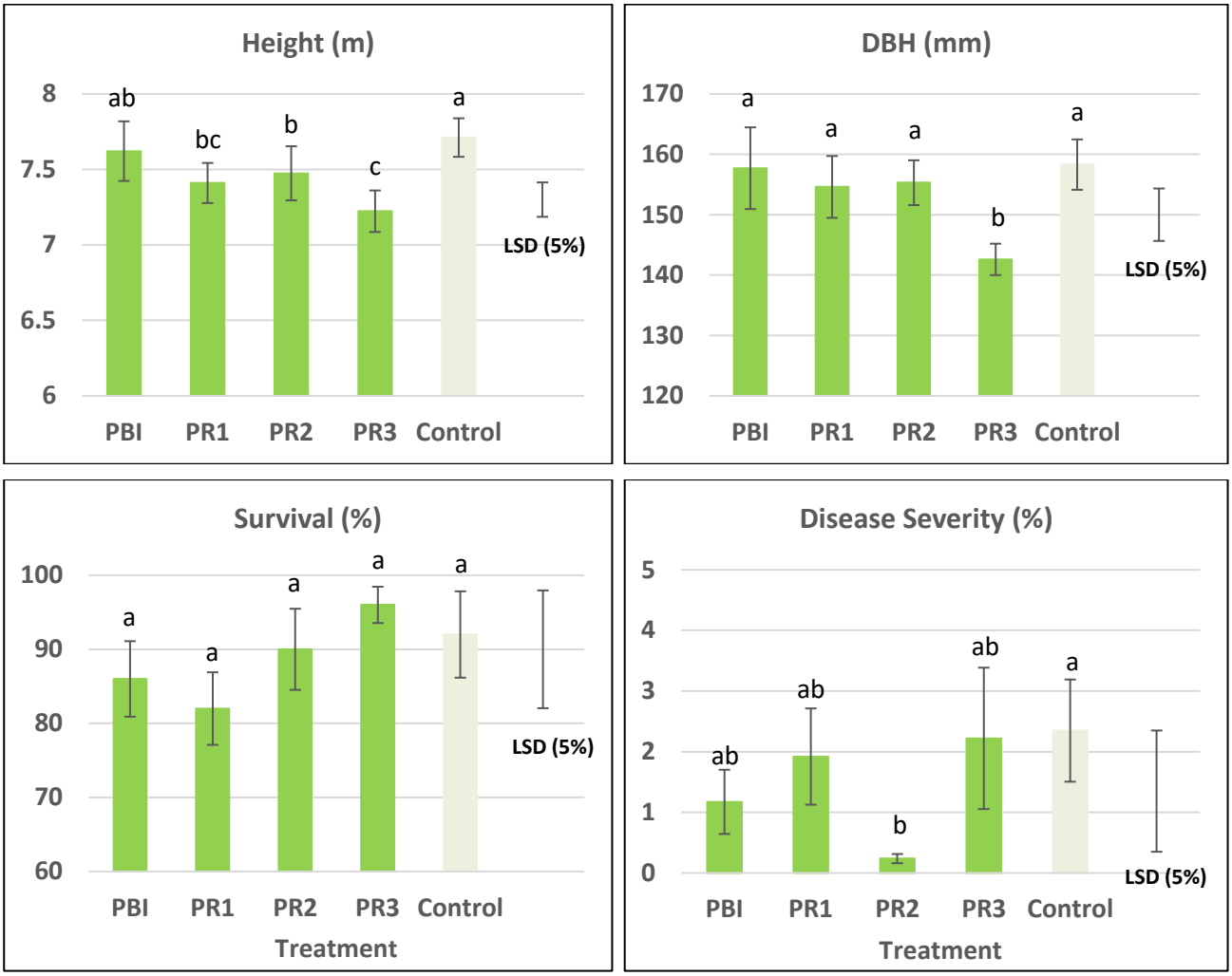

Figure 9: Effect of *Trichoderma* treatments on tree height, DBH, survival and disease severity approximately 5.5 years after planting, in the Waiau 2014 trial.

2.3.3.2 Nelson Forests Kohatu 2014 trial

Trial assessed at Year 5 (8 July 2019; Figure 10):

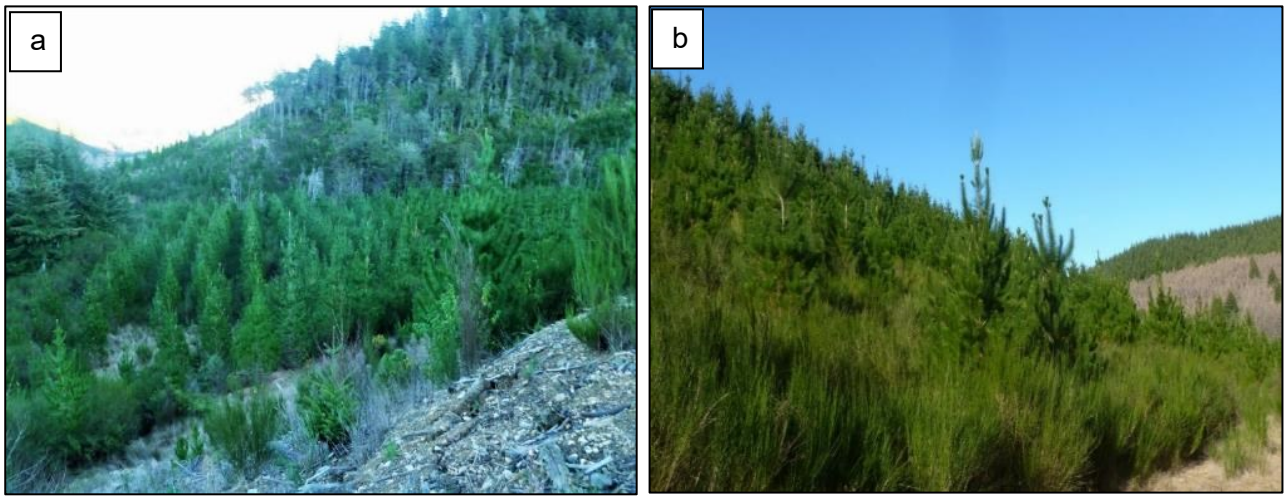

Figure 10: Trees in the valley flat (a) and hillside (b) replicates in the Kohatu 2014 trial on 8 July 2019.

- Height was similar (between 6.2 and 6.4m) in all treatments (not significantly different at P<0.05, Figure 11).
- DBH was similar (between 94 and 98mm) in all treatments (not significantly different at P<0.05, Figure 11).
- Survival levels in the treatments were highly variable (range of 77 to 93%), but were not significantly different (P<0.05) from each other (Figure 11).

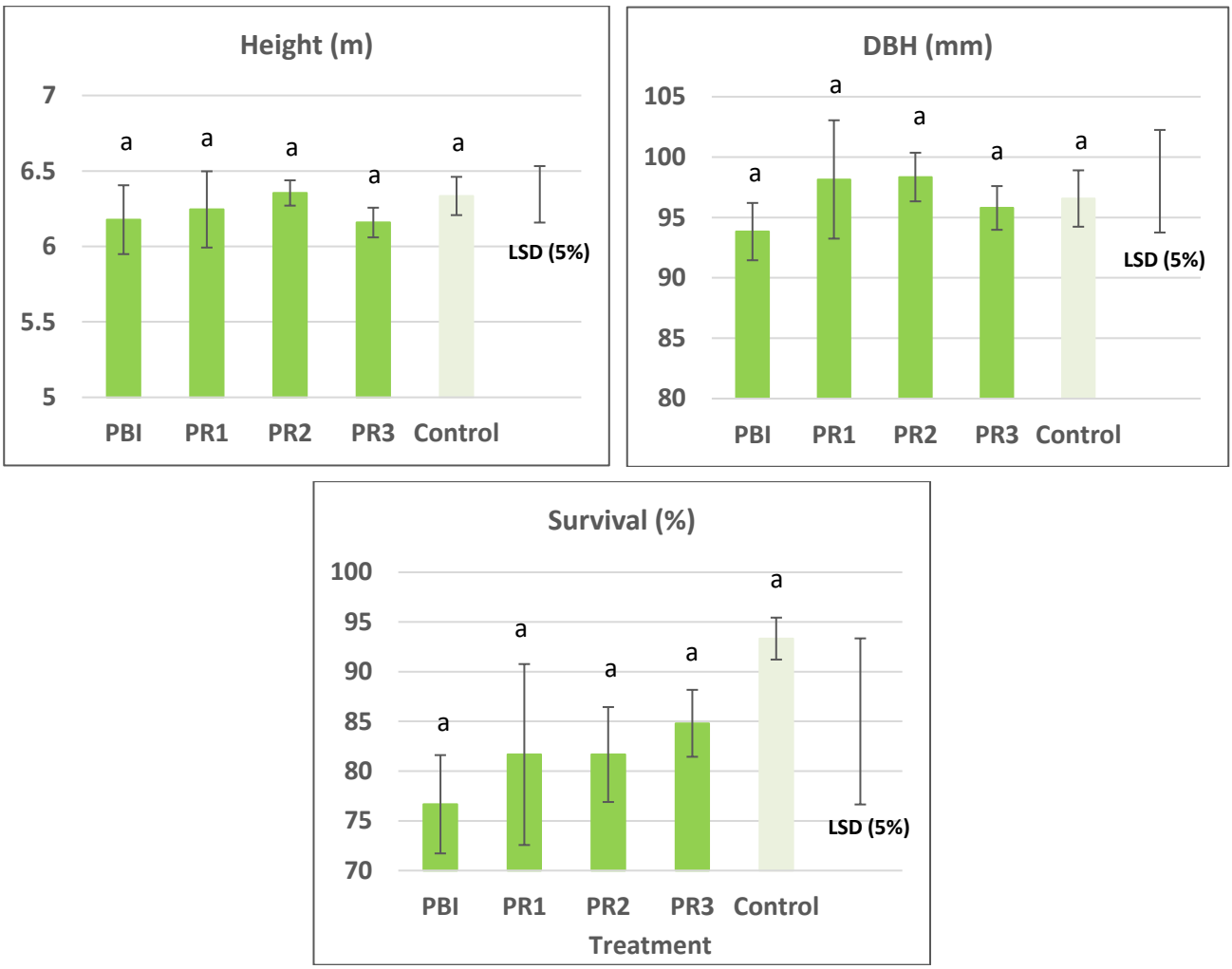

Figure 11: Effect of *Trichoderma* treatments on tree height, DBH and survival (%) five years after planting, in the Kohatu 2014 trial.

2.3.3.3 Hancock Natural Resources Group Giles Rd, Kinleith Forest 2015 trial

Trial assessed at Year 4.5 (2 December 2019):

Figure 12: The Giles Rd 2015 trial 3 years after establishment on 2 May 2018.

- Treatments had similar height measurements, apart from PR6 that was significantly (P<0.05) greater than PR5 and FCC320 (Figure 13).
- Treatments had similar DBH measurements apart from PR6 that was significantly (P<0.05) greater than PR5 (Figure 13).
- Tree survival was high (92 to 98%) and was not significantly (P<0.05) different between treatments (Figure 13).
- In December 2019, Dothistroma needle blight (*D. septosporum*) was present in the trial at moderate levels (11 to 18% disease severity) but was not significantly different (P<0.05) between treatments (Figure 13).
- Of the four *Trichoderma* treatments, PR6 was the most beneficial to growth and disease suppression, but was not significantly (P<0.05) different compared to the untreated Control. In comparison, PR5 was the least beneficial *Trichoderma* treatment in this trial.

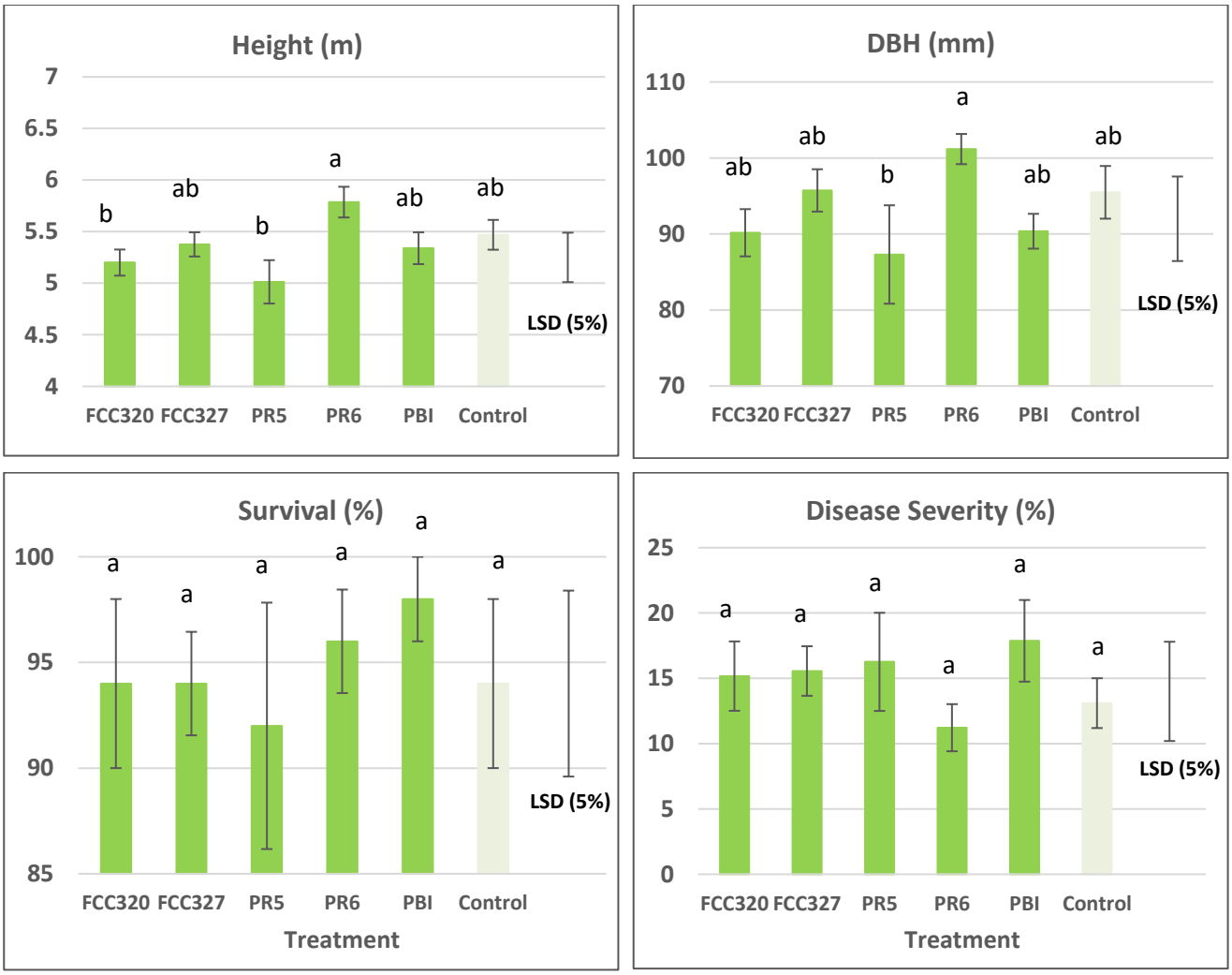

Figure 13: Effect of *Trichoderma* treatments on tree height, DBH, survival and disease severity of Dothistroma needle blight four years after planting in the Giles Rd 2015 trial.

2.3.3.4 Hancock Natural Resources Group Phoenix Rd, Horohoro Forest 2015 trial

Trial assessed at Year 4.5 (3 December 2019):

Figure 14: The Phoenix Rd 2015 trial 3.5 years after establishment on 29 October 2018.

- Treatments had similar height measurements (approximately 4m) apart from FCC320 that was significantly greater than PR5 (Figure 15).
- DBH (between 63 to 70mm) was not significantly ($P<0.05$) different between treatments (Figure 15).
- Tree survival was relatively high (88 to 98%) and was not significantly ($P<0.05$) different between treatments (Figure 15).
- Dothistroma needle blight was present in the trial in December 2019 at moderate levels (12 to 18% disease severity) but was not significantly different ($P<0.05$) between treatments (Figure 15).

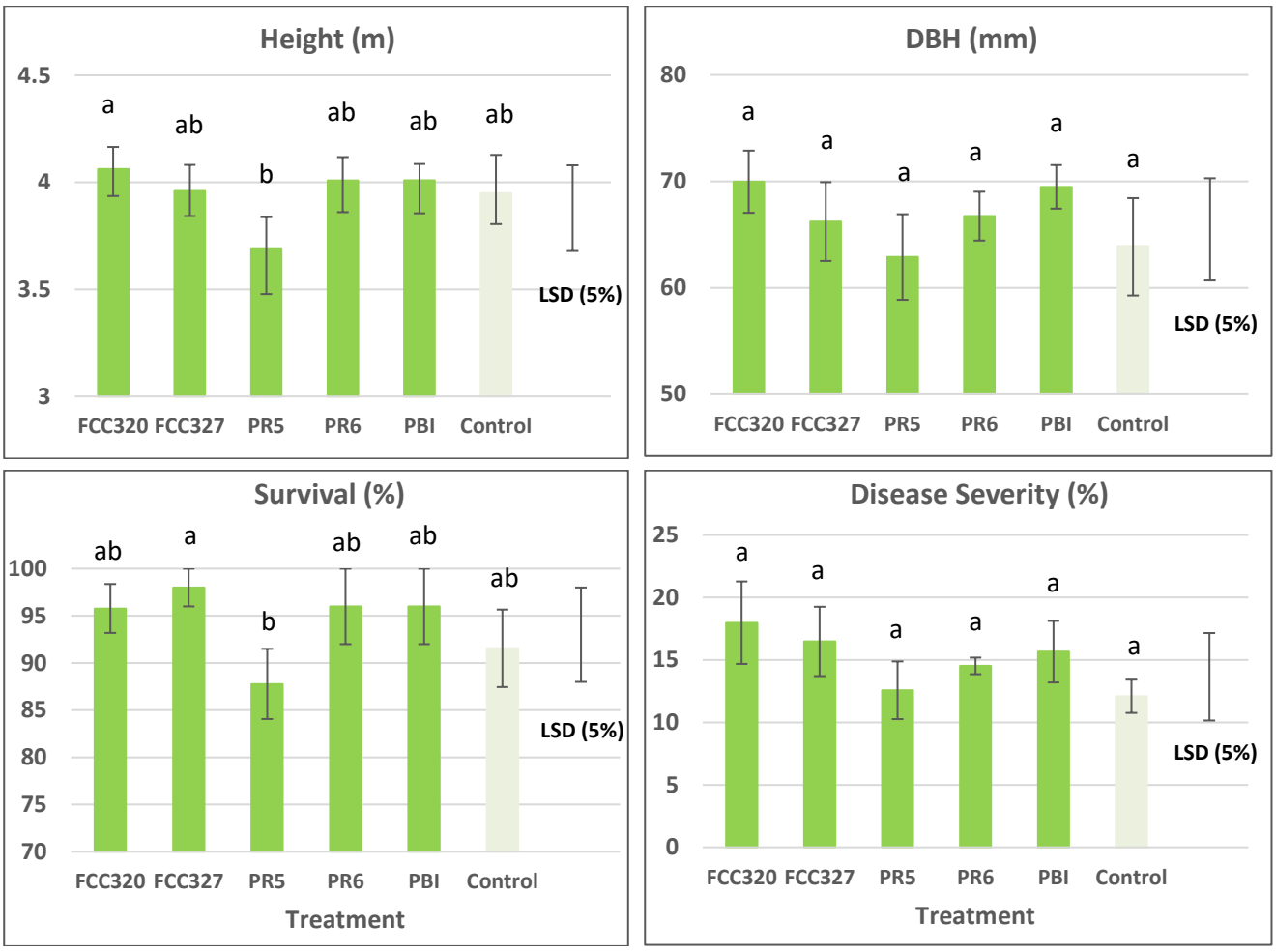

Figure 15: Effect of *Trichoderma* treatments on tree height, DBH, survival and disease severity of Dothistroma needle blight 4.5 years after planting in the Phoenix Rd 2015 trial.

2.3.3.5 Hancock Natural Resources Group Otaenga 2014 trial

Trial assessed at Year 5 (13 August 2019; Figure 16):

- Height ranged from 7.5 to 8m and was not significantly (P<0.05) different between treatments (Figure 17).
- DBH ranged from 131 to 142mm and was not significantly (P<0.05) different between treatments (Figure 17).
- Tree survival was very high (94 to 98%) and was not significantly (P<0.05) different between treatments (Figure 17).

Figure 16: The Otaenga 2015 trial (foreground; a) and measuring height and truck diameter at breast height (DBH; b) on 14 August 2019.

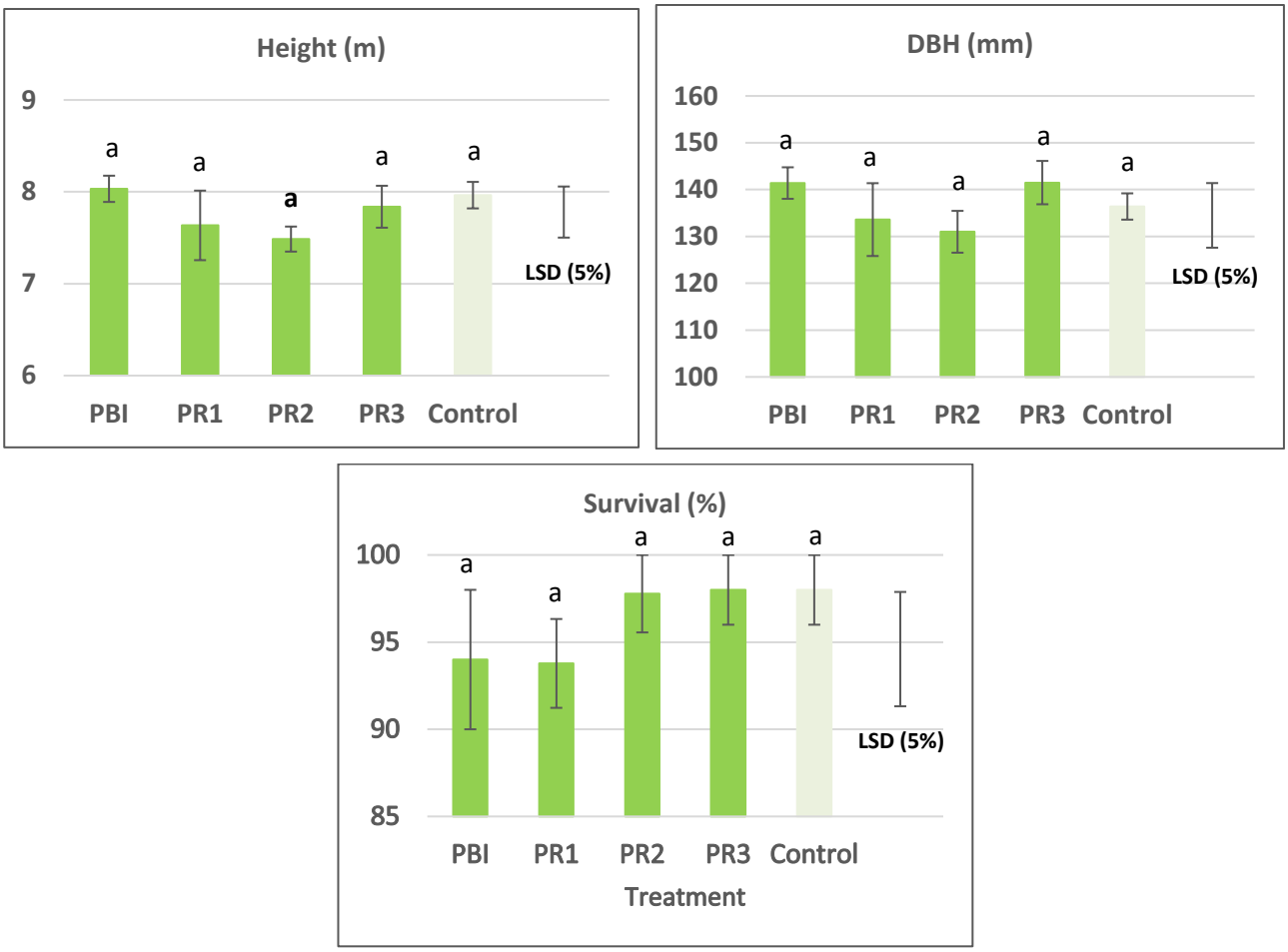

Figure 17: Effect of *Trichoderma* treatments on tree height, DBH and survival five years after planting, in the Otaenga 2014 trial.

2.3.3.6 Rayonier Matariki Forests Glenbervie 2015 trial

Trial assessed at Year 4 (15 August 2019; Figure 18):

Figure 18: The Glenbervie trial on 15 August 2019.

- No statistically significant interactions were observed between isolate and fertiliser treatment for any of the measured parameters at Year 4 (Table 7). Therefore, results are discussed in terms of main effects.
- Height ranged from 5.12m (Control, PBI and FCC327) to 5.35m (PR6) in the isolate treatments but were not significantly different ($P<0.05$) due to the large amount of variability in the trial (two replicates only were established; Table 7).
- Application of fertiliser 3 months after planting had a significant ($P<0.05$) effect on tree height at Year 4 (Table 7). Fertilised trees were 6% taller compared to unfertilised trees.
- DBH ranged from 77.0 (FCC320) to 82.7mm (PR6) in the isolate treatments but were not significantly different ($P<0.05$; Table 7).
- Application of fertiliser 3 months after planting had no significant ($P<0.05$) effect on DBH at Year 4 (Table 7).
- Tree survival was at moderate levels, ranging from 86.0% (Control) to 92.9% (PR6) in the isolate treatments and 87.6% and 90.4% in the fertiliser treatments (Table 7). Survival was not significantly ($P<0.05$) different in either of the main factors.

Table 7: Effect of *Trichoderma* isolate and fertiliser treatments on tree height, diameter at breast height (DBH) and survival four years after planting, in the Glenbervie Northland trial.

	Height (m) [a]	DBH (mm)	Survival (%)
MAIN EFFECT MEANS:			
Isolate (main plot treatment factor):			
PR6	5.35 a	82.7 a	92.9 a
FCC320	5.15 a	77.0 a	90.0 a
PR5	5.14 a	79.5 a	87.0 a
FCC327	5.12 a	81.0 a	89.1 a
PBI	5.12 a	77.6 a	89.0 a
Control	5.12 a	80.6 a	86.0 a
LSD (5%)	1.01	17.7	13.2
Fertiliser (sub plot treatment factor):			
F (Fertiliser)	5.31 a	83.3 a	90.4 a
N (No fertiliser)	5.02 b	75.8 a	87.6 a
LSD (5%) **Significance**	0.28 P<0.05	8.0 NS	6.1 NS
ISOLATE X FERTILISER INTERACTION MEANS:			
F:			
PR6	5.50	86.5	92.0
FCC320	4.99	79.8	96.0
PR5	5.24	82.9	84.0
FCC327	5.33	85.3	90.2
PBI	5.06	77.9	90.0
Control	5.44	87.2	90.0
N:			
PR6	5.20	76.9	93.8
FCC320	5.30	74.1	84.0
PR5	5.03	76.1	90.0
FCC327	4.91	76.6	88.0
PBI	5.18	77.2	88.0
Control	4.80	74.0	82.0
LSD (5%) for comparing means for same level of isolate (main plot treatment factor)	0.76	16.9	14.9
LSD (5%) for all other comparisons	1.04	19.0	14.9
Significance of Isolate x Fertiliser Interaction	NS	NS	NS

[a] Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. NS = not significant.

Generally, *Trichoderma* treatments were not beneficial for tree growth in the trials described in sections 2.3.3.1 to 2.3.3.6, compared to untreated Controls. This result was in contrast to the results found in the other trials established at the same time and measured between September 2017 and March 2019 (Whelan, 2019a).

2.3.4 Feasibility of treating established trees with *Trichoderma* root bioprotectants to mitigate disease problems

In New Zealand, promising results have been obtained in *Trichoderma* bioprotection trials, with the most effective *Trichoderma* treatments increasing tree height in one- to 5½- year-old stands by up to 20% (Hill and Whelan, 2016, Hill *et al.,* 2015, Whelan, 2019a and see section 2.3.2). Application of *Trichoderma* inoculum was by seed-coat in the nursery; this being a practical, effective, low-cost and socially acceptable method for application of bioprotection agents. However, the majority of the approximately 1.5 million hectares of New Zealand's radiata pine plantations have not had *Trichoderma* applied. Three trials in Bay of Plenty/Waikato were set-up to investigate whether it was feasible to inoculate established plantation trees with *Trichoderma* bioprotectants in order to induce disease resistance and growth benefits (Table 8).

Table 8: Experimental design in the three established tree trials.

Features	**Trial**		
Forest and trial establishment date	Tarawera Forest, Kawerau 2017	Kaingaroa Forest, Kaingaroa 2019	Kinleith Forest, Kinleith 2019
Location	38°09'50.2"S 176°44'20.3"E	38°44'23.3"S 176°29'06.6"E	38°25'16.5"S 176°03'15.0"E
Planting date and stand age	1996; 23 years	2008; 11 years	2017; 2 years
Trial establishment date	November 2017	February 2019	December 2019
Experimental design	Randomised complete block design		
Treatments	5	5	7
Number of replicates	5	6	5
Number of trees per treatment	1 (Figure 19)	1 (Figure 19)	20 (2 adjoining rows of 10 trees)
Number of Control treatments per replicates	1	2	1
Spore concentrations and application methods	Refer to Whelan 2018c	Refer to Whelan 2018c	5.4E+6 spores/ml. Spore suspensions included Penetra (an organosilicone surfactant, 0.1% v/v) and were applied with a 15 litre knapsack
Buffer trees	Only selected between soil drench and neighbouring trees	1 to 3 untreated trees were selected between the majority of treated trees to provide a buffer between treatments	none
Tree pruning	yes	First pass in 2016; unthinned trees were selected as buffer trees	none
Average tree DBH and height at trial establishment	552mm; approximately 35m	180mm; 13.9m	no DBH; 2.1m
Average disease severity at trial establishment	70% Red needle cast (*Phytophthora pluvialis)*	1.3% Dothistroma needle blight (*D. septosporum*)	<2% *D. septosporum*

Trichoderma treatments in the Kawerau 2017 and Kaingaroa 2019 trials used a single mixture (PR6 comprising isolates FCC55, FCC318, FCC327 and FCC340) and included:

1. *Trichoderma* infused-root dowels (Figure 20)
2. trunk injection with spore suspension (Figure 20)
3. trunk spray with spore suspension
4. soil drench around the base of the trunk (1.5m radius), and
5. an untreated control.

Methods were described in Whelan (2018c).

Figure 19: Individual trees within a replicate of the Kawerau 2017 (a) and Kaingaroa 2019 (b) trial at establishment on 30 November 2017 and February 2019 respectively.

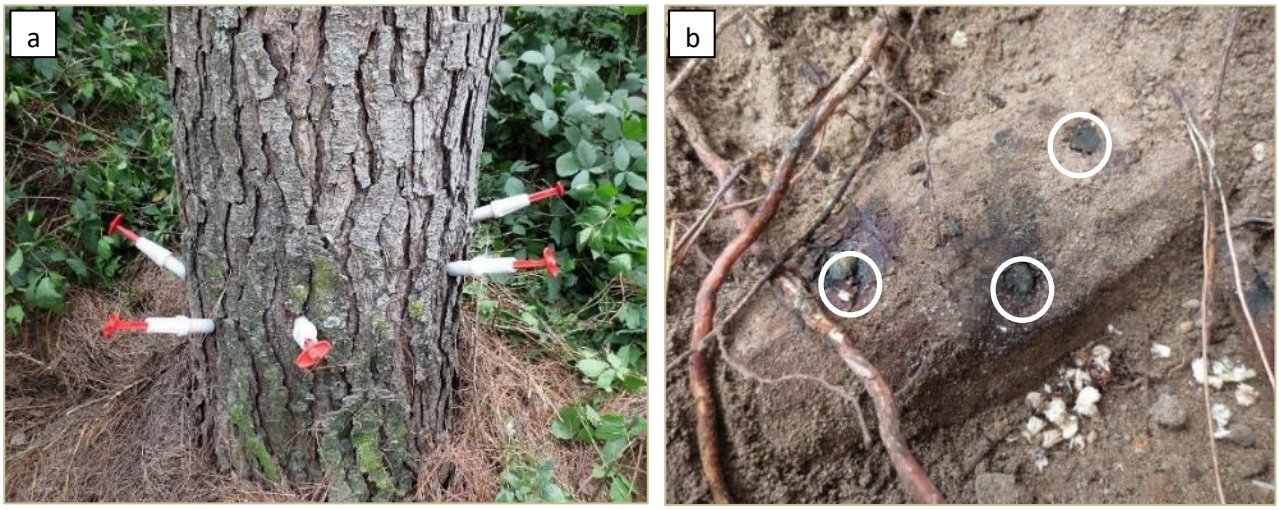

Figure 20: Inserted a) injectors containing *Trichoderma* spore suspension in trunks, and b) *Trichoderma* spore-infused wooden dowels in roots of radiata pine trees in the Kawerau 2017 trial.

In the Kinleith 2019 trial (Figure 21), *Trichoderma* treatments were:

1. PR6 mixture, applied to foliage (40ml per tree)
2. PR3a mixture, applied to foliage (40ml per tree)
3. PR6 mixture, applied to the ground (80ml per tree in an approximately 1.8m radius around the truck)
4. PR3a mixture, applied to the ground (80ml per tree in an approximately 1.8m radius around the truck)
5. PR6 mixture, applied to the foliage and ground (40ml foliage + 80 ml ground per tree)
6. PR3a mixture, applied to the foliage and ground (40ml foliage + 80 ml ground per tree), and
7. an untreated control.

Trichoderma spore suspensions were applied with a 15-litre knapsack on 11 December 2019.

Figure 21: Trees in the Kinleith 2019 trial

In all trials, *Trichoderma* root colonisation was measured using MRB agar plates, according to established protocols (Whelan, 2018c). Data were analysed for significance by either analysis of variance (ANOVA) or covariance of variance (ANCOVA) and unprotected LSD tests at the 5% level (Genstat, v19).

Kawerau 2017 Trial:

The presence of natural *Trichoderma* fungi, before treatment application in November 2017, was estimated at 6%, by sampling small diameter, shallow roots from the five trees allocated treatments in replicate 5 and another three trees within or near replicate 5 (Table 9). Eleven months after treatment, mean *Trichoderma* root colonisation levels had increased by 136% in the treated trees, compared to the untreated trees. Root colonisation levels in the untreated trees remained relatively low at 9%. Root colonisation levels increased in all treatments 24 months after treatment, but no significant differences were found, due to large variation in each treatment (Table 9).

Table 9: *Trichoderma* colonisation (%) of radiata pine roots in trees measured prior to, eleven months and 24 months after treatment application in the 2017 Kawerau trial.

Treatment	Measurement Date		
	30 Nov 2017 (before treatments applied) [a]	30 Oct 2018 (11 months after treatment) [a]	7 November 2019 (24 months after treatment) [b]
Treated Trees:			
Trunk Injection	0	18	22.4 ab
Root Dowel	0	25	28.3 ab
Trunk Spray	4	15	25.8 ab
Soil Drench	8	25	40.3 a
Mean of treated trees	**4**	**21**	**28.0**
Untreated Trees:			
Control	4	5	22.5 ab
neighbouring tree	8	5	na
neighbouring tree	20	13	na
neighbouring tree	0	13	na
Mean of untreated trees	**6**	**9**	**22.5**
LSD (5%)	na	na	20.5

[a] root colonisation measured only in replicate five and the surrounding area (approximately 15 random root pieces per tree were sampled, then 40 random root pieces selected for testing).
[b] root colonisation measured in all trees (approximately 10 root pieces per tree, then 24 random root pieces selected for testing). Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. na = not applicable.

The effect of *Trichoderma* on tree growth and disease expression was measured eleven months after application by trunk diameter at breast height (DBH) and disease severity (%). Change in DBH was found to be influenced by the initial DBH (*ie*: larger trees grew more than smaller trees irrespective of the treatment applied), therefore initial DBH was used as a covariate to calculate the adjusted change in DBH in ANCOVA analysis.

DBH increased in all five treatments eleven months after treatment application (Table 10). The root dowel treatment resulted in approximately twice the increase (P<0.05) in DBH increment, compared to the untreated Control. No disease was present at the October 2018 assessment; therefore, the growth response in the root dowel treatment may have been due to increased disease resistance and quicker recovery of canopy green tissue, compared to the Control trees. DBH increased in all treatments 24 months after application (Table 10). Similarly to the eleven-month measurement, the root dowel treatment had the largest growth response, but was not significantly (P<0.05) different from the Control (Table 10).

No disease was present in November 2019, therefore, the effect of *Trichoderma* on disease expression was not determined.

Table 10: Adjusted change in DBH (mm) increment eleven and 24 months after treatment application in the Kawerau 2017 trial.

Treatment	Adjusted Change in DBH (mm) Increment [a]	
	Eleven month period from Nov 2017 to October 2018	24 month period from Nov 2017 to Nov 2019
Root Dowel	10.2 a	22.7 a
Trunk Injection	7.8 ab	18.9 ab
Trunk Spray	6.4 ab	16.2 b
Soil Drench	6.1 b	16.8 b
Control	5.3 b	17.6 ab
LSD (5%)	4.1	5.5

[a] Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05.

Kaingaroa 2019 Trial:

Root colonisation levels were initially high at trial establishment (average of 40%; Table 11), but had reduced in all treatments twelve months after treatment application. Soil drench and trunk spray treatments had significantly (P<0.05) higher colonisation levels compared to the Control (Table 11).

Table 11: *Trichoderma* colonisation (%) of radiata pine tree roots measured prior to, and twelve months after, treatment application in the Kaingaroa 2019 trial [a].

Treatment	Measurement Date	
	21 Feb 2019 (before treatments applied) [b]	**14 Feb 2020 (12 months after treatment) [b]**
Root Dowel	47.7 a	16.7 abc
Trunk Injection	35.5 a	7.6 bc
Trunk Spray	40.7 a	17.2 ab
Soil Drench	43.0 a	24.4 a
Control	35.1 a	5.7 c
LSD (5%) for comparison of double-replicated Control with any other treatment	15.7	12.9
LSD (5%) for comparison of any two non-Control treatments	13.4	11.0

[a] approximately 10 small diameter shallow root pieces per tree were sampled, then 30 random root pieces were selected for testing

[b] Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05

Change in height increment, twelve months after treatment application, was significantly (P<0.05) greater (by 29%) in the trunk injection treatment, compared to the Control (Table 12). However, *Trichoderma* had no effect on DBH. *D. septosporum* presence was low (1% disease severity) in February 2020, therefore, the effect of *Trichoderma* on disease expression was not determined.

Table 12: Change in height (m) and DBH (mm) increment twelve months after treatment application in the Kaingaroa 2019 trial.

Treatment	Change in Height (m) Increment [a]	Change in DBH (mm) Increment [a]
Root Dowel	1.82 ab	16.4 a
Trunk Injection	1.97 a	15.6 a
Trunk Spray	1.62 ab	16.8 a
Soil Drench	1.67 ab	13.8 a
Control	1.53 b	14.1 a
LSD (5%) for comparison of double-replicated Control with any other treatment	0.43	4.1
LSD (5%) for comparison of any two non-Control treatments	0.42	3.5

[a] Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at $P<0.05$.

In the Kawerau 2017 and Kaingaroa 2019 trials, inoculation of *Trichoderma* spore suspensions directly into the tree (by root impregnated dowel or trunk injection), as opposed to application to the tree surface (by trunk spray and soil drench), appeared to result in increased growth. This may be because the spores had greater probability of germination and development inside the tree tissues.

In both trials, measurement of root colonisation levels was a good predictor of tree growth when comparing *Trichoderma* and non-*Trichoderma* (*ie*. Control) treatments. However, when comparing individual *Trichoderma* treatments and tree growth it was less accurate. This may have been due to the potential spatial variation of *Trichoderma* in the roots and/or the amount sampled may have been too small to measure accurately the relative amount in each tree.

Kinleith 2019 Trial

Application methods used in the Kawerau 2017 and Kaingaroa 2019 trials are unpractical for large-scale application of bioprotectants. An additional trial using foliar and ground sprays was established in 2019 to determine if *Trichoderma* could be inoculated into established trees in an efficient and effective manner to generate a growth and disease suppression effect. Root colonisation, height, DBH and disease will be measured at the start of 2021.

2.4 Milestone 4 – Nursery and forest plantation trials in Cypresses

Cypress is an important timber and shelter species for small-scale foresters, lifestylers and farmers. It can be strongly affected by cypress canker disease, caused by *Seiridium cardinale* and *Lepteutypa cupressi,* and trees may not reach maturity.

The potential for BPRC's *Trichoderma* root endophytes to control cypress canker is being assessed in two projects:

1. inoculated plants (Whelan 2018c) were distributed to members of the New Zealand Cypress Development Group (an interest group of the New Zealand Farm Forestry Association comprising foresters, landowners and lifestylers) in multiple locations where canker is prevalent. Measurement of canker will commence in 2020 and beyond.
2. a pot trial where inoculated plants will be infected with canker at the end of 2020 or early 2021.

An additional containerised trial was established at Southern Cypresses Nursery, Ohoka, Canterbury, to determine the effect of *Trichoderma* mixtures on cypress seedling growth of two species (*C. macrocarpa* and *C. lusitanica* var. *lusitanica*). The trial comprised a RCBD with six replicates.

Six *Trichoderma* treatment (no fungicide), including:

1. GenMix (FCC320, FCC327, LU633)
2. PR6 (FCC55, FCC318, FCC327 and FCC340; mixture used in radiata pine 2018 validation trials)
3. ModArb (LU655, LU659, LU660, LU661 and LU663)
4. PBI (LU132, LU140, LU584, LU633)
5. Murchison A (*T. harzianum* isolate)
6. Murchison B (*T. polysporum* isolate)

were compared to two controls:

1. Control with no fungicide (Control – fungicide) and
2. Control with fungicide (Control + fungicide).

No fungicide was used in the nursery in the 2018/2019 growing season, therefore fungicide was not applied to the Control + fungicide trays. Data were analysed with two control - fungicide treatments per replicate. Two of the *Trichoderma* treatments (Murchison A and B) were isolates collected from a susceptible *C. macrocarpa* clonal (Kukupa) stand in Murchison (a region prone to canker infection) with a history of low canker levels and high levels of natural *Trichoderma* (Whelan 2018c).

Seeds were mechanically planted into 6x8 cell plastic trays containing nursery potting mix (4 and 2 seeds per cell for *C. macrocarpa* and *C. lusitanica* respectively) on 30 November 2018. Multiple seeds were planted in each cell because of low germination and extra plants were removed four weeks after initial planting. Seeds were covered with a thin layer of vermiculite. *Trichoderma* treatments (5ml of 5.0 x 10^6 spores/ml spore suspensions) were pipetted into each cell at seeding and tap water was applied to the control trays. Trays were placed in a covered area for six months then placed outside. Trays were watered daily or when required.

Trichoderma root colonisation was measured on 11 November 2019 in 36 random root pieces from 6 plants per treatment, using MRB agar plates, according to established protocols (Whelan 2018c). Seedling height, RCD and dry weight of roots and shoots were measured for eighteen plants from the centre of the tray on 6 December 2019 (twelve months after seedling and inoculation; Figure 22). Data were analysed for significance by analysis of variance (ANOVA) and unprotected LSD tests at the 5% level (Genstat, v19).

Figure 22: The trial at harvest on 6 December 2019.

Root colonisation (%) ranged from an average of 10 to 53% in the *Trichoderma* treatments (Figure 23) with both seedlots susceptible to colonisation. No *Trichoderma* was found in the Control seedlings, therefore, environmental sources of *Trichoderma* had no impact on the result**.**

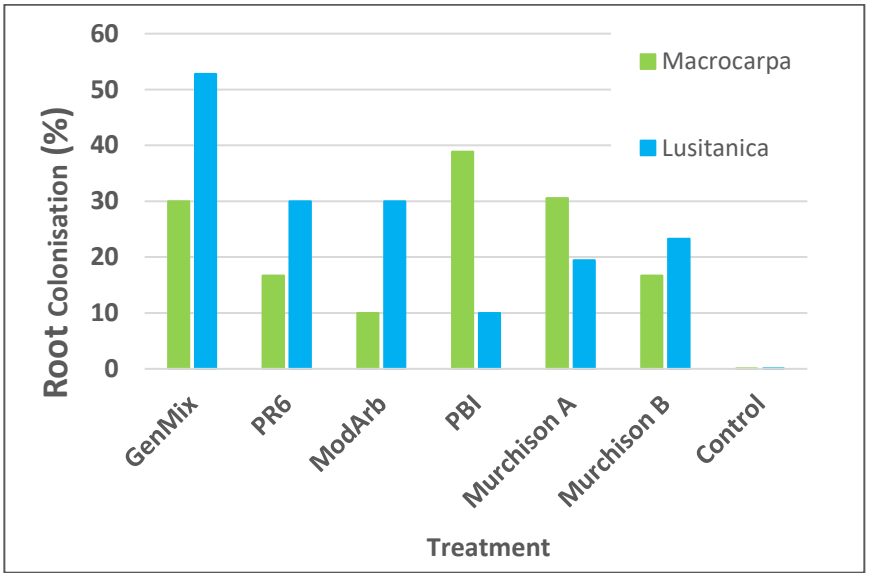

Figure 23: *Trichoderma* colonisation (%) of cypress seedling roots in treatments, sampled eleven months after seeding and treatment applications, based on MRB plating data.

No statistically significant interactions were observed between cypress species and *Trichoderma* treatment for any of the measured parameters (Table 13). Consequently, all results are discussed in terms of main effects. *C. macrocarpa* had significantly (P<0.05) larger seedlings, compared *to C. Lusitanica* var. *lusitanica* (Table 13). In one or more treatments, *Trichoderma* significantly (P<0.05) increased RCD, height and plant dry weight by 7%, 7% and 12% respectively, compared to the Control. *Trichoderma* had the biggest impact on root dry weight. *Trichoderma* increased root dry weight by 27% and resulted in a higher root/shoot ratio (by 26%), compared to the Control (Table

13). Therefore, application of *Trichoderma* to cypress seedlings may result in trees with improved establishment in the field, due to increased nutrient and water uptake and less socketing and windthrow, compared to untreated trees.

Trichoderma had no effect on shoot dry weight (Table 13). No individual *Trichoderma* treatment consistently increased seedling growth parameters, however ModArb was the least beneficial treatment. The two isolates selected from the Murchison stand did not increase seedling growth, compared to the BPRC isolates. However, the ability of the Murchison A isolate to supress canker disease will be tested in the 2019 potting trial (see below).

2019 Potting Trial:

On 6 December 2019, approximately 34 plants were randomly selected from the GenMix, PR6, Murchison A and Control - fungicide treatments in both seedlots and planted into 1.5 litre plastic pots containing potting mix (refer to Whelan 2018c for recipe). GenMix, PR6, and Murchison A spore suspensions (50ml of $5x10^6$ spores/ml) were applied to appropriate pots on 20 December 2019. Pots were placed in a shade house and watered daily (Figure 24). The potential for *Trichoderma* to reduce canker infection in these plants will be investigated at the end of 2020 or early 2012.

Figure 24: Inoculated cypress pot trial on May 2020.

Table 13: Effect of *Trichoderma* on root collar diameter (RCD), height and root, shoot and plant dry weight of cypress seedlings twelve months after seeding and inoculation on 6 December 2019.

	RCD (mm) [a]	Height (cm)	Mean Dry Weight (g)			Root / Shoot Ratio
			Root	Shoot [c]	Plant	
MAIN EFFECT MEANS:						
Species:						
C. Lusitanica	3.33 b	38.5 b	1.52 b	2.86 b	4.38 b	0.537 a
C. Macrocarpa	3.75 a	45.8 a	1.77 a	3.64 a	5.43 a	0.497 b
LSD (5%)	0.12	1.3	0.11	0.20	0.27	0.032
***Trichoderma*:**						
GenMix	3.70 a	42.5 abc	1.84 a	3.30 a	5.14 ab	0.565 a
PR6	3.61 ab	43.1 ab	1.71 ab	3.49 a	5.25 a	0.509 a
ModArb	3.49 ab	41.2 bc	1.62 bc	3.17 a	4.79 ab	0.516 a
PBI	3.53 ab	43.9 a	1.66 ab	3.32 a	4.99 ab	0.511 a
Murchison A	3.57 ab	42.4 abc	1.73 ab	3.11 a	4.84 ab	0.563 a
Murchison B	3.54 ab	43.3 ab	1.72 ab	3.11 a	4.84 ab	0.569 a
Control [b]	3.45 b	40.4 c	1.45 c	3.25 a	4.70 b	0.451 b
LSD (5%) for comparison of double-replicated control with any other treatment:						
	0.21	2.3	0.19	0.34	0.46	0.056
LSD (5%) for comparison of any two non-control treatments:						
	0.24	2.6	0.22	0.40	0.53	0.064
SPECIES x *TRICHODERMA* INTERACTION MEANS:						
C. Lusitanica:						
GenMix	3.53	40.0	1.72	2.95	4.67	0.582
PR6	3.49	39.5	1.61	3.23	4.84	0.498
ModArb	3.22	37.9	1.46	2.75	4.21	0.534
PBI	3.36	39.4	1.62	3.07	4.69	0.529
Murchison A	3.30	39.8	1.62	2.76	4.38	0.589
Murchison B	3.28	38.7	1.65	2.60	4.25	0.639
Control	3.24	36.4	1.26	2.75	4.01	0.462
C. Macrocarpa:						
GenMix	3.87	45.0	1.96	3.66	5.62	0.549
PR6	3.73	46.6	1.80	3.75	5.67	0.510
ModArb	3.76	44.5	1.78	3.45	5.37	0.498
PBI	3.70	48.3	1.70	3.58	5.28	0.493
Murchison A	3.83	45.1	1.84	3.45	5.29	0.537
Murchison B	3.80	48.0	1.80	3.63	5.43	0.499
Control	3.66	44.4	1.64	3.74	5.38	0.440
LSD (5%) for comparison of double-replicated control with any other single-replicated treatment:						
	0.29	3.2	0.27	0.49	0.65	0.079
LSD (5%) for comparison of double-replicated control with another double-replicated control for a different species:						
	0.24	2.6	0.22	0.40	0.53	0.064
LSD (5%) for comparison of any two non-control treatments:						
	0.34	3.7	0.31	0.56	0.75	0.091
Significance of Species x *Trichoderma* Interactions:						
	NS	NS	NS	NS	NS	NS

[a] Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. NS = not significant.

[b] No fungicide was used in the Control + fungicide treatment, therefore data for Control + fungicide and Control - fungicide treatments were analysed as two separate control - fungicide treatments in each replicate.

[c] Shoot dry weight was assessed from potting mix level to tip of seedling.

2.5 Milestone 5 – Nursery and forest plantation trials in Douglas-fir

Douglas-fir (*Pseudotsuga menziesii)* is the second most widely planted forestry plantation crop in New Zealand and can be affected by nursery and plantation foliar diseases, including Swiss needle cast (*Phaeocryptopus gaeumannii).* The effect of *Trichoderma* root isolates on Douglas-fir seedling growth was assessed in two containerised trials (2017 and 2018) at the Lincoln University Nursery, Canterbury. The potential for *Trichoderma* to control Swiss needle cast will be determined in plantation trial(s) in winter 2021.

A containerised trial at Lincoln University Nursery was established in September 2018 to verify the seedling growth benefits of *Trichoderma* found in the 2017 trial. The trial comprised a RCBD with eight replicates. Seed source, stratification, sowing and establishment methods of seedlings in 2018 were similar to that of the 2017 trial (Whelan, 2018c).

Eight *Trichoderma* treatments including:

- GenMix (FCC320, FCC327, LU633)
- PR3a (FCC13, FCC14, FCC15, FCC180; mixture used in radiata pine 2018 validation trials)
- PR6 (FCC55, FCC318, FCC327, FCC340; mixture used in radiata pine 2018 validation trials)
- ModArb (LU655, LU659, LU660, LU661 and LU663)
- Isolate FCC327
- PBI (LU132, LU140, LU584, LU633)
- D-fir A and B; two root isolates collected from healthy, actively growing Douglas-fir trees sampled in Rayonier Matariki Forests Glendhu Forest, South Otago

were compared to an untreated control.

Seedlots were:

- 12/663 with California provenance
- 12/662 with Oregon provenance.

Trichoderma root colonisation was measured at harvest (12 September 2019) in 36 random root pieces from 24 plants per treatment, using MRB agar plates, according to established protocols (Whelan 2018c). Seedling height, RCD and dry weight of roots and shoots were measured for either 14 or 21 plants in the centre of the tray (depending on the tray size). Data were analysed for significance by analysis of variance (ANOVA) and unprotected LSD tests at the 5% level (Genstat, v19).

Seedling germination and emergence were at expected levels in the Oregon seedlot (89% for seedlot germination and 87% emergence for the Control treatment) but very low (35% for seedlot germination and 27% emergence for the Control treatment) in the California seedlot (Table 14). Data were analysed as two separate RCBD ANOVA's due to the potential confounding effects of seed germination and plant density on plant growth.

Both seedlots were highly susceptible to *Trichoderma* colonisation, with levels ranging from 30 to 97% in the root pieces tested twelve months after seeding and inoculation (Figure 25). Similar results in the GenMix and PR3a treatments were found in a 2017 trial, when applied to three seedlots (average of 66%; Whelan, 2018c). In the 2018 trial, persistence was particularly high

(>80%) in the PR6 mixture. The Glendhu Forest isolates D-fir A and B were the poorest at colonising the seedling roots, although still at adequate levels (an average of 37%). The selection process the BPRC isolates undertook may have selected isolates that were more endophytic in nature and/or more aggressive at colonising roots, whereas the Glendhu isolates were not pre-tested.

The untreated Control seedlings had low (<6%) levels of *Trichoderma* from environmental sources (*eg.* potting mixture and/or airborne spores in the nursery), which had only had a minimal impact on the results.

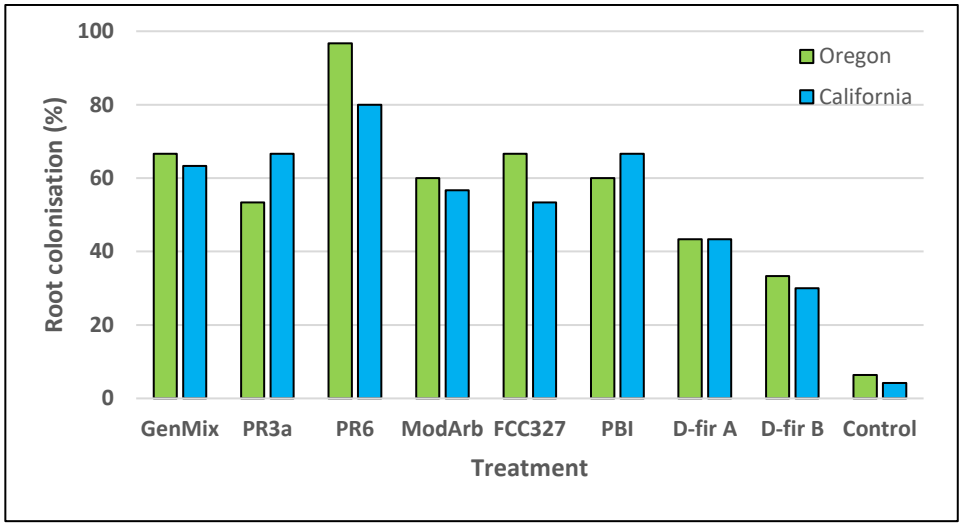

Figure 25: *Trichoderma* colonisation (%) of Douglas-fir roots in treatments, sampled twelve months after seeding and treatment application, based on MRB plating data.

Figure 26: Control and PR6 *Trichoderma* treatment trays in the Oregon seedlot twelve months after seedling and inoculation.

In the Oregon seedlot, five *Trichoderma* treatments significantly (P<0.05) increased seedling survival by an average of 7.7% (93.2%) at twelve months after seedling and inoculation, compared to the untreated Control (86.5%, Table 14). All *Trichoderma* treatments significantly (P<0.05) increased RCD by up to 14%, whilst four treatments significantly (P<0.0.5) increased height by up to 25%, compared to the Control (Table 14). In addition, three or more *Trichoderma* treatments significantly (P<0.05) increased root, shoot and plant dry weight, by up to 45%, 38% and 42% respectively, compared to the Control (Table 14). *Trichoderma* had no effect on the root/shoot ratio, compared to the Control. Overall, PR6 (Figure 26), GenMix and PBI were the most effective

treatments for increased growth in the Oregon seedlot. PR3a was also beneficial to growth, apart from it had a survival value similar to the Control.

In the California seedlot, a number of *Trichoderma* treatments were found to significantly ($P<0.05$) increase RCD, height, root, shoot and plant dry weight, by up to 13%, 22%, 38%, 35% and 29% respectively, compared to the Control (Table 14). PR3a was the only treatment to significantly ($P<0.05$) increase the root/shoot ratio, compared to the Control (Table 14). Overall, GenMix and D-fir B were the most effective treatments for increased growth, whilst PR6 was similar to the Control.

These results and those found in a 2017 trial, suggest that *Trichoderma* could reduce overall costs in nurseries due to increased production values and improve establishment of seedlings due to larger initial plant size once planted in the field. The PR6 mixture (a treatment used in the radiata pine validation 2018 trials) had very high levels of root colonisation (>80%), survival and growth, particularly in the Oregon seedlings, and may be a strain suitable for containerised Douglas-fir nursery production. Further research is required to confirm the suitability of this mixture in other commercially available plant genotypes and nursery production systems (*eg*. seedbed).

Approximately 3500 *Trichoderma* inoculated and untreated Douglas-fir plants were lined out in an industry nursery in March 2018 for use in plantation trials to investigate the potential of *Trichoderma* to control Swiss needle cast. A large number of the seedlings were damaged during undercutting, and, after discussion with relevant parties, the plants were abandoned. Replacement seedlings were produced in late 2019/early 2020 at Lincoln University Nursery and await bedding in the nursery.

Table 14: Effect of *Trichoderma* on survival, root collar diameter (RCD), height and root, shoot and plant dry weight of Oregon and California Douglas-fir seedlings twelve months after seeding and inoculation on 12 September 2019.

Seedlot and *Trichoderma* treatment	Survival (%) [a]	Root Collar Diameter (mm) [b]	Height (cm)	Mean Dry Weight (g)			Root /Shoot Ratio
				Root	Shoot [c]	Plant	
Oregon: [d]							
GenMix	95.5 a	2.77 a	22.5 a	0.95 ab	1.33 ab	2.28 abc	0.75 a
PR6	92.5 ab	2.81 a	22.3 a	1.16 a	1.53 a	2.69 a	0.81 a
PR3a	89.0 bc	2.75 a	23.0 a	1.13 a	1.47 a	2.61 a	0.83 a
ModArb	92.9 ab	2.75 a	21.6 ab	1.05 a	1.34 ab	2.39 ab	0.84 a
FCC327	89.8 bc	2.69 a	20.7 ab	1.07 a	1.29 ab	2.35 ab	0.88 a
PBI	93.0 ab	2.74 a	22.6 a	1.09 a	1.50 a	2.59 ab	0.76 a
D-fir A	92.3 ab	2.64 a	20.8 ab	0.92 ab	1.32 ab	2.23 bc	0.76 a
D-fir B	91.7 abc	2.67 a	19.8 ab	1.05 a	1.27 ab	2.32 abc	0.90 a
Control	86.5 c	2.46 b	18.4 b	0.80 b	1.11 b	1.90 c	0.75 a
LSD (5%)	**5.2**	**0.18**	**3.3**	**0.25**	**0.27**	**0.44**	**0.21**
California:							
GenMix	26.1 a	2.91 a	20.0 ab	1.30 a	1.72 a	3.02 a	0.82 cd
PR6	29.2 a	2.63 cd	17.3 bc	1.03 c	1.42 ab	2.46 bc	0.77 d
PR3a	24.4 a	2.67 bcd	17.3 bc	1.46 a	1.40 ab	2.86 ab	1.14 a
ModArb	25.6 a	2.86 ab	16.4 c	1.28 ab	1.22 b	2.50 bc	1.08 ab
FCC327	30.6 a	2.73 abcd	18.9 abc	1.27 ab	1.42 ab	2.69 abc	0.93 bcd
PBI	31.5 a	2.81 abc	17.2 bc	1.33 a	1.37 ab	2.70 abc	1.00 abc
D-fir A	30.0 a	2.79 abcd	19.0 abc	1.25 abc	1.42 ab	2.67 abc	0.90 bcd
D-fir B	30.5 a	2.93 a	20.7 a	1.30 a	1.52 ab	2.82 abc	0.87 bcd
Control	26.8 a	2.60 d	17.0 c	1.06 bc	1.27 b	2.35 c	0.89 bcd
LSD (5%)	**7.1**	**0.20**	**2.8**	**0.23**	**0.35**	**0.50**	**0.21**

[a] Low germination (27%) was found in the California seedlot and contributed to low emergence and survival rates.

[b] All seedlings were harvested in the California trays. In the Oregon seedlot, 14 or 21 plants were harvested in each tray (depending on the size of tray).

[c] Shoot dry weight was assessed from potting mix level to tip of seedling

[d] The two seedlots were statistically analysed in separate analyses. Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at $P<0.05$.

3.0 COMMERCIALISATION OF *TRICHODERMA* ISOLATES

Two isolate mixtures PR6 and PR3a were found to have growth promotion effects in young radiata pine plantations and in radiata pine, Douglas-fir and cypress nursery trials. The potential of these mixtures for commercial product development will be evaluated by Agrimm Technologies Ltd, Lincoln, Canterbury during 2020.

4.0 PROJECT OUTPUTS (JULY 2019 TO APRIL 2020)

Bergmann, G Barge, E, Whelan, H. and Busby, P. 2019. Exploring the identity and function of fungal seed endophytes in Douglas-fir *(Pseudotsuga menziesii* var*. menziesii)*. 87th Annual Meeting of the Mycological Society of America. Poster.

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Whelan, H. 2020. Summary of assessment for the Timberlands Ltd. Kaingaroa 2018 *Trichoderma* trial. Report to Kaingaroa Ltd. April 2020. 6p.

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5.0 CONCLUSIONS

Research at BPRC continues to improve our understanding of the health and growth benefits of *Trichoderma* bioprotectants in forestry nurseries and plantations. Results from the majority of large plantation trials established in 2018 confirmed that two *Trichoderma* mixtures (PR6 and PR3a), selected from previous national trials, could increase tree height at twelve months of age. In addition, *Trichoderma* significantly reduced disease severity of *D. septosporum* in trees fifteen months of age, in one trial, although did not eliminate the presence of disease. Benefits of *Trichoderma* to nursery production were also reaffirmed in recent trials, including a 75% increase in radiata pine cuttings suitable for plantation planting, a 10 and 42% increase in plant survival and dry weight in Douglas-fir and a 27% increase in root dry weight of cypress plants. In addition, the two mixtures being evaluated in the large plantation 2018 trials also performed well in the nursery trials suggesting that they are good contenders for commercial consideration.

This study provides the foundation for the development of effective fungal bioprotectant agents in New Zealand forestry. A commercial partner has been approached to determine the viability of the proposed bioprotectants. Availability of bioprotectants will ultimately contribute to healthier forests with fast growth and lead to increased productivity and economic gains for the forestry industry.

6.0 PROPOSED FUTURE RESEARCH

Priorities for research in 2020 most likely to lead to beneficial outcomes for the forestry industry were submitted and approved by FGR in December 2019.

7.0 REFERENCES

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