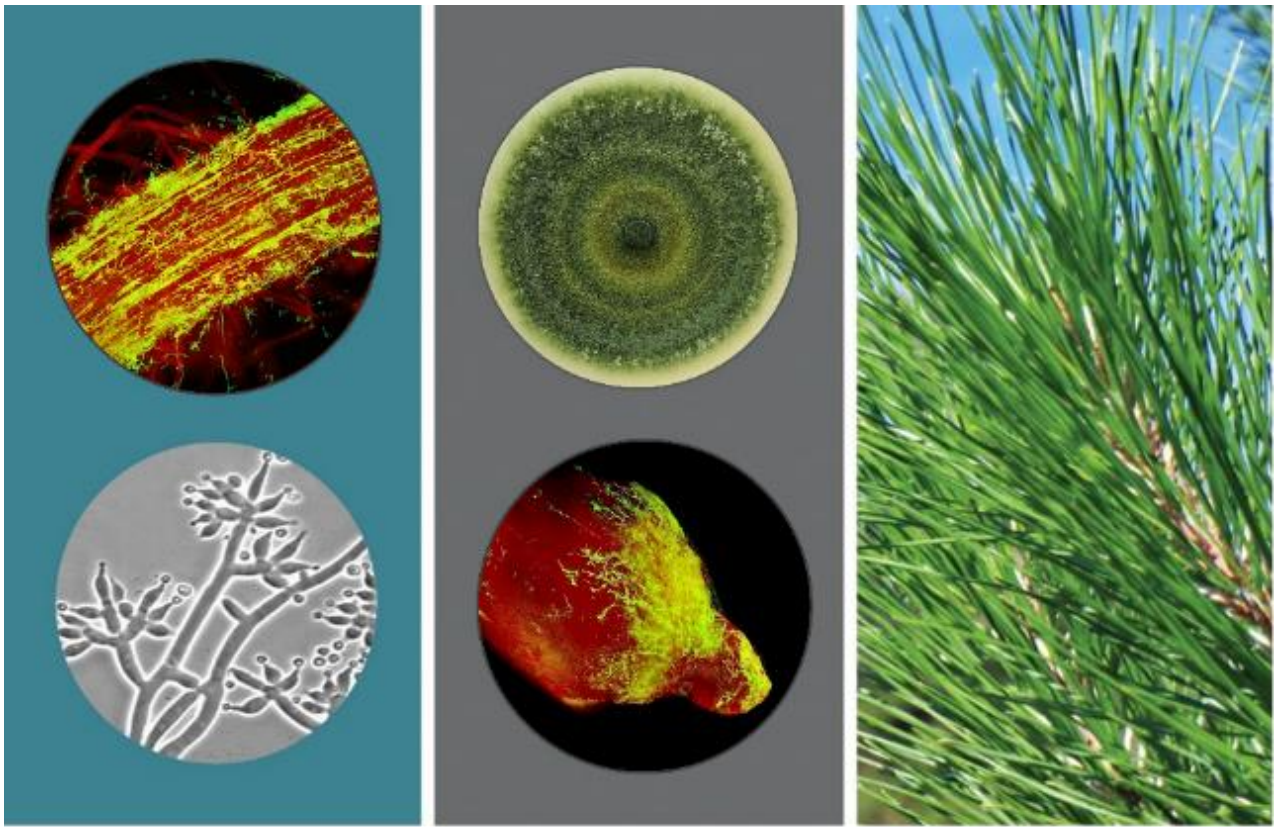
Bioprotection - enhancing growth and health Research Programme

LINCOLN UNIVERSITY
TE WHARE WĀNAKA O AORAKI

Bioprotection for foliar diseases and disorders of radiata pine

Project Overview
January 2022 to December 2022

Report prepared for New Zealand Forest Growers Research



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FOREST GROWERS RESEARCH

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EXECUTIVE SUMMARY

Plantation growth promotion and disease reduction with nursery applied *Trichoderma*:

This research programme has shown that nursery *Trichoderma* inoculation with PR6 and PR3a mixtures is an important management tool for radiata pine tree cultivation. Both inoculations were equally effective at significantly enhancing young tree growth and reducing foliar disease in a wide range of growing conditions in six forestry regions (Northland, Gisborne, Bay of Plenty/Waikato, Nelson, Canterbury and Otago, Table 1). Another Nelson trial with an additional *Trichoderma* treatment (PBI) also resulted in a significantly ($P<0.05$) increased tree height by 13%, compared to an untreated control, in year two.

Table 1: Effect of *Trichoderma* treatments PR6 and PR3a on mean radiata pine survival (%), height (m), diameter at breast height (DBH, mm), stand volume ($m^3.ha^{-1}$) and disease incidence and severity (%), including expressed as a percentage difference compared to the control (in brackets), in eight and five plantation trials established in 2018 and 2021 respectively.

Treatment	2018 Trials (year four)						2021 Trials (year one)	
	Survival (%)	Height (m)	DBH (mm)	Stand Volume ($m^3.ha^{-1}$) [b]	Disease		Survival (%)	Height (m)
					Incidence [c]	severity		
PR6	94.3 ns [a]	4.70 (7.1) *	76.5 (7.0) *	14.5 (12.3) *	51 (17) *	3.0 (38%) *	98.2 (ns)	59.8 (10.3) *
PR3a	95.0 *	4.69 (6.8) *	77.0 (7.7) *	14.7 (14.1) *	47 (23) *	2.6 (46%) *	97.1 (ns)	58.4 (7.8) *
Control	92.6	4.39	71.5	12.9	61	4.8	97.6	54.2

[a] significance of relationships $P<0.05$ (*) and not significant (ns).

[b] stand volume was estimated by summing the under-bark stem volume of the trees within the plot and adjusting to a per-hectare basis based on the size of the plot.

[c] mean foliar disease incidence and severity were measured in six trials where predominantly *Dothistroma septosporum* was present (the maximum level of disease severity in the control treatment was 13.1% in the Kaingaroa 660_2 trial).

Gain in productivity and nutrient response from *Trichoderma* inoculation:

Trichoderma treatments significantly ($P<0.05$) increased year four productivity (stem volume per hectare) by up to 40.2% across the eight sites. Stand productivity followed an optimum response relationship, where *Trichoderma* treatments contributed to gains at different abiotic stress levels and tree growth potentials. Inoculation with *Trichoderma* resulted in volume gains of:

- -5.0 to 19.9% in the low growth potential stands (four trials each in Nelson and Bay of Plenty/Waikato, ranging from 0.65 to 8.2$m^3.ha^{-1}$)
- 25.1 to 40.2% in the medium growth potential stands (four trials in Northland, ranging from 11.2 to 18.7 $m^3.ha^{-1}$) and
- 0.9 to 14.6% in the high growth potential stands (four trials in Gisborne, ranging from 29.9 to 34.6 $m^3.ha^{-1}$).

The gain in productivity from *Trichoderma* was found to be significantly ($P<0.05$) higher in sites with warmer air temperatures and more rainfall and was inversely correlated to the altitude of the site.

New Zealand's planted forest soils are now supporting rotations at higher stocking with genotypes that grow faster and have greater nutrient demands. Silviculture management practices that conserve accumulated nutrients will enable the uptake of faster and greater nutrient supplies in the following rotation. Nutrient analysis in needle and soil samples was undertaken in seven trials to determine if the previously observed growth benefits in trees inoculated *with Trichoderma* influenced the uptake of soil nutrients into the foliage. Across all nutrients, *Trichoderma* treatments did not significantly ($P<0.05$) improve uptake from the soil into the needles. However, the samples were collected in winter (delayed from the planned date of February/March because of Covid-19 travel restrictions) and so potentially reflect optimal soil moisture, nutrient availability and low foliage nutrient demand conditions. However, the efficiency of phosphorus uptake increased by 5.6-10.4% for PR3a and PR6 on average across sites respectively. Future sampling at the more critical and typical time of February or March when trees are under high nutrient stress may provide greater treatment contrasts.

Vector analysis techniques were used to interpret the important influences of tree volume (surrogate of biomass), foliar nutrient concentration, and predicted nutrient content of individual nutrients across sites and treatments. The responses showed that sites with volume gains associated with *Trichoderma* inoculation absorbed larger amounts of nutrients than uninoculated trees, with trends suggest a current accumulation phase or that individual nutrients were previously limited. Part of this response may also have been that growth increased needle biomass to the extent that nutrient concentration was diluted relative to that in the control trees, supporting the hypothesis that an improved nutrient efficiency did occur in some inoculated plots.

These data show complex relationships between inoculated *Trichoderma* species and radiata pine trees grown in New Zealand's soil and climatic conditions. The best responses were found across mid to low growth sites and this may help to target *Trichoderma* applications better in the future.

Impact of *Trichoderma* inoculants on tree uniformity:

Practical methods to improve tree structure uniformity are important to forestry managers when the goal is to develop, manage and harvest a forest with low levels of variability in tree height and trunk diameter. The coefficient of variation (CV) and the Lorenz Curve and Gini Coefficient (GC) statistics were used to identify that both *Trichoderma* PR6a and PR3a mixtures increased the uniformity of tree height, DBH, basal area and stem volume by 9.2 to 13.9% when meaned across all the 2018 sites. These mixtures are therefore recommended as practical and effective tools for foresters that want to reduce production or harvest costs and produce timber of more uniform size. This technique is relevant to both intensive or low cost, extensive management regimes and will ultimately lead to more efficient and sustainable use of forest resources.

Use of *Trichoderma* to boost new nursery areas being brought into production:

In this research programme, nursery application of *Trichoderma* inoculants has been during the production stages of stock material (as part of a seed coat, a soil or potting media drench or a root dip). An alternative method for the introduction of *Trichoderma* into the nursery system could be a spore suspension spray onto fallow land to boost soil *Trichoderma* levels prior to land preparation for stock production.
Three *Trichoderma* treatments (PR6, PR3a and GM), applied by a commercial sprayer almost two years before the harvest of lined-out cuttings, did not significantly ($P<0.0.5$) affect soil *Trichoderma* levels at three sampling times, apart from seven months after application, when the GM treatment was significantly higher than the untreated area. However, at cutting harvest, root *Trichoderma* levels, survival, and the number of plants with high root scores (3 or 4 quadrants with strongly growing roots) or root collar diameters of >6.9mm, were significantly ($P<0.05$) higher in the *Trichoderma* treatments compared to the cuttings planted in the untreated areas. This led to significantly ($P<0.05$) more cuttings passing the production standard for field planting (ranging from 48 to 54%) compared to the control (30%). The spraying of *Trichoderma* onto fallow land was an efficient and effective method for improving production outputs.

Potential for *Trichoderma* to control of Swiss needle cast in Douglas-fir:

Douglas-fir (*Pseudotsuga menziesii)* is the second most widely planted forestry plantation crop in New Zealand and can be affected by foliar diseases, including Swiss needle cast (*Phaeocryptopus gaeumannii*; SNC). The potential for *Trichoderma* to control SNC will be determined in a Kaingaroa Forest plantation trial established on 18 August 2022. The trial comprised of eight treatments and seven replicates and measurements of tree height, disease and root colonisation will be performed in winter 2023.

Promotion of *Trichoderma* inoculants in the NZ forestry industry:

In 2022, *Trichoderma* inoculum was supplied to twelve New Zealand forestry nurseries for the treatment of approximately 65 million radiata pine seeds. The supply of inoculum is to help nurseries gain experience using *Trichoderma* in their production systems and to build a business case for commercialisation of a forestry specific mixture. Two nurseries now inoculate all their radiata pine seed with *Trichoderma*. Additional inoculum will be offered to the industry in 2023.

1.0 INTRODUCTION

Foliar diseases are the most significant cause of economic loss for the New Zealand Forestry industry (Hill 2016). To alleviate losses caused by existing diseases and to reduce potential impacts of biosecurity threats, ongoing work is being conducted to establish a long-term symbiotic relationship between *Pinus radiata* (radiata pine) and other forestry tree species, and beneficial native *Trichoderma* root endophytes. *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 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 septosporum* (Dothistroma needle blight), *Colletotrichum acutatum* (terminal crook disease) or *Sphaeropsis sapinea* (diplodia canker) in radiata pine seedlings. The 24 most effective isolates were then tested, as an 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 2019b). The two most effective mixtures, PR6 and PR3a were then tested in fourteen large-scale trials in six forestry regions. The project is looking to validate and commercialise a *Trichoderma* inoculant for forestry bioprotection and sustainable production in nurseries and plantations.

NZ Forest Growers Research using recurring 12-month contracts funded this project. Recent project tasks completed for the period August 2019 to December 2021 were detailed in Whelan (2019b, 2020 and 2021). This report summarises research results from January 2022 to December 2022.

2.0 BIOPROTECTION PROJECT MILESTONES

2.1 Production of *Trichoderma* inoculum (Milestone 1)

Trichoderma inoculum was supplied to the following companies in 2022: Appletons Tree Nursery, ArborGen Te Teko, Kaikohe, Puha and Tokoroa Nurseries, Leithfield Nursery, PF Olsen Ltd Waiuku and Seddon Nurseries, Rangiora Nursery and Kauri Park Nursery, for coating of approximately 65 million seeds or drench and root applications to cuttings or ramets. Distribution of spores will contribute to information gain for the business case for commercialisation of a specific forestry mixture. Appletons Tree Nursery treated all their radiata pine seeds in September 2022 and stock was used for the whole estate plantings of OneFortyOne. Similarly, PF Olsen Waiuku treated all their radiata pine seed, as they have done consistently for the past twenty years.

2.2 Nursery and forest plantation trials in radiata pine (Milestone 3.2)

2.2.1 Forestry plantation validation trials for the two most effective treatments PR6 and PR3a

Following laboratory, greenhouse and plantation screening programmes (Hill 2016, Whelan 2019a) two *Trichoderma* mixtures, PR6 and PR3a, were selected for validation of previously observed growth promotion and foliar disease suppression effects in fourteen large-scale plantations trials.

2.2.1.1 Plantation validation trials established in 2018

The trial series established in 2018 included two trials in each of four important forestry regions (Gisborne, Northland, Bay of Plenty/Waikato and Nelson, Table 2). In brief, the two *Trichoderma* mixtures were applied as part of a seed coat recipe to a single radiata pine seed-lot and the seedlings were raised under standard containerised tray management practices in a commercial nursery. Treated and untreated (control) seedlings were then out-planted in commercial plantation forests that coincidentally had different site preparation methods and tree stocking rates (ranging from 800 to 1190 stems per hectare). Herbicide management was also variable in the first two years of growth, but no insecticides, fungicides or fertilisers were applied. Experimental design consisted of randomised complete blocks with 7 to 10 replications within each trial and plots contained 81 trees in a 9 x 9 grid pattern. Tree height (measured with a height pole or Haglöf Vertex 5 Ultrasonic Hypsometer (https://haglofsweden.com), diameter at breast height (DBH, at 1.4m, measured with a diameter tape) and foliar disease (percentage incidence and severity, method according to Whelan 2019a) were measured in the central 25 plants (5 x 5) approximately four years after trial establishment on 29 May and 1 June (Berrymans and Sherry), 4 and 7 July (Topuni and Whatoro), 15 and 16 August (Kaingaroa 660_2 and 209_4) and 6 and 8 September 2022 (Tauwhareparae and Patunamu, Figure 2).

Under-bark stem volume (v) for each individual tree was calculated using the following formula:

$$v=h*ba*(B1*(h-1.4)**(-B2)+ B3 \quad (1)$$

where h = height of an individual tree (m) in measurement plot, ba = basal area of an individual tree in measurement plot ($ba = 7.85\times10^{-5}\times DBH^2$, m^2) and f = individual-tree breast height volume form factors (derived from $f = v/(ba\times h$, m^3; B1=0.86, B2=0.972 and B3=0.304; Kimberley and Beets 2007). Then, productivity at stand level was scaled by summing the volume of the trees within the plot and adjusting to a per-hectare basis (stand volume, $m^3.ha^{-1}$) based on the size of the plot.

Disease severity (%) was scored as a combination of current infection and previous infection where needles had been cast ("defoliation"; Figure 1).

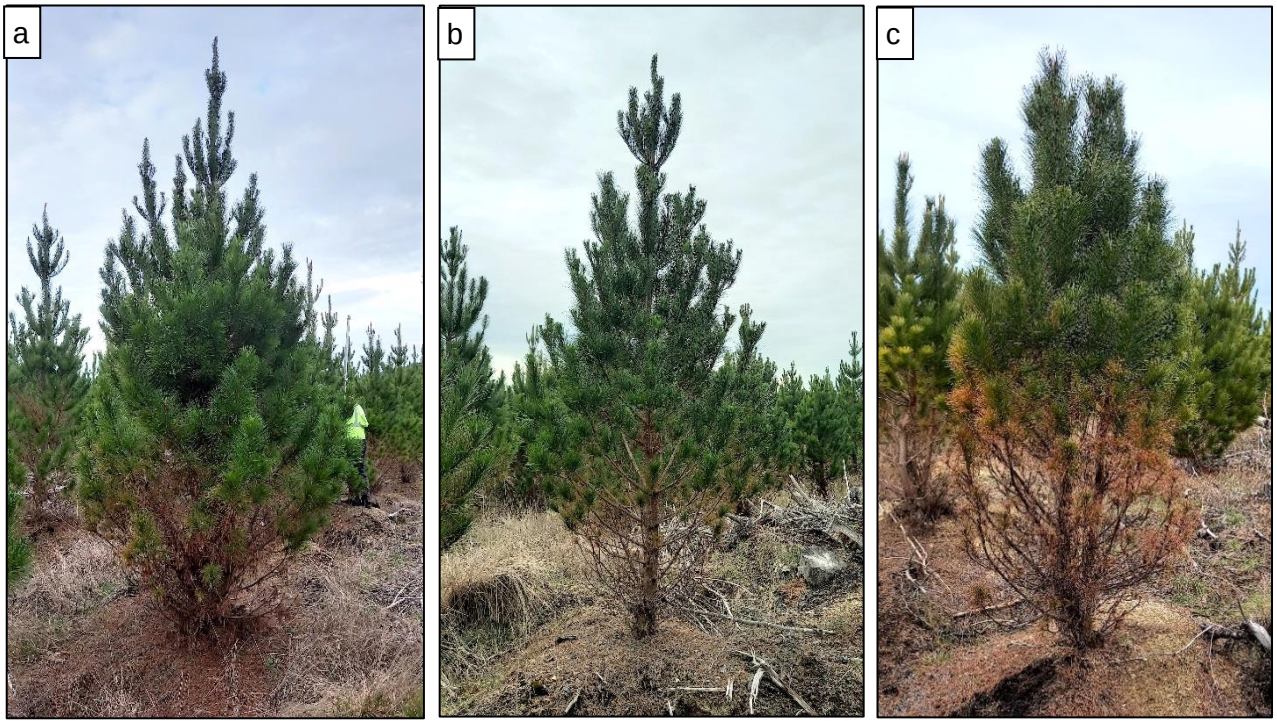

Figure 1: Examples of *Dothistroma septosporum* infection in the Kaingaroa 660_2 trial on 17 August 2022. Tree a) had 15% disease severity (10% current and 5% defoliation), b) had 25% severity (5% current and 20% defoliation) and c) had 40% severity (15% current and 25% defoliation).

Table 2: Establishment details and comments in winter 2022 for the 2018 radiata pine trials.

Region	Company	Forest / Trial Name	Location	Altitude (m asl)	Planting Date	No Replicates	Planting Stocking Rate and spacing
Bay of Plenty / Waikato	Timberlands	Kaingaroa 209_4	-38.559711 176.445696	180	24/07/18	10	925 (4m x 2.7m)
			Spot-mounded, flat, free-draining soil. Blackberry was beginning to regrow from the pre-plant herbicide but was not limiting tree growth. *D. septosporum* was present at moderate levels in 2020 but had reduced in 2022 possibly due to reduced inoculum load caused by a nearby operational fungicide spray.				
		Kaingaroa 660_2	-38.868971 176.280092	240	23/07/18	10	925 (4m x 2.7m)
			Spot-mounded, flat free-draining soil. No weeds of importance were present four years after planting. Pigs had rooted the ground but did not cause damage to the trees. *D. septosporum* had further developed since the previous assessment in winter 2020.				
Northland	Rayonier Matariki Forests Hancock	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. At the year four assessment gorse, cutty grass (*Gahnia lactera*), pampas grass and rushes had established strongly, particularly in the lower half of the trial, however, trees were beginning to outgrow these weeds.				
		Whatoro	-35.708955 173.676602	330-350	08/08/18	10	833 (4m x 3m)
			10km from coast, flat. At the year four assessment, annual grasses and broadleaf weeds were the dominant weeds with patches of pampas grass established. Inkweed (*Phytolacca octandra*) had developed over larger areas since winter 2020 but was found to be generally desiccated in July 2022. Many trees with socketed trunks observed in earlier measurements had stabilised and grown vertically.				
Gisborne	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. Grasses were the dominant weed, with rushes, broadleaf weeds and hawthorne also present, at the year four assessment on 8 September 2022. Blackberry, pampas grass and cutty grass had begun to establish. Pig, deer and goats were in the area but generally had not damaged the trial trees. Red needle cast *(Phytophthora pluvialis)* was present in the Gisborne area in winter 2022 and had developed in this trial.				
	PFOlsen	Tauwhareparae	-38.198800 178.099317	400-425	11/08/18	10	1010 (3.3m x 3m)
			Variable slope (flat to 10°) and aspect (south, NE and west). Trees with yellow stunted growth in earlier assessments had either recovered (but were short) or had died. Overall, survival was reduced, particularly in the control plots. Soil continues to move in the trial with slippage found in five plots; one plot was not measured. Annual grasses and broadleaf weeds were prevalent, but no disease was present in the year four assessment.				
Nelson	OneFortyOne	Golden Downs Sherry	-41.448233 172.651675	310-380	03/09/18	8	800 (5m x 2.5m)
			Ex-Douglas-fir. West slope (7-10° bottom half and 17-32° top half of trial) with replicates placed across the slope. Cold, snow-prone site but more sheltered from winds compared to Berrymans. The bottom 3 replicates had low survival (mean 86%) due to frost damage. Trees were strongly growing and generally not impacted by the grasses, Himalayan honeysuckle, bracken and blackberry. A strip of land that did not receive a pre-plant herbicide had large broom plants and plots in this zone were not measured. Relatively low levels of *D. septosporum* were observed.				
		Golden Downs Berrymans	-41.458333 172.90833	450	05/09/18	7	800 (5m x 2.5m)
			South 8° slope with internal gullies (plots were arranged to avoid these). A range of slash levels but plots arranged to have similar levels in each replicate. The trees were very small compared to the other sites, due to the cold, frosty, wind-exposed nature of the site and poor soils. The bottom 3 replicates had low survival (mean of 83%) due to frost damage. *D. septosporum* was present at low levels.				

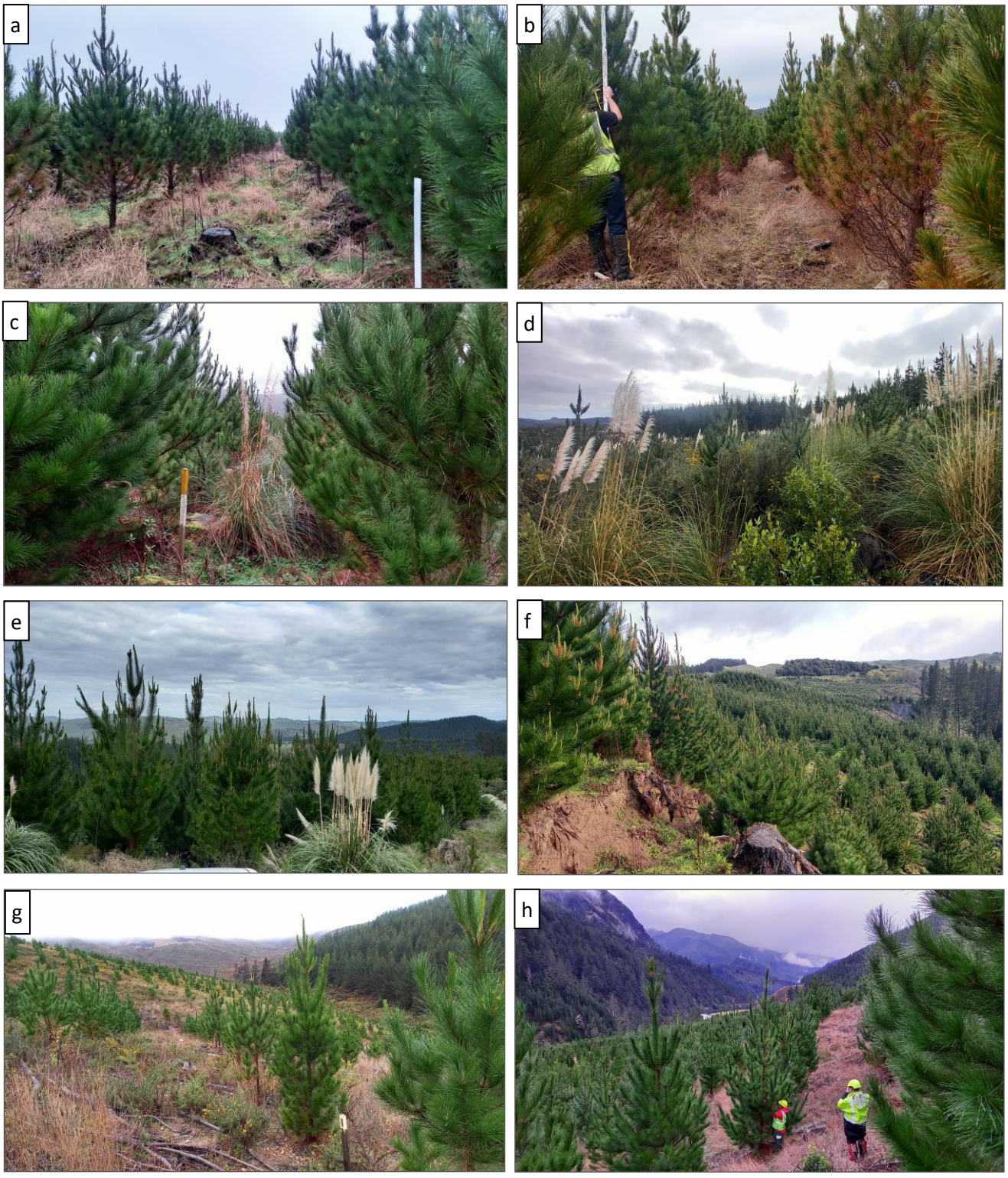

Figure 2: Validation trials a) Kaingaroa 209_4 and b) 660_2, Bay in Plenty/Waikato, c) Whatoro and b) Topuni in Northland, e) Patanamu and f) Tauwhareparae in Gisborne and g) Berrymans and h) Sherry in Nelson at the year four assessment in winter 2022.

Establishment of trees in the trials was relatively high, with mean survival of 94% in year four (Table 3; down from 96.7% in year one). *Trichoderma* treatment PR3a had significantly ($P<0.05$) higher mean survival (95%) compared to the control (92.6%) and was particularly evident in Topuni (99.5% vs 91.5%). This inoculant also trended higher in the Nelson trials (Sherry 94.8% vs 91.4% and Berrymans 92% vs 87.4%).

Trichoderma treatments had significantly (P<0.05) increased tree height by up to 15.9% in six of the eight 2018 trials in year four (Table 3). The largest increases in height due to *Trichoderma* were in the Northland (9.1% to 15.9%), followed by the Bay of Plenty/Waikato (6.4% to 9.1%) and Gisborne (4.9% and 10.1%) trials. Similar trends in DBH to height were found in the *Trichoderma* treatments, with Northland trials having a 14.7 to 18.0%, Bay of Plenty/Waikato 8.6% and 9.2% and Gisborne 3.7 to 8.4% increase in DBH (Table 3). In the Nelson Golden Downs trials, the PR6 and PR3a mixtures had no effect on height and DBH compared to the controls (Table 3). When analysed over all sites, *Trichoderma* treatments significantly (P<0.05) increased tree height by 7.1% (4.7m, PR6) and 6.8% (4.7m, PR3a) compared to the control (4.4m), and DBH by 7.0% (76.5cm, PR6) and 7.7% (77cm, PR3a) compared to the control (71.5cm, Table 3).

Trichoderma increased trunk basal area and volume by larger amounts compared to height and DBH because these variables had additional mathematical derivations (ie. the exponential treatment of DBH and the multiplication of basal area and height in the volume calculation). When analysed over all sites, *Trichoderma* treatments significantly (P<0.05) increased basal area by 11.7% (0.00547m^2, PR6) and 12.7% (0.00552m^2, PR3a) compared to the control (0.0049m^2, Table 3). Tree and stand volume of *Trichoderma* inoculated trees were also significantly (P<0.05) increased by 12.5% (0.0162m^3) and 12.3% (14.5$m^3.ha^{-1}$) for PR6, and 16.1% (0.0167m^3) and 14.1% (14.7$m^3.ha^{-1}$) for PR3a respectively (Table 3), compared to the controls (0.0144m^3 and 12.9$m^3.ha^{-1}$). These early gains in young tree production from *Trichoderma* are important for the successful establishment of the stand and also provide environmental, economic and social opportunities for rapid carbon sequestration. The effect of *Trichoderma* on the gain in productivity under different environmental conditions is discussed in section 2.3.

Trichoderma was measured in the tree roots at each site (except Tauwhareparae due to resource constraints), with the Gisborne sites having high natural levels at each measurement (Table 4). When analysed over all sites within each year, root colonisation in the *Trichoderma* treatments was significantly (P<0.05) higher compared to the controls (Table 4). Generally, *Trichoderma* treatments that generated growth responses had higher levels of root colonization than the control. Therefore, the inoculated isolates appeared to have a more dominant role in the growth response compared to the naturally occurring strains.

In the winter 2022 assessments*, Dothistroma septosporum* (present in Kaingaroa 660_2 and 209_4, Whatoro, Berrymans and Sherry) and *Phytophthora pluvialis* (present in Patunamu) were found on between 16 to 100% of the control trees, but disease severity was at relatively low levels (between 1 to 13%; Figure 3). In all sites with the presence of disease, mean incidence and severity were significantly (P<0.05) reduced from 61.4% (control) to 50.8% (PR6) and 47.3% (PR3a), and from 4.8% (control) to 3.0% (PR6) and 2.6% (PR3a) respectively.

Table 3: Effect of *Trichoderma* treatments on tree survival (%), height (m), trunk diameter (mm), basal area (m^2) and mean tree (m^3) and stand volume ($m^3.ha^{-1}$), including percentage difference compared to the untreated controls, in the 2018 trials approximately four years after planting.

Region and Site	Treatment	Survival [a] (%)	Height [b] (m)	Trunk Diameter (mm) [c]	Basal Area (m^2) [d]	Volume [d]	
						Tree (m^3)	Stand ($m^3.ha^{-1}$)
Bay of Plenty / Waikato Kaingaroa 209_4	PR6	98.8 a	3.95 (9.1) a	62.5 (9.1) a	0.00324 (16.1) a	0.00853 (17.7) a	7.80 (19.9) a
	PR3a	96.8 a	3.72 (2.8) b	58.9 (2.8) b	0.00289 (3.6) b	0.00750 (3.4) b	6.72 (3.4) b
	Control	97.2 a	3.62 b	57.3 b	0.00279 b	0.00725 b	6.51 b
	LSD (5%)	**2.4**	**0.15**	**3.1**	**0.000301**	**0.00081**	**0.70**
Bay of Plenty / Waikato Kaingaroa 660_2	PR6	98.4 a	3.87 (7.8) a	58.1 (8.6) a	0.00278 (15.4) a	0.00723 (15.9) a	6.56 (16.4) a
	PR3a	97.2 a	3.82 (6.4) a	58.4 (9.2) a	0.00281 (16.6) a	0.00732 (17.3) a	6.59 (16.8) a
	Control	98.0 a	3.59 b	53.5 b	0.00241 b	0.00624 b	5.64 b
	LSD (5%)	**2.3**	**0.14**	**3.4**	**0.000294**	**.00079**	**0.75**
Northland Topuni	PR6	96.1 a	4.74 (15.9) a	69.5 (14.7) a	0.00415 (25.4) a	0.01167 (28.7) a	11.22 (35.0) a
	PR3a	99.5 a	4.73 (15.6) a	68.8 (13.5) ab	0.00403 (21.8) ab	0.01170 (29.0) a	11.64 (40.2) a
	Control	91.5 b	4.09 b	60.6 b	0.00331 b	0.00907 b	8.31 b
	LSD (5%)	**4.2**	**0.3**	**8.7**	**0.00081**	**0.00235**	**2.69**
Northland Whatoro	PR6	95.2 a	4.91 (9.1) a	98.9 (18.0) a	0.00855 (32.3) a	0.02441 (33.4) a	18.74 (33.3) a
	PR3a	94.4 a	5.01 (11.3) a	98.5 (17.5) a	0.00828 (28.2) a	0.02604 (42.3) a	17.59 (25.2) a
	Control	93.6 a	4.50 b	83.8 b	0.00646 b	0.01830 b	14.06 b
	LSD (5%)	**4.7**	**0.31**	**8.9**	**0.00125**	**0.0054**	3.01
Gisborne Patunamu	PR6	92.3 a	6.85 (4.9) a	109.4 (3.7) a	0.00967 (7.0) a	0.03173 (10.2) a	32.81 (0.9) a
	PR3a	94.2 a	6.74 (3.2) ab	110.4 (4.6) a	0.00981 (8.5) a	0.03180 (10.5) a	34.61 (6.4) a
	Control	95.6 a	6.53 b	105.5 b	0.00904 b	0.02879 b	32.52 a
	LSD (5%)	**6.5**	**0.26**	**3.7**	**0.00062**	**0.0025**	**4.55**
Gisborne Tauwhareparae	PR6	91.5 a	6.10 (6.8) ab	113.5 (4.2) ab	0.0106(3.8) b	0.0329 (3.5) b	29.85 (11.5) b
	PR3a	90.7 a	6.29 (10.1) a	118.1(8.4) a	0.0113 (11.1) a	0.0356 (11.9) a	30.72 (14.6) a
	Control	86.4 a	5.71 b	108.9 b	0.0102 b	0.0318 b	26.80 b
	LSD (5%)	**11.7**	**0.41**	**8.1**	**0.00091**	**0.0036**	**4.00**
Nelson Sherry	PR6	92.0 a	4.48 (2.1) a	68.5 (-0.4) a	0.00388 (-3.5) a	0.01055 (-4.0) a	7.41 (-5.0) a
	PR3a	94.8 a	4.51 (2.7) a	69.7 (1.3) a	0.00403 (0.3) a	0.01101 (0.0) a	8.18 (4.7) a
	Control	91.4 a	4.39 a	68.8 a	0.00402 a	0.01099 a	7.81 a
	LSD (5%)	**6.6**	**0.37**	6.8	**0.00127**	**0.00218**	**2.00**
Nelson Berrymans	PR6	89.7 a	2.70 (0.0) a	31.9 (-3.3) a	0.000895 (-6.2) a	0.00236 (-6.0) a	1.65 (-4.1) a
	PR3a	92.0 a	2.73 (1.1) a	33.4 (1.2) a	0.001002 (5.0) a	0.00263 (4.8) a	1.90 (10.4) a
	Control	87.4 a	2.70 a	33.0 a	0.000954 a	0.00251 a	1.72 a
	LSD (5%)	**9.3**	**0.19**	**4.9**	**0.000353**	**0.00063**	**0.41**
Mean	PR6	94.3 ab	4.70 (7.1) a	76.5 (7.0) a	0.00547 (11.7) a	0.0162 (12.5) a	14.5 (12.3) a
	PR3a	95.0 a	4.69 (6.8) a	77.0 (7.7) a	0.00552 (12.7) a	0.0167 (16.1) a	14.7 (14.1) a
	Control	92.6 b	4.39 b	71.5 b	0.00490 b	0.0144 b	12.9 b
	LSD (5%)	**2.1**	**0.28**	**3.3**	**0.000431**	**0.00155**	**1.14**

[a] letters were assigned according to a Fisher's 5% level unprotected LSD procedure. For each site and variable, values followed by different letters are significantly different at $P<0.05$.

[b] *Trichoderma* treatment measurement expressed as a percentage difference compared to the control within each site (in brackets).

[c] trunk diameter was measured at 1.4m above ground level (DBH).

[d] basal area (m^2) and under-bark stem volume of an individual tree (m^3) were according to Formula (1). Productivity at stand level was calculated by summing the volume of the trees within the plot and adjusting to a per-hectare basis ($m^3.ha^{-1}$) based on the size of the plot.

Table 4: *Trichoderma* colonisation (%) of radiata pine roots in the 2018 trials, sampled one, two and four years after planting, based on MRB plating data.

Region	Site	*Trichoderma* root colonisation [a]								
		2019 (Year One)			2020 (Year Two)			2022 (Year Four)		
		PR6	PR3a	Control	PR6	PR3a	Control	PR6	PR3a	Control
Gisborne	Patunamu	64.3	81.0	51.1	52.8	43.3	21.4	98	100	97.6
	Tauwhare-parae	59.5	92.9	72.2	66.7	61.1	56.7	-	-	-
Bay of Plenty / Waikato	Kaingaroa 209_4	88.6	36.7	22.2	43.8	29.2	20.4	69	73.8	64.3
	Kaingaroa 660_2	43.5	38.9	13.5	37.8	33.4	12.3	86.1	76.2	61.9
Northland	Topuni	47.5	41.7	16.7	30.0	44.4	33.3	61.9	77.8	47.2
	Whatoro	34.6	70.1	32.3	70.8	33.3	12.5	76.2	73.8	48.8
Nelson	Sherry	30.0	26.7	4.2	33.3	41.7	11.1	44.4	47.2	44.4
	Berrymans	14.0	20.0	6.7	42.3	26.9	26.0	27.8	25.0	33.3
Mean [b]		47.8 (8.2) a	51.0 (9.5) a	28.1 (8.7) b	47.2 (5.3) a	39.2 (3.9) a	24.2 (5.4) b	66.2 (9.1) a	67.7 (9.2) a	56.8 (7.9) b
LSD (5%)		16.5			11.4			9.0		

[a] root pieces for each treatment were bulked together at each site. *Trichoderma* was then isolated from approximately 48 sterilised random pieces of roots per treatment, using malt yeast extract and rose bengal agar plates, according to established protocols (Whelan 2018b).

[b] letters were assigned according to a Fisher's 5% level unprotected LSD procedure. For each site and year, values followed by different letters are significantly different at P<0.05. Standard error of means in brackets.

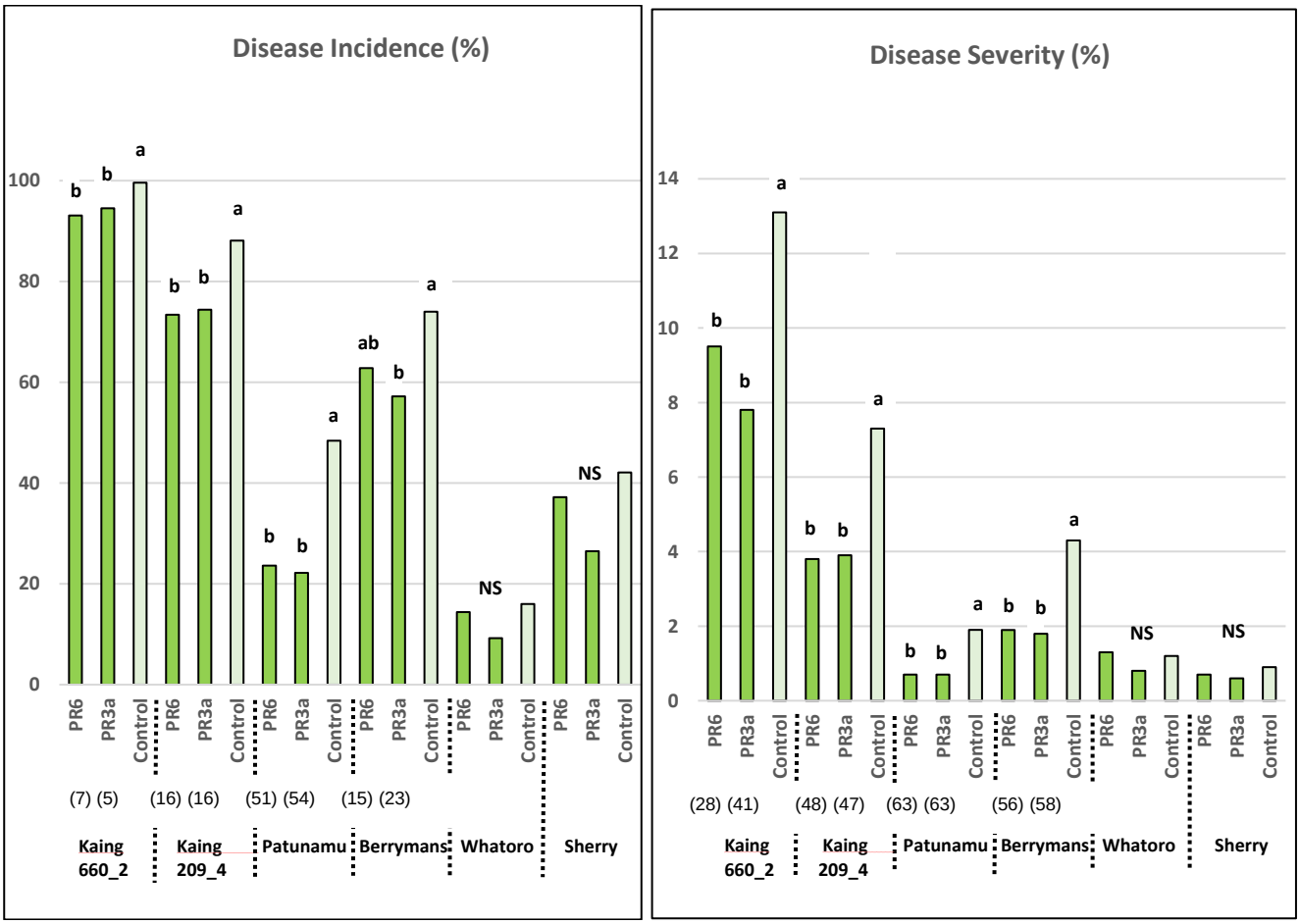

Figure 3: Effect of *Trichoderma* treatments on *Dothistroma septosporum* (Kaingaroa 660_2 and 209_4, Whatoro, Berrymans and Sherry) and *Phytophthora pluvialis* (Patunamu) incidence and severity (%), including percentage difference from the control (in brackets), in winter 2022. Letters were assigned according to a Fisher's 5% level unprotected LSD procedure. For each site and variable, values followed by different letters are significantly different at P<0.05.

2.2.1.2 Plantation validation trials established in 2020 and 2021

The positive results in the 2018 trials encouraged forestry companies to develop additional trials in the South Island. One plantation trial, testing three *Trichoderma* mixtures (PR6, PR3a and PBI - consisting of LU132, LU140, LU584 and LU633 *T. atroviride* isolates) against an untreated control, was established in Nelson in 2020 (Table 5, Figure 4a). Bare root seedlings (Seedlot 19/505, 1/0) inoculated with *Trichoderma* spores as part of a seed coat, were supplied by Appletons Tree Nursery.

Five other trials were established in Canterbury and Otago in 2021 to test the *Trichoderma* PR6 and PR3a mixtures in cooler, and sometimes drier, locations than that of the earlier sites (Table 5 and Figures 4b to f). Rangiora Nursery (RNL 17/203) supplied bare root cuttings (1/0) for the Rayonier Matariki, Port Blakely, City Forests and Wenita trials, whilst Leithfield Nursery (Seedlot 20/785) supplied bare root seedlings (1/0) for the Ernslaw One Ltd trial. In each nursery, spore suspensions ($3.0E10^6$ spores/ml) were applied to stock using a watering can on 2 September (Rangiora) and 6 November (Leithfield) 2020.

Trials comprised a randomised complete block design (RCBD) with large plots (49, 50 or 56 trees in a 7 x 7, 5 x 10, or 8 x 7 grid pattern respectively) and six or ten replicates at each site. Data were analysed for significance by analysis of variance (ANOVA) and the Fisher's unprotected least significant difference (LSD) test at the 5% level using Genstat, v21 (Genstat 2021).

Tree survival was high in all trials, apart from in Dusky where *Hylastes ater* (black pine bark beetle) or *Costelytra zealandica* (grass grub) beetles and wet soils reduced survival to a mean of 92.5% (Table 6). *Trichoderma* treatments had no effect on survival (mean of >97%) apart from in Kings Ridge, where PR3a (97.9%) was significantly ($P<0.05$) higher than the control (92.4%, Table 6). However, *Trichoderma* had a large effect on tree height in year one or two and was significantly greater in five of the six sites compared to the control (Table 6). Kakahu had the largest increase of all trials (19% in the PR6 treatment) and approximately a 10% increase was observed in Hillend, an ex-pasture site with potentially high fertility soils. Over all sites planted in 2021, *Trichoderma* significantly ($P<0.05$) increased height by 10.3% and 7.8% in the PR6 and PR3a treatments compared to the control (Table 6). Root colonisation in the inoculated trees was approximately double that of the controls (Table 7).

At Kings Ridge, the PBI *Trichoderma* mixture (consisting of four *T. atroviride* isolates) was able to thrive and induce a very large growth response (a 21% and 13% increase in height in year one and two respectively). This mixture is recommended for the promotion of radiata pine growth in the Nelson Golden Downs Forest region, instead of PR6 and, to a lesser extent PR3a due to its ability to improve survival. Future studies to determine the mechanisms of this strong response would be helpful.

Table 5: Establishment details and updated comments for the 2020 and 2021 radiata pine trials.

Region	Company and Forest / Trial Name	Location	Altitude (m asl)	Planting Date	No Replicates	Planting Stocking Rate and Spacing
Nelson	OneFortyOne Golden Downs **Kings Ridge**	-41.638371, 172.899004	480-600	30/07/20	6	800 (2.5m x 5m)
		Located on a cool, elevated, steep 30° westerly slope site, 35km south-south-west of Richmond. The cutover site was windrowed, and pre-plant herbicide sprayed. Plots were overlayed over the windrows. Replicates were generally arranged across the slope to minimise variation in soil type and altitude. Mean tree height was similar irrespective of the altitude, however, survival decreased down the slope. Low levels of weeds (mainly fleabane, broadleaf weeds, bracken and Himalayan honeysuckle) and no disease were present at the year two assessment.					
Canterbury	Rayonier Matariki Forests **Okuku**	-43.099028, 172.487361	580-640	23/07/21	6	833 (4m x 3m)
		A north facing, summer-dry, elevated site with a uniform slope of 25 - 30°. The cutover site was windrowed, and pre-plant herbicide sprayed. An ex-Douglas-fir site with regenerating seedlings. Each plot had a central windrow with 4 trees on either side, and a windrow on each downhill boundary. Few weeds (mainly fleabane, a few pasture species and woolly mullein) and no disease were present at the year one assessment. Deer had recently eaten many tips and will need to be controlled in the future.					
Canterbury	Port Blakely Ltd **Kakahu**	-44.128841, 171.067654	320-370	13/07/21	10	800 (5m x 2.5m)
		Site between Fairlie and Geraldine. Rolling top site with a range of aspects (northerly, easterly- and south-easterly). Plots were placed between the windrows. Weed pressure (mainly broom, fleabane and a little blackberry) was generally low, and no disease was present, at the year one assessment. The growth of 21% of trees was severely reduced by compacted soil caused by machinery and log movement during the previous crop harvest. Trees affected were mainly in the PR6, but also the PR3a and control treatments. This was considered during statistical analysis. Potential wallaby damage in the future.					
Otago	City Forests **Hillend**	-46.110606, 169.775399	130	08/07/21	10	833 (4m x 3m)
		Ex-sheep and beef farm inland of Milton. Located on a cool, relatively low (130metres asl), rolling-top site 12km west of Milton, South Otago. Weed pressure was generally low at the year one assessment due to the Release KT spot spray (control of grass and broadleaf weeds) applied in October 2021. The trees had grown strongly in the first year, but some tops and trunks (3%) had been eaten by cattle and deer. No disease was present in June 2022. Future risk to the trial is cattle and deer damage.					
Otago	Ernslaw One Ltd **Dusky**	-45.852581 169.180736	170	10/09/21	10	1000 (3.3m x 3m)
		Trial is a fourth rotation planting on a relatively flat site inland of Tapanui. Plots were arranged over windrows that were burnt pre-planting, however, most trees measured were not in these zones. An aerial spray of Release KT herbicide was applied in November 2021. In June 2022, the dominant weed was dense, very short broom seedlings. There was no disease. Survival was relatively low (92.5%) due to wet soils and *Hylastes ater* (black pine bark beetle) or grass grub (*Costelytra zealandica)* damage at planting. Some tree tips (3%) were eaten by cattle and deer during the first year. Risks to the trial are future cattle, deer and insect damage.					
Otago	Wenita Forest Products Ltd **Akatore**	-46.1232861, 170.122282	160-180	07/07/21	10	1000 (2.5m x 4m)
		Seaward of Milton and 4km from the east coast. The rolling top, cut-over site is exposed to wind, occasional snow and some frosts. Plots were placed between windrows. At the year one assessment, there were few weeds due to a release herbicide spray in November 2021 and no disease or animal damage. The growth of 8% of trees was impacted by machinery compaction of the soil that occurred during the previous crop harvest. Trees affected were mainly in treatments PR3a and PR6 but not in the control treatment. This was considered when doing statistical analysis. Future risks include regenerating pine seedlings and gorse and broom growth, but a regen pull and spring 2022 herbicide spray are being considered. The site is planned to receive an aerial application of Yorkshire fog grass in the future.					

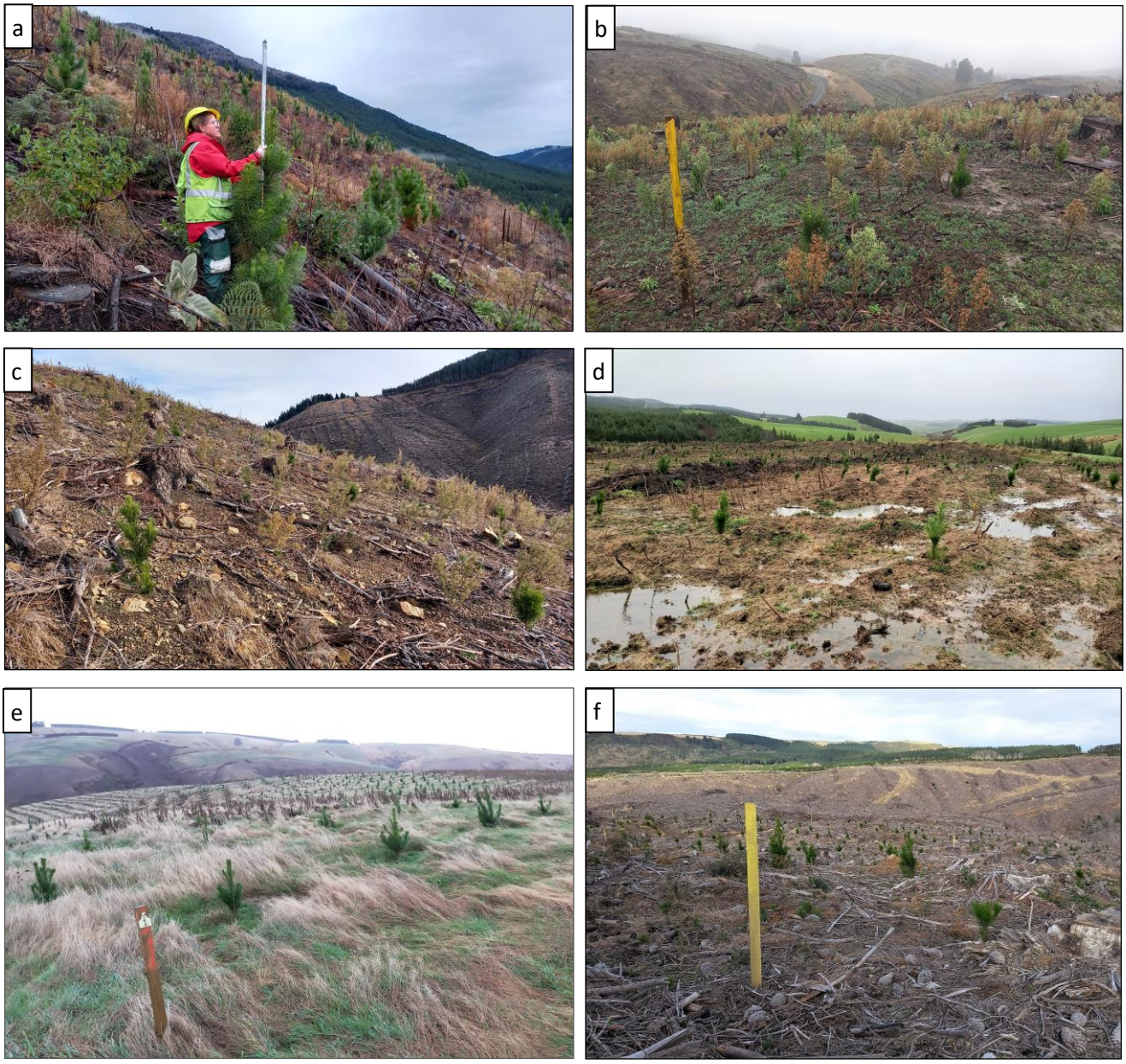

Figure 4: Kings Ridge trial in Golden Downs Forest, Nelson at the year two assessment on 30 May 2022 (a), and the Kakahu (b) and Okuku Forests (c), Canterbury, and Dusky Forest (d) Hillend region (e) and Akatore Forest (f), Otago, at the year one assessment in June 2022.

Table 6: Effect of *Trichoderma* treatments on tree survival (%) and height (cm), including percentage difference compared to the untreated controls, in the 2020 and 2021 trials approximately twelve months or two years after planting.

Region and site	Establishment Year	Treatment	Survival (%) [a]	Height (cm) [b]
Nelson Kings Ridge, Golden Downs	2020	PR6	90.8 b	128.0 (0.1) b
		PR3a	97.9 a	130.5 (2.0) b
		PBI	95.8 ab	144.5 (13.0) a
		Control	92.4 ab	127.9 b
		LSD (5%)	**7.0**	**10.5**
Canterbury Kakahu [c]	2021	PR6	98.8 a	63.9 (19.0) a
		PR3a	97.9 a	58.6 (9.1) ab
		Control	99.2 a	53.7 b
		LSD (5%)	**2.5**	**7.8**
Canterbury Okuku	2021	PR6	100.0 a	54.8 (7.7) ab
		PR3a	98.9 a	57.7 (13.4) b
		Control	99.4 a	50.9 a
		LSD (5%)	**1.8**	**4.7**
Otago Hillend	2021	PR6	98.8 a	72.2 (10.1) b
		PR3a	98.8 a	72.6 (10.6) b
		Control	99.2 a	65.6 a
		LSD (5%)	**2.9**	**6.4**
Otago Akatore	2021	PR6	98.8 a	50.5 (6.5) a
		PR3a	98.0 a	48.8 (3.0) a
		Control	99.2 a	47.3 a
		LSD (5%)	**2.7**	**3.2**
Otago Dusky	2021	PR6	94.8 a	57.5 (7.5) b
		PR3a	92.0 a	54.4 (1.7) ab
		Control	90.8 a	53.5 a
		LSD (5%)	**6.1**	**3.8**
Mean of the 2021 trials		PR6	98.2 a	59.8 (10.3) a
		PR3a	97.1 a	58.4 (7.8) a
		Control	97.6 a	54.2 b
		LSD (5%)	**1.4**	**3.1**

[a] letters were assigned according to a Fisher's 5% level unprotected LSD procedure. For each site and variable, values followed by different letters are significantly different at P<0.05.

[b] *Trichoderma* treatment measurement expressed as a percentage difference compared to the control within each trial (in brackets).

[c] in Kakahu, tree height was severely impacted by compacted soil conditions (15% of total trees). Height data for severely stunted trees (defined as stunted growth, yellow needles and cast needles on lower trunk) was removed from the dataset and the variable "percent severely stunted trees" in each plot was used as a covariate to calculate adjusted height in an analysis of covariance (ANCOVA).

Table 7: *Trichoderma* colonisation (%) of radiata roots in the 2020 and 2021 trials, sampled one or two years after planting, based on MRB plating data.

Region and Site	Establishment Year	*Trichoderma* root colonisation (%) [a]			
		PR6	PR3a	PBI	Control
Nelson, Kings Ridge	2020	52.4	38.1	73.8	40.5
South Otago, Hillend	2021	73.8	97.6	-	55.6
South Otago, Dusky	2021	59.5	69	-	33.3
South Canterbury, Kakahu	2021	60.4	87.8	-	35.4
North Canterbury, Okuku	2021	80.6	94.4	-	25
South Otago, Akatore	2021	92.9	50	-	40.5
Mean of the 2021 trials [b]		73.4 (6.3) a	79.8 (8.9) a	-	38.0 (5.1) b
LSD (5%)			**23.8**		

[a] root pieces for each treatment were bulked together at each site. *Trichoderma* was then isolated from approximately 48 sterilised random pieces of roots per treatment, using malt yeast extract and rose bengal agar plates, according to established protocols (Whelan 2018b).

[b] letters were assigned according to a Fisher's 5% level unprotected LSD procedure. Values followed by different letters are significantly different at P<0.05. Standard error of means in brackets.

2.3 Can *Trichoderma* increase nutrient uptake in plantation trees? (Milestone 2A)

Plantation forests in New Zealand are typically on low fertility or steep terrain land that is less suitable for agriculture. Soils are generally young and naturally acidic with low levels of nitrogen and phosphorus (Parfitt et al. 2008). Silviculture management practices that conserve accumulated nutrients will enable the uptake of faster and greater nutrient supplies in the following rotation and this will become increasingly important as increased carrying capacity and productivity is a key goal of the forest industry. New Zealand's planted forest soils are now supporting rotations at higher stocking with genotypes that grow faster and have greater nutrient demands. In extensive or low-cost, and intensive management systems, priority should be given to silviculture techniques that facilitate more efficient nutrient uptake and are cost-effective and practical to use. *Trichoderma* spp. are endophytic plant symbionts that have been used successfully in this research programme to enhance young tree growth and control foliar diseases in radiata pine. The objective of Milestone 2A was to determine if the growth benefits of radiata pine trees inoculated with nursery-applied *Trichoderm*a influenced the uptake of soil nutrients into tree foliage once out-planted in plantations. Details of this study are in the New Zealand Forest Growers Research report BIO-T028 (Whelan 2023).

Soil and foliar samples were taken on 29 and 30 May (Berrymans and Kings Ridge), 4 and 7 July (Topuni and Whatoro), 15 and 16 August (Kaingaroa 660_2 and 209_4) and 8 September 2022 (Patunamu). Because of Covid-19 travel restrictions, the sampling dates were delayed from the typical sampling time of February or March (autumn) when trees have high growth demands for nutrients, are exposed to environmental stress and foliage nutrient concentrations are relatively stable (Knight, 1978, Mead and Will, 1976), to winter 2022. Two additional trials (Sherry in Nelson and Tauwhareparae in Gisborne) in the 2018 series were not sampled due to resource constraints.

Foliage samples consisted of current season fully-grown needles (approximately 10 fascicles per tree) exposed to full sunlight on the youngest second-order branches in the top third of the crown (method according to Will, 1985). Samples were collected from 20 to 25 unstressed trees in the centre of each plot. Hands were covered with nitrile laboratory gloves and gloves were replaced when sampling each plot. Needles were bulked for each plot, placed in a clean paper bag, and sent to the Veritec Laboratory in Rotorua (https://www.scionresearch.com/services/laboratory-services) within two days of collection. At all stages after harvest, the needles were kept chilled. Soil samples were collected from the same plots that were sampled for foliage concentrations. The overlying soil organic layer was removed at each sample point and mineral soil was collected using a 17mm diameter Hoffer tube from 0 to 10 cm depth. Sampling points were within 500mm of the tree trunk, and approximately 20 samples were collected in each plot. Soil from the same treatment was bulked together, mixed, and approximately 500g was subsampled, placed in a clean plastic bag and sent to Veritec Laboratory for analysis. Full laboratory analysis techniques are detailed in Whelan (2023) and results are expressed on an oven dry (104°C) basis and reported as percentage or parts per million (ppm).

Soil types were estimated using the New Zealand Manaaki Whenua – Landcare Research soil digital mapping facility (https://soils-maps.landcareresearch.co.nz) but no soil profile pits were dug to confirm soil types.

Gain in productivity from *Trichoderma*

The *Trichoderma* treatments significantly ($P<0.05$) increased productivity (estimated using stem volume x tree stocking in winter 2022) by up to 40.2% in the eight trials established in 2018 (see Table 3). Gains in productivity followed an optimum response relationship (Figure 5a) where the *Trichoderma* treatments contributed to gains at different levels of growth potential. The gains in volume to *Trichoderma* were between -5 to 19.9% in the lowest productivity trials (ranging from 0.65 to 8.2$m^3.ha^{-1}$ at Berrymans, Sherry, Kaingaroa 209_4 and 660_2) and between 0.9 to 14.6% in the highest productivity trials (ranging from 29.9 to 34.6$m^3.ha^{-1}$ at Tauwhareparae and Patunamu). In comparison, the trials with medium productivity (ranging from 11.2 to 18.7$m^3.ha^{-1}$ at Topuni and Whatoro) had the greatest gains of between 25.1 to 40.2%.

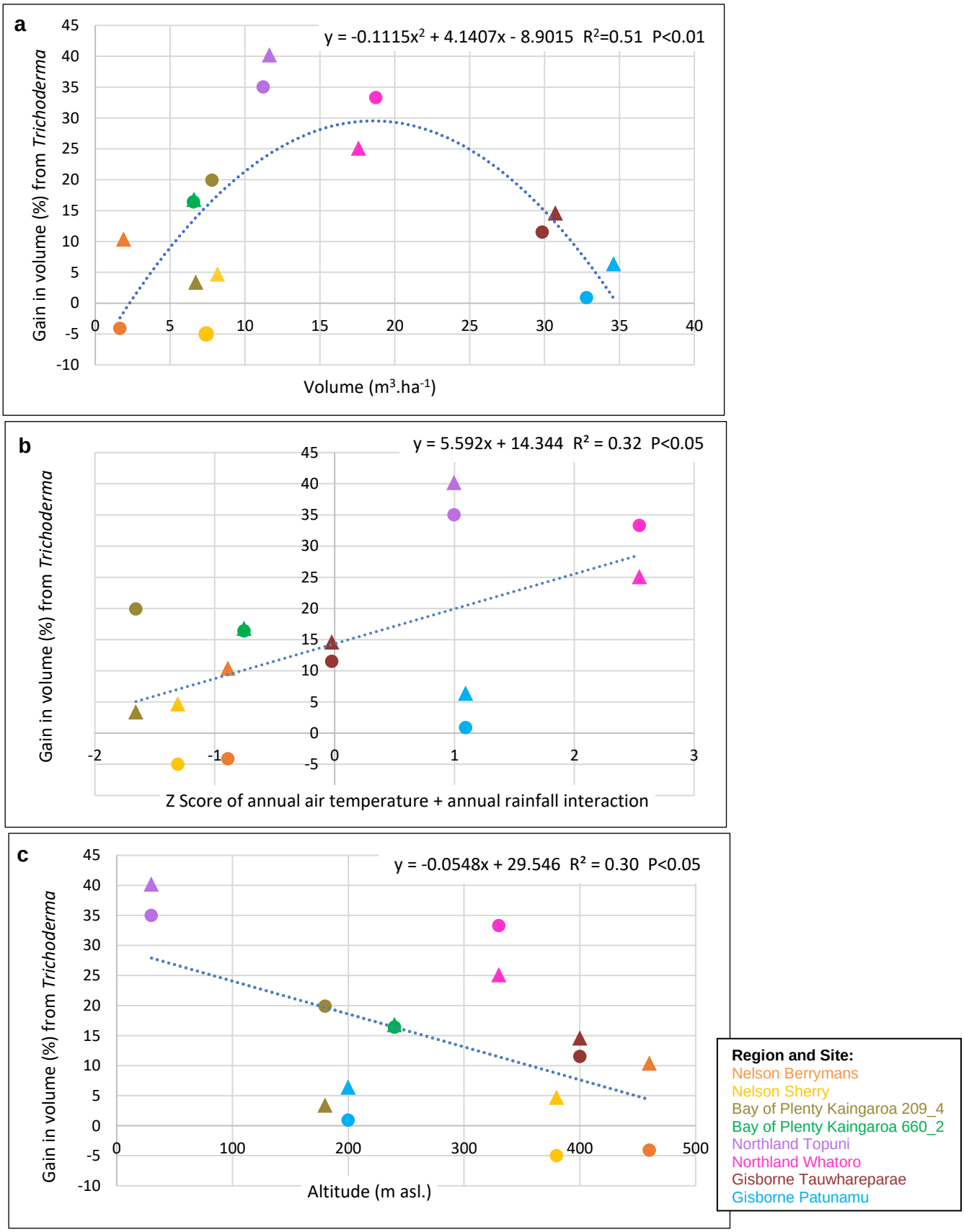

Figure 5: The relationship between a) 2022 stem volume ($m^3.ha^{-1}$), b) Z Score of annual air temperature and annual rainfall (data obtained from Table 3) or c) altitude (m asl.) and percentage gain in volume in the *Trichoderma* treatments (PR6 circle, PR3a triangle) compared to the untreated controls in the eight trials.

The tree productivity response to *Trichoderma* at each site may have been dependent on factors including the interaction between the nutritional status of the soil as well as environmental conditions.

Recently in New Zealand, a 300 Index model for radiata productivity of sites showed that productivity is strongly regulated by available soil water, air temperature and site fertility (Watt et al. 2021). The effect of climate variables on the productivity of *Trichoderma* inoculated trees was determined using two summary climate variables, annual air temperature and annual rainfall (being a surrogate for available soil water because few sites had this data measured). Similarly to Watt et al. (2021), the gain in productivity from *Trichoderma* was found to be significantly ($P<0.05$) higher in sites with warmer air temperatures and more rainfall (Figure 5b) and was also inversely correlated to the altitude of the site (Figure 5c).

Site characteristics and impact on available soil and foliar nutrients

a) High productivity site:

The most productive site was the Gisborne Patunamu trial with a mean four-year volume of 33.3$m^3.ha^{-1}$ across all treatments. The gains to *Trichoderma* treatments were small (PR6 0.9% and PR3a 6.4%) possibly because the trees were near the expected maximum growth potential for the region (35-40$m^3.ha^{-1}.yr^{-1}$; Palmer et al. 2010). At Patunamu, the Orthic Pumice soil reserves (Table 8) and foliar nutrient levels (Table 9) were at sufficient levels for strong tree growth (interpretations according to Davis et al. 2015) and an optimal balance of most essential foliar nutrients was present. For example, the soil carbon: nitrogen ratio (17) suggested that carbon in the organic matter did not greatly restrict the availability of nitrogen, and Total N (0.29%) and P (Mehlich-3 extraction method; 15.3ppm) were balanced with an N/P ratio of 10 (Davis et al. 2015). The site also had few climatic limitations to growth, in comparison to some other sites, with warm temperatures and sufficient spring, summer and autumn rainfall (Appendix B) and low weed pressure.

The inability of *Trichoderma* to strongly enhance growth in sites with low, or no abiotic stress, due to their services not being required by the plant, has been observed in other studies (Maherali 2014, Huey et al. 2020). The fungi can create an environment in which they do not germinate or grow but when plant roots release sugars and hormones and form an association with mycorrhizal spores, the spores can reactivate and commence ecological services (Huey et al. 2020). This ongoing relationship between the plant and endomycorrhizal fungi may be important as soil and environment conditions flux both seasonally and long-term, particularly with the potential impact of future climate change (Bauer et al. 2020).

b) Medium productivity sites:

The Northland sites consisted of strongly weathered Ultic clay (Topuni) and Granular (Whatoro) soils with significant chemical and physical limitations that can severely restrict root and stem growth (Ross et al. 2009). The soils were the most acidic in the trial series (pH of 4.9 and 5, Table 8) and this may have contributed to low soil availability of P (3.9 or 4.6ppm) due to P being fixed by the soil clay components. In Topuni, the luxurious uptake of N (possibly due to large plant numbers of the legume gorse) may have further reduced the availability of P and led to a potential deficiency (Davis et al. 2015). Topuni had a very high level of weeds (mainly gorse and pampas grass - *Cortaderia selloana*) that had developed since trial establishment, and this may have caused plant stress. Root development may also have been restricted in control plots due to clayey subsoils with poor permeability. This was the only site that received remediation (ripping) to improve water drainage.

The Whatoro and Topuni trials were the most responsive (25.1 and 40.2% increase, Figure 5a) to *Trichoderma*, suggesting that these treatments substantially contributed to the trees overcoming the poor-quality and low-fertile nature of the soils. *Trichoderma* spp are known to generate a symbiotic relationship with host plants in soil with low nutrients, including P (Huey et al. 2020) and to enhance tolerance to abiotic stresses during plant growth (Yildirim et al. 2006). This is in part due to induced root growth in the plant, increasing the root absorption surface area and enhancement of nutrient uptake by solubilization of phosphates and micronutrients in soil (Altomare et al. 1999).

c) Low productivity sites:

In the trials with low productivity (Berrymans, Sherry and Kaingaroa 209_4 and 660_2) *Trichoderma* contributed to gains in growth by up to 19.9% (Figure 5a) but a full response may have been restricted due to many nutrients and other environmental limitations. The Berrymans trial was situated on Brown soils that have low natural levels of numerous nutrients (including P (3.9ppm), Ca (365ppm), K (98ppm), Mg (72ppm), Mn (9.1ppm), Na (9.7ppm) and Zn (1.4ppm, Table 8)) and being inland, little opportunity for coastal nutrient inputs. Foliar N was relatively high (1.46%, Table 9) and likely to be a limitation for uptake of P and K and other nutrients. Foliage analysis indicated that P (0.11%), Mg (0.08%), Na (42.5ppm), B (9ppm) and Cu (3.4ppm) were near or at deficiency levels and likely to limit growth (Table 9, Adams and Walker, 1973).

The Kaingaroa Forest is primarily on pumice soils that have low reserves of macro nutrients, including N (0.12 and 0.19%), P (4.3 and 7.2ppm) and B (0.14 and 0.18ppm). They also have shallow topsoils with low C% and a low water holding capacity, although rainfall generally maintains adequate plant available water during the year. Production was limited in the two Kaingaroa sites mainly due to a likely deficiency in magnesium (foliar Mg at 0.06ppm, Table 9) because of very low magnesium in the soil (10 and 16 ppm) and high K, particularly in Kaingaroa 209_4 (270ppm, Table 8). Severe deficiency symptoms (needle tips golden yellow, remainder of needle normal green, Davis et al. 2015) were observed in some trees in the Kaingaroa 660_2 trial in August 2022. The Kaingaroa and Nelson trials also have lower growth potential compared to the Northland and Gisborne sites due to colder temperatures, particularly in spring and autumn (Appendix B).

In the three Nelson trials established in 2018 (Berrymans and Sherry) and 2020 (Kings Ridge), the least responsive *Trichoderma* treatment was PR6. Productivity was 4.1 and 5.0% less in Berrymans and Sherry respectively (Figure 5a) although this was not significantly different ($P<0.05$) from the control (Table 6). The repeated inability of PR6 to stimulate growth in the Nelson forests suggests that the four isolates that make up the mixture (3 x *T. harzianum* and 1 x *T. atrobrunneum*) were not suited to the Golden Downs Forest soil environment or could not activate a growth response. Soil temperature was unlikely to be a limiting factor (Appendix B) in isolate survival or development because both *Trichoderma* species have a broad range of tolerance (Whelan 2018a). In addition, the PR6 mixture has performed well in other sites with colder temperatures in Southern Otago and Canterbury (at year one the mean tree height increase was 10.2% in the PR6 treatment compared to the control, Table 6). The survival rate of the PR6 isolates in the tree roots is unknown because of the unavailability of specific molecular identification tests.

The Kings Ridge trial was not considered in the volume productivity data because the two-year-old trees were too small for DBH measurement, but they were measured for nutrients. Although the site was mapped as having an Othic Brown soil type (similar to the Berrymans site) the soil test suggested an Allophanic Brown soil type may be more appropriate. Allophanic Brown soils can retain high levels of phosphorus due to the large amounts of iron oxides weathered from the soil parent material. This soil was considerably different from those in the other sites with adequate levels of available P (46.2ppm), Ca (1905ppm), Mg (318ppm), Mn (16ppm), Fe (216ppm) and Co (0.14ppm, Table 8). Although foliar N was high (1.71%), the trees were able to absorb sufficient P from the soil (0.23%) for growth not restricted substantially by P supply. Foliar magnesium may also be at adequate levels for growth at 0.1% (Davis et al. 2015).

Available soil nutrients

The wide spread of the soil nutrient concentrations across sites shows the broad range in site types explored (Table 8). No significant (P<0.05) difference in soil nutrient concentrations was found in any treatment plots.

Table 8: Mean total C and N, pH and available soil nutrients across sites.

Region	Site	Soil Parameters														
		Total C (%)	Total N (%)	pH	Mehlich 3 Extraction (ppm)											
					K	Ca	P	Mg	Al	Mn	Na	Zn	Fe	B	Cu	Co
Gisborne	Patunamu	4.8	0.29	5.7	198	1024	15.3	186	1924	26	24	12.5	96	0.30	0.37	0.07
Northland	Topuni	3.5	0.15	5.0	149	1076	4.6	431	1354	41	79	2.2	307	0.19	0.88	0.11
	Whatoro	8.4	0.53	4.9	132	452	3.9	113	1676	6	41	0.9	175	0.31	2.00	0.02
Bay of Plenty/ Waikato	Kaingaroa 660_2	4.0	0.19	5.3	83	49	4.3	10	2186	4	19	0.8	137	0.14	2.59	0.04
	Kaingaroa 209_4	1.9	0.12	5.8	270	115	7.2	16	2032	5	43	1.8	70	0.18	0.85	0.01
Nelson	Berrymans	2.9	0.13	5.3	98	365	3.9	72	1493	9	10	1.5	243	0.23	0.38	0.07
	Kings Ridge	3.5	0.17	5.7	198	1905	46.2	318	981	16	7	2.7	216	0.18	0.63	0.14

Winter foliage nutrient concentrations

Foliage nutrient concentrations sampled in winter varied substantially across nutrients and sites (Table 9). The soil nutrient analysis shows potential for limitations that would impact growth of the tree during late spring conditions when growth demand increases.

Table 9: Mean foliage nutrient concentration (% and ppm) across sites.

Site	Nutrient Concentration															
	%					ppm										
	N	K	Ca	P	Mg	Al	Mn	Zn	Fe	B	Cu	Co	Li	Sr	Ba	Ni
Patunamu	1.21	0.85	0.28	0.12	0.08	325	242	56	25	16	2.5	0.08	0.089	8.9	4.2	0.08
Topuni	1.54	0.81	0.24	0.11	0.11	365	647	57	41	18	3.6	0.13	0.065	3.8	2.2	0.36
Whatoro	1.58	0.71	0.18	0.12	0.09	450	236	39	58	16	3.8	0.09	0.015	1.5	0.2	0.50
Kaingaroa 660_2	1.33	0.66	0.20	0.12	0.06	497	351	43	30	9	4.2	0.07	0.048	2.3	1.9	0.74
Kaingaroa 209_4	1.37	0.80	0.24	0.14	0.06	478	303	57	30	10	6.0	0.02	0.070	4.9	4.0	0.11
Berrymans	1.46	0.80	0.21	0.11	0.08	454	332	43	37	9	3.4	0.12	0.024	8.4	1.5	0.22
Kings Ridge	1.71	0.98	0.29	0.23	0.10	156	146	49	35	13	3.9	0.09	0.015	9.8	3.1	0.77

Nutrient Uptake efficiency

Nutrient uptake efficiency reflects a plant's absorption of nutrients from the soil and the internal transport, storage and remobilisation of nutrients, and might be used to determine the effects of *Trichoderma* inoculation. The ratio of foliage to soil nutrient concentrations varied across all nutrients and all sites (Figure 6). The uptake ratio of B, K, Mn, P and Zn (Table 10) was significantly greater than all other nutrients. This demonstrates the effort of the radiata pine trees to accumulate these nutrients despite the low concentrations in the soil relative to plant demand. Accumulation of phosphorus was the greatest compared with requirements of other nutrients, indicating its fundamental importance in large amounts for plant structural development. On average across the sites, approximately 200 parts per million (Table 10) of phosphorus was accumulated in the foliage for every ppm as measured by soil available phosphorus (Mehlich-3 extraction method). Boron accumulation (important for cell wall synthesis and plant metabolism) was also high at approximately 65 parts per million accumulated for every ppm of soil available boron. This may have approached excessive levels at some sites, like Topuni, due to the wet soil conditions and the narrow range of concentration from essential to toxic levels (Landi et al. 2018).

There was no statistical evidence at P=0.05 level to support the view that the *Trichoderma* treatments improved nutrient uptake based on this dataset (Table 10). However, the samples were collected in winter and so reflect sub-optimal conditions. The efficiency of P uptake improved by 5.6-10.4% for PR3a and PR6

on average across sites respectively. Low concentrations of the foliar nutrients N, P, B and Cu in radiata pine may occur in spring and summer (Knight, 1978) when rapid growth is taking place but before the autumn and winter rainfall commences that increase the availability of these nutrients. Future sampling of these trials during the summer months, after most reserves of nutrients have been mobilised for growth, may increase the likelihood of detecting treatment differences in foliage N, P, B and Cu.

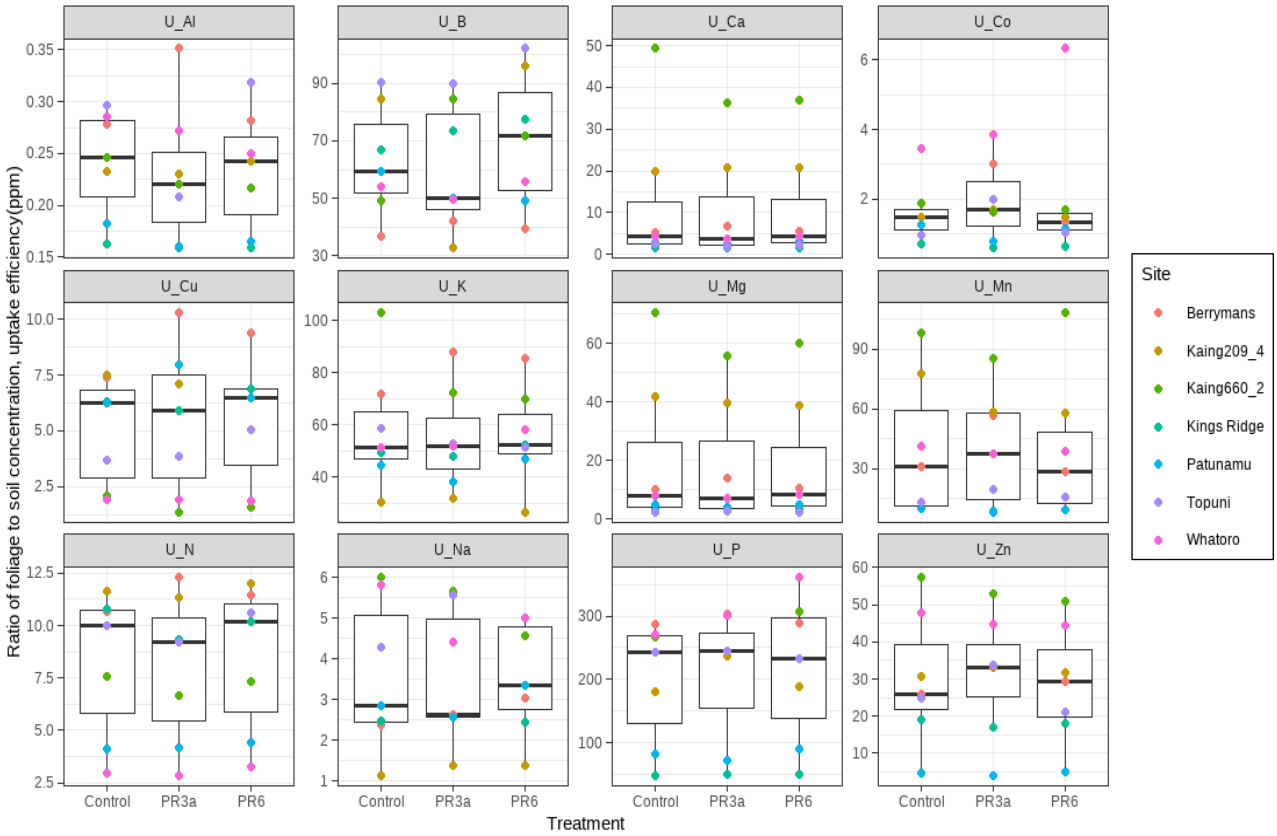

Figure 6: Boxplots of the ratio of foliage to soil nutrient concentrations across sites (indicated by coloured dots), split by treatment of Control, PR3a and PR6. Horizontal lines represent the lower 25%, upper 75% quartile and median (dark line). Whisker lines represent scores outside the middle 50% with outliers above or below the whisker lines.

Vector analysis, a bivariate model to simultaneously depict changes in plant yield (often needle dry mass) and nutritional response in a single diagram (Isaac and Kimaro 2011) was used to show the effect of the two *Trichoderma* treatments on needle nutrient concentration. Responses were expressed relative to the control (that is normalised to 100) to facilitate comparisons between various treatments and nutrients. Year four under-bark stem volume per hectare (Formula 1) in six trials, and year two tree height in the Kings Ridge trial were selected as suitable proxies for the needle dry mass because these variables are often correlated with long-term stand stem-wood and the plot data was available from previous measurements of height and diameter at breast height.

Contrasting trends of different nutrients were apparent over the sites (Figure 7). Nutrient contents were lowest in Kings Ridge, Patunamu and Berrymans, and highest, in Whatoro and Topuni, largely reflecting the impact of *Trichoderma* on tree size. Kaingaroa 209_4 had the largest difference in nutrient content between the two *Trichoderma* treatments, suggesting the PR3a mixture in this situation, had better ability to utilise soil nutrients than PR6. Some plots had an increase in nutrient concentration as well as tree volume (eg. PR6 in Berrymans) indicating these nutrients were approaching or at deficiency levels. In contrast, some plots had increased growth without nutrient addition (ie. values below the nutrient concentration horizontal line of 100), and resulted in a dilution in needle nutrient concentration. Dilution of nutrients was observed in both *Trichoderma* treatments including examples of reduced P and Ca concentration in the PR6 treatment in Topuni and the PR3a treatment in Kaingaroa 660_9 (Figure 7). These data support the hypothesis that an

improved nutrient efficiency did occur in some plots but the dilution of nutrient concentration in the needles possibly masked the effects of *Trichoderma*. In addition, datapoints reflected a luxurious uptake of Mg in both *Trichoderma* treatments in Kings Ridge (increase in concentration and no change in volume), excessive uptake of P, K, N and Ca also in Kings Ridge (reduction in concentration and volume) or sufficiency trends in the rest (no change in concentration).

Table 10: Mixed-model ANOVA of mean foliage and soil concentration ratios (*n* = 4) per site (*n* = 7).

Fixed effects	Estimate	Standard Error	Degrees of Freedom	t-value	P-value	Significance
Intercept	0.24	13.01	210	0.018	0.9853	
PR3a	-0.01	16.94	210	-0.001	0.9994	
PR6	-0.01	16.94	210	0.000	0.9997	
B	62.80	16.94	210	3.707	***0.0003***	***
Ca	11.96	16.94	210	0.706	0.4810	
Co	1.37	16.94	210	0.081	0.9358	
Cu	4.77	16.94	210	0.281	0.7787	
K	58.19	16.94	210	3.435	***0.0007***	***
Mg	19.81	16.94	210	1.169	0.2436	
Mn	40.03	16.94	210	2.363	***0.0190***	*
N	8.01	16.94	210	0.473	0.6369	
Na	3.33	16.94	210	0.197	0.8443	
P	196.53	16.94	210	11.600	***0.0000***	***
Zn	29.80	16.94	210	1.759	***0.0801***	.
PR3a:B	-2.65	23.96	210	-0.111	0.9121	
PR6:B	7.18	23.96	210	0.300	0.7648	
PR3a:Ca	-1.67	23.96	210	-0.070	0.9446	
PR6:Ca	-1.56	23.96	210	-0.065	0.9481	
PR3a:Co	0.35	23.96	210	0.015	0.9883	
PR6:Co	0.35	23.96	210	0.015	0.9882	
PR3a:Cu	0.48	23.96	210	0.020	0.9841	
PR6:Cu	0.44	23.96	210	0.018	0.9854	
PR3a:K	-3.73	23.96	210	-0.156	0.8764	
PR6:K	-2.67	23.96	210	-0.111	0.9115	
PR3a:Mg	-2.10	23.96	210	-0.088	0.9303	
PR6:Mg	-1.70	23.96	210	-0.071	0.9437	
PR3a:Mn	-0.73	23.96	210	-0.030	0.9759	
PR6:Mn	-1.72	23.96	210	-0.072	0.9427	
PR3a:N	-0.24	23.96	210	-0.010	0.9920	
PR6:N	0.22	23.96	210	0.009	0.9928	
PR3a:Na	-0.02	23.96	210	-0.001	0.9994	
PR6:Na	-0.02	23.96	210	-0.001	0.9994	
PR3a:P	11.01	23.96	210	0.460	0.6462	
PR6:P	20.40	23.96	210	0.852	0.3954	
PR3a:Zn	1.26	23.96	210	0.053	0.9579	
PR6:Zn	-1.38	23.96	210	-0.058	0.9540	

Significance of relationships P< 0.001 (***), P< 0.05 (*) and P less than 0.1 ('.').

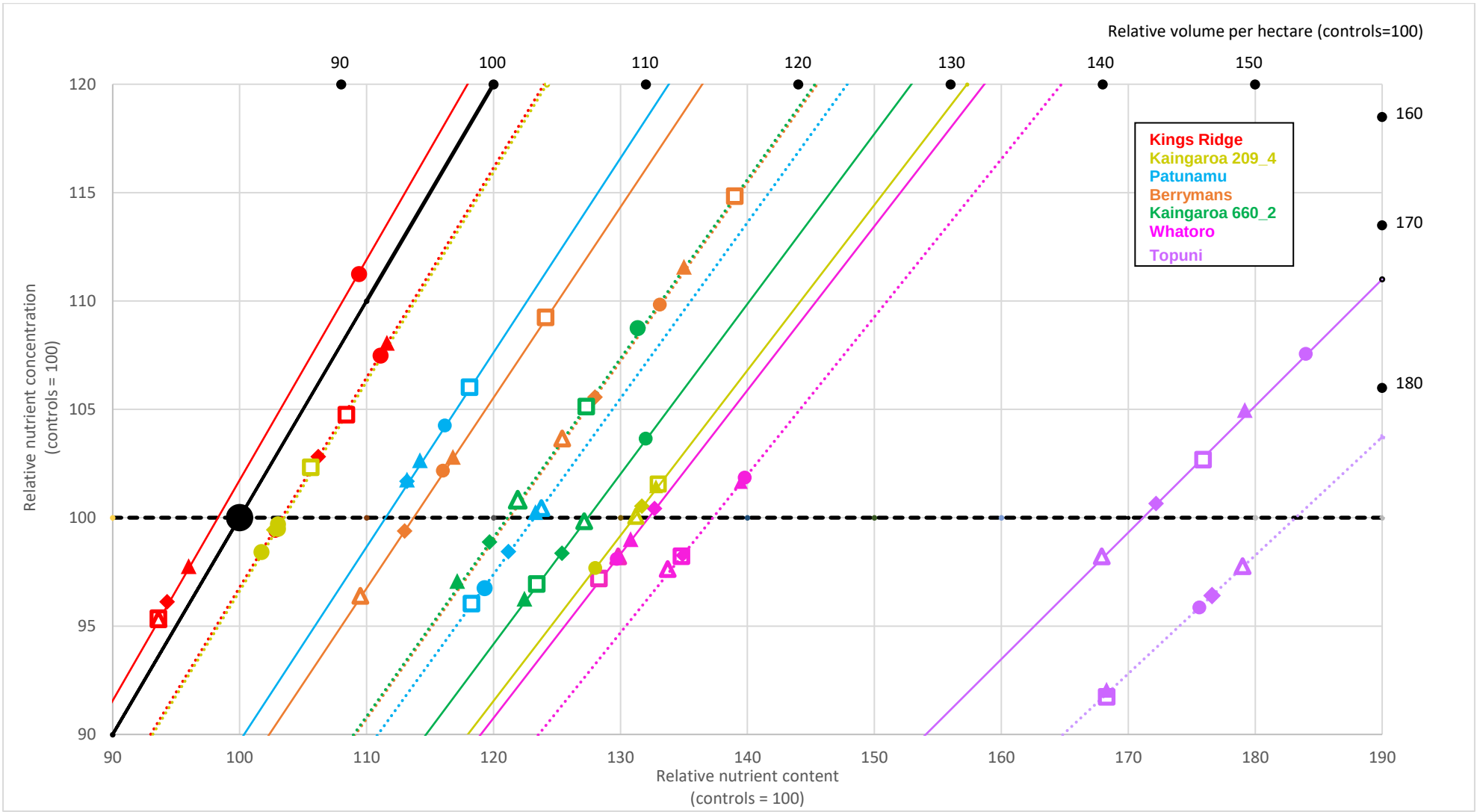

Figure 7: Diagram of relative response in volume.ha^{-1} and nutrient content (N△, P▲, K ◆, Ca □ and Mg ●) of foliage in radiata pine for the *Trichoderma*-treated plots (PR3a ····· , PR6 ———) in Kings Ridge, Kaingaroa 209_4, Patunamu, Berrymans, Kaingaroa 660_2, Whatoro and Topuni trials. Responses are relative to the untreated control plots that were normalised to 100 (●). For simplicity vector arrows from the reference point were not drawn on this diagram. Height in the Kings Ridge trial and volume.ha^{-1} in the other trials were used to represent biomass.

2.4 Can Nursery Application of *Trichoderma* Inoculants Improve Stand Uniformity? (Milestone 2B)

Stand uniformity is a desired trait in many radiata pine timber production systems in New Zealand. Variability in plantation stands may alter the growth of individual trees and decrease productivity due to an unequal division of resources amongst competing plants (Bourdier et al. 2016). Stand uniformity and tree productivity may be increased with the planting of improved genetic stock and intensive silvicultural practices such as site preparation, weed control and fertilisation (Allen et al. 2005). In this research programme the application of *Trichoderma* inoculants to radiata pine nursery stock significantly improved growth once planted in the plantation (see Tables 3 and 6) but the impact of this silviculture technique on tree size variability has not been studied.

Data from the eight radiata pine plantation trials established in 2020 (Whelan, 2019b) was used to assess forest uniformity at the sampling plot level using two summary statistics, the coefficient of variation (CV) and the Lorenz Curve and Gini Coefficient statistics (hereafter referred to as GC). Each plot contained 81 trees and the central 25 trees were measured for year four height, DBH and foliar disease (percentage incidence and severity, method according to Whelan 2019a). Details of this study are in the New Zealand Forest Growers Research report BIO-T026 (Whelan 2022).

GC can be used to measure stand uniformity as a derivation from a perfectly uniform population for a given growth trait (Katholnig 2012). In brief, the trees within a plot are sorted in ascending order for the response variable of interest (eg. DBH). Then, the cumulative proportion of this variable is plotted against the cumulative proportion of the trees, which generates the Lorenz curve (Katholnig 2012). The GC is calculated as the ratio of the area that lies between the line of equality (the one-to-one line) and the Lorenz curve, divided by the total area under the line of equality. Perfect stand uniformity (where all trees have equal size) would have a value of 0 whereas the lowest stand uniformity, in practice may have a maximum value of up to 0.8 (Keren et al. 2020). An example of the calculation of the GC for two plots with the same mean DBH of 10.0cm but different levels of uniformity is presented in Appendix C.

Both statistics were found to quantify the relative inequality in size among the trees growing in proximity. At each site, the CV for height (ranged from 10.9 to 25.9%) was lower than for DBH (ranged from 14.6 to 38.9%) indicating less variability was present in the height data (Table 11). CV increased sharply when the calculations involved one or more variables or more complex steps, and therefore additional ways to introduce variability to the results. CV for basal area had an exponential treatment of DBH and resulted in mean values at least double (ranged from 27.5 to 67.2%) that of CV for height or DBH (Table 11). Similarly, CV volume which had an additional step of multiplication of the height variable, increased values to a range of 31.8 to 69.1%. The only exception was in the Nelson Berrymans trial where the inclusion of the height variable in the CV volume calculation lowered the values from a range of 56.3 to 67.2% to a range of 50.7 to 61.2%, due to height having proportionately lower variability than the DBH values. When meaned over all sites, CV values progressively increased as height (16.9 to 19.2%), DBH (24.3 to 28.0%), basal area (44.9 to 50.8%) and volume (47.3 to 53.0%) were considered (Table 11).

A similar pattern to CV was observed in GC for basal area and volume. At each site, the GC for basal area ranged from 15.5% to 35.7% (mean of 24.2 to 27.3% in all trials), whilst GC for volume ranged from 17.9% to 37.9% (mean of 25.4 to 28.3% in all trials, Table 11). GC for volume was generally higher than the GC basal area, again due to the additional variation caused by the inclusion of the height dataset in the calculation. The Nelson Berrymans trial was also the exception, where GC volume was slightly lower than the GC basal area values.

One or both *Trichoderma* treatments of PR6 and PR3a had significantly ($P<0.05$) reduced CV and GC statistics in three (Bay of Plenty/Waikato Kaingaroa 660_2, Northland Whatoro and Gisborne Tauwhareparae) of the eight trials compared to the control (Table 11). In addition, the Gisborne Patunamu trial had CVs for basal area and volume that were significantly lower in the PR3a treatment. Tree variability, when statistics were meaned over all trials, was significantly ($P<0.05$) lower in both *Trichoderm*a treatments by 10.3 to 13.9% (CV) and 9.2 to 11.4% (GC) compared to the control (Table 11). Therefore, this management tool had a

Table 11: Effect of *Trichoderma* treatments on the CV (%) of tree height, diameter at breast height, basal area and under-bark stem volume and Gini Coefficient of basal area and under-bark stem volume in the 2018 trials approximately four years after planting.

Region and Site		CV (%) [a]				Gini Coefficient [b]	
	Treatment	Height	DBH	Basal Area [b]	Volume [b]	Basal Area	Volume
Bay of Plenty / Waikato Kaingaroa 209_4	PR6	17.4 a	23.9 a	45.6 a	47.1 a	24.4 a	25.6 a
	PR3a	16.7 a	24.2 a	47.6 a	48.6 a	25.5 a	26.4 a
	Control	17.7 a	27.8 a	52.8 a	53.8 a	27.4 a	28.2 a
	LSD (5%)	**2.8**	**4.3**	**8.0**	**8.4**	**4.0**	**4.1**
Bay of Plenty / Waikato Kaingaroa 660_2	PR6	14.8 b	21.2 b	40.8 b	42.7 b	22.0 b	22.8 b
	PR3a	15.3 ab	22.4 b	42.7 b	44.3 b	23.1 b	23.9 b
	Control	16.9 a	26.6 a	50.7 a	52.0 a	27.4 a	27.9 a
	LSD (5%)	**2.1**	**2.9**	**5.7**	**5.8**	**2.9**	**2.9**
Northland Topuni	PR6	20.3 a	28.5 a	51.9 a	56.4 a	28.4 a	30.5 a
	PR3a	22.7 a	32.1 a	60.0 a	66.5 a	31.7 a	34.6 a
	Control	22.8 a	35.7 a	64.8 a	69.1 a	34.3 a	36.3 a
	LSD (5%)	**4.5**	**8.7**	**14.8**	**14.3**	**6.7**	**6.4**
Northland Whatoro	PR6	21.1 b	31.3 b	51.4 b	55.2 ab	28.1 b	30.2 b
	PR3a	18.3 b	28.1 b	49.2 b	50.7 b	26.7 b	28.9 b
	Control	25.9 a	38.9 a	63.6 a	67.4 a	35.0 a	37.0 a
	LSD (5%)	**4.3**	**6.4**	**11.5**	**13.1**	**5.9**	**6.0**
Gisborne Patunamu	PR6	10.9 a	16.0 a	30.2 ab	35.0 ab	16.6 a	18.9 a
	PR3a	12.1 a	14.6 a	27.5 b	31.8 b	15.5 a	17.9 a
	Control	12.1 a	17.4 a	32.8 a	37.6 a	18.0 a	20.2 a
	LSD (5%)	**2.3**	**3.1**	**5.3**	**5.8**	**3.1**	**3.4**
Gisborne Tauwhareparae	PR6	15.7 b	19.1 b	35.7 ab	40.0 ab	19.0 ab	21.7 ab
	PR3a	14.4 b	17.4 b	31.8 b	36.3 b	17.1 b	19.5 b
	Control	23.0 a	26.0 a	43.4 a	49.1 a	23.7 a	26.0 a
	LSD (5%)	**5.9**	**6.6**	**9.6**	**9.2**	**4.3**	**4.5**
Nelson Sherry	PR6	16.3 a	21.4 a	40.8 a	44.2 a	21.9 a	23.6 a
	PR3a	15.0 a	19.5 a	37.4 a	40.6 a	20.1 a	21.7 a
	Control	17.5 a	22.7 a	42.0 a	44.1 a	22.7 a	23.8 a
	LSD (5%)	**4.6**	**5.3**	**10.1**	**9.5**	**5.0**	**4.6**
Nelson Berrymans	PR6	18.9 a	32.9 a	62.9 a	57.8 a	32.8 a	30.2 a
	PR3a	20.5 a	35.8 a	67.2 a	61.2 a	35.7 a	32.5 a
	Control	17.6 a	29.0 a	56.3 a	50.7 a	29.8 a	26.7 a
	LSD (5%)	**3.7**	**8.1**	**17.5**	**14.2**	**8.2**	**6.9**
Mean	PR6	16.9 (-11.8) b	24.3 (-13.3) b	44.9 (-11.6) b	47.3 (-10.7) b	24.2 (-11.4) b	25.4 (-10.0) b
	PR3a	16.9 (-12.0) b	24.3 (-13.9) b	45.4 (-10.6) b	47.5 (-10.3) b	24.4 (-10.5) b	25.7 (-9.2) b
	Control	19.2 a	28.0 a	50.8 a	53.0 a	27.3 a	28.3 a
	LSD (5%)	**2.2**	**3.0**	**4.7**	**5.1**	**2.5**	**2.5**

[a] Letters were assigned according to a Fisher's 5% level unprotected LSD test. For each site and variable, values followed by different letters are significantly different at P<0.05. The percentage decrease in means for *Trichoderma* treatments compared to the untreated controls is in brackets.

[b] Formulae for basal area and under-bark stem volume of an individual tree are according to Formula (1).

homogenizing effect on the size of the trees in the plot, with fewer trees being very small and more trees in the central size classes (for example, the Whatoro data, Figure 8). There was also no significant ($P<0.05$) difference in CV or GC statistics between the two *Trichoderma* treatments (Table 11). Therefore, both mixtures are recommended for forest companies that aim to produce timber of similar size and/or want to reduce production or harvest costs. Production costs could be reduced by refinement of the number or type of thinning and pruning operations or the number of inventory plots to obtain a desired level of precision in growth traits measurements. Harvest may be more effective and efficient due to better definition of harvest dates, less waste of small-diameter stems, and increased yield of similar-sized stems. Forests operating on low, or no management inputs would also benefit from *Trichoderma* inoculation by taking advantage of reduced stem variability at harvest.

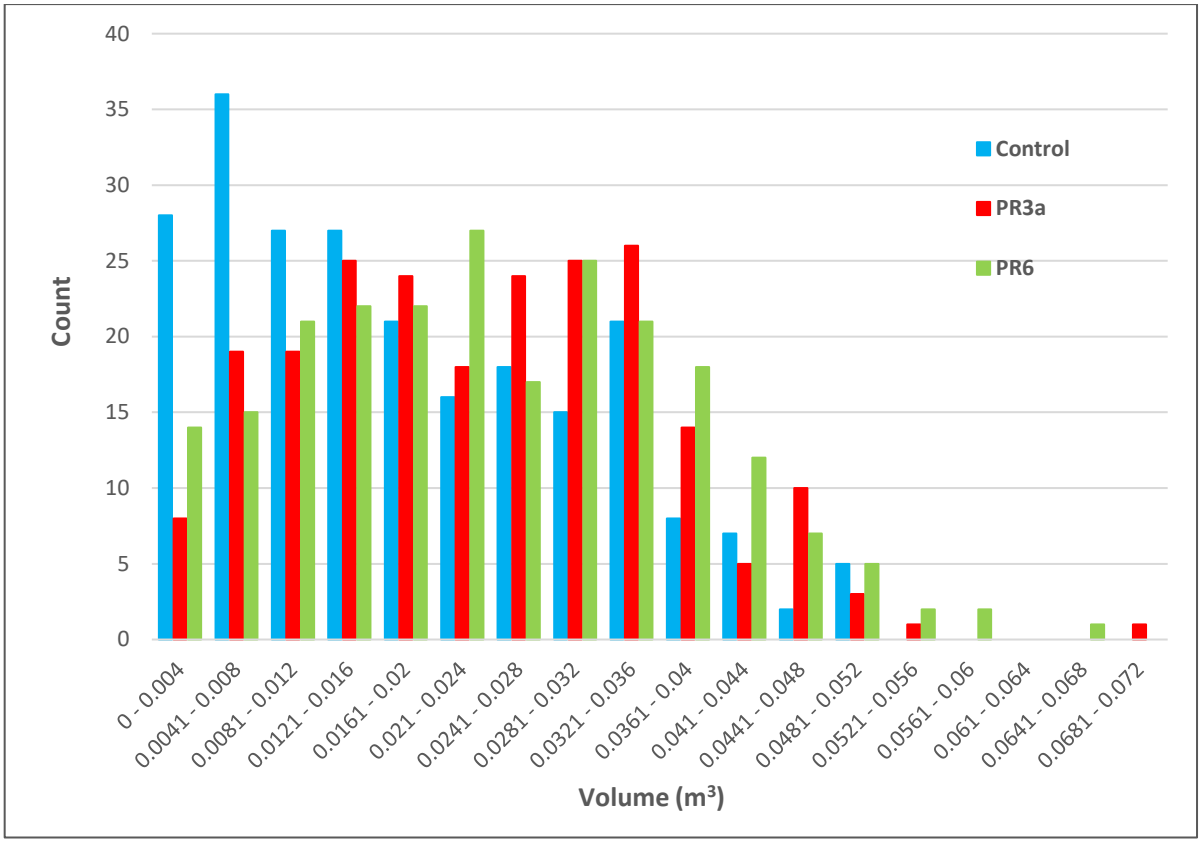

Figure 8: Frequency distribution of all individual tree volumes (m^3) in the control, PR3a and PR6 treatment plots in the Northland Whatoro trial four years after establishment.

Both statistics were useful by providing an overview of plot tree size variability using simplistic and concise descriptions. The data required for both statistics, where an interaction between neighbouring trees has occurred, is often available using existing forest company resources (eg. from inventory plots that have a minimum of approximately 20 trees). In addition, no arbitrary classes for tree variables were required in both statistic types, increasing the accuracy of the results. The main advantage of the CV statistic was the ease of calculation in statistical programmes (often a standard procedure in statistical analysis). However, CV is obtained from the standard deviation, which calculates the dispersion of tree variables around their mean, so describes a simplistic measure of variability. In comparison, GC is a more robust and efficient indicator of tree size variability due to its consideration of the differences among individual tree pairs.

GC has other advantages compared to CV, including being insensitive to datasets with small sample sizes, the presence of anomalously low or high values, or if the data are approaching a non-normal distribution (ie. the presence of skewness or kurtosis). Overall, GC is recommended for the quantification of the effect of silviculture treatments (eg. nursery *Trichoderma*) on tree size uniformity because it provides a robust, concise

and efficient statistic that is directly related to size hierarchy and therefore a good indicator of asymmetric competition between trees.

This study was undertaken in trees four years after establishment (but prior to canopy closure) when tree size uniformity is expected to be the highest during the life of the stand (Valbuena et al. 2016). At canopy closure, competition for light is generally considered the main competition process and can be characterized by strong asymmetry in stands, ie. larger trees monopolise light resources proportionally more than their size (Bourdier et al. 2016). The reduction in variability of height in the *Trichoderma* inoculated trees may lead to these trees reaching canopy closure in a more condensed timeframe, compared to untreated trees, resulting in less light competition, greater use efficiency and ultimately higher production (Stape et al. 2010). Future monitoring of these trials will determine if the improvement in tree uniformity from *Trichoderma* is maintained at canopy closure and beyond.

The definition of the effect of *Trichoderma* on tree size variation was confounded by the presence of disease at some sites. Foliar disease, mainly *Dothistroma septosporum*, was measured at moderate levels (incidence of >93% and severity of 8 to 13%) in the Kaingaroa 660_2 trial and at low levels at five other sites (incidence of 9 to 88% and severity of 1 to 7%; Figure 3). Disease may have contributed to tree size variation when present in the plots. For example, a significant (P<0.01) positive linear correlation between GC for plot tree volume and disease severity was found in the Kaingaroa 660_2 trial (Figure 9). However, there was insufficient disease in the other trials to confirm this relationship.

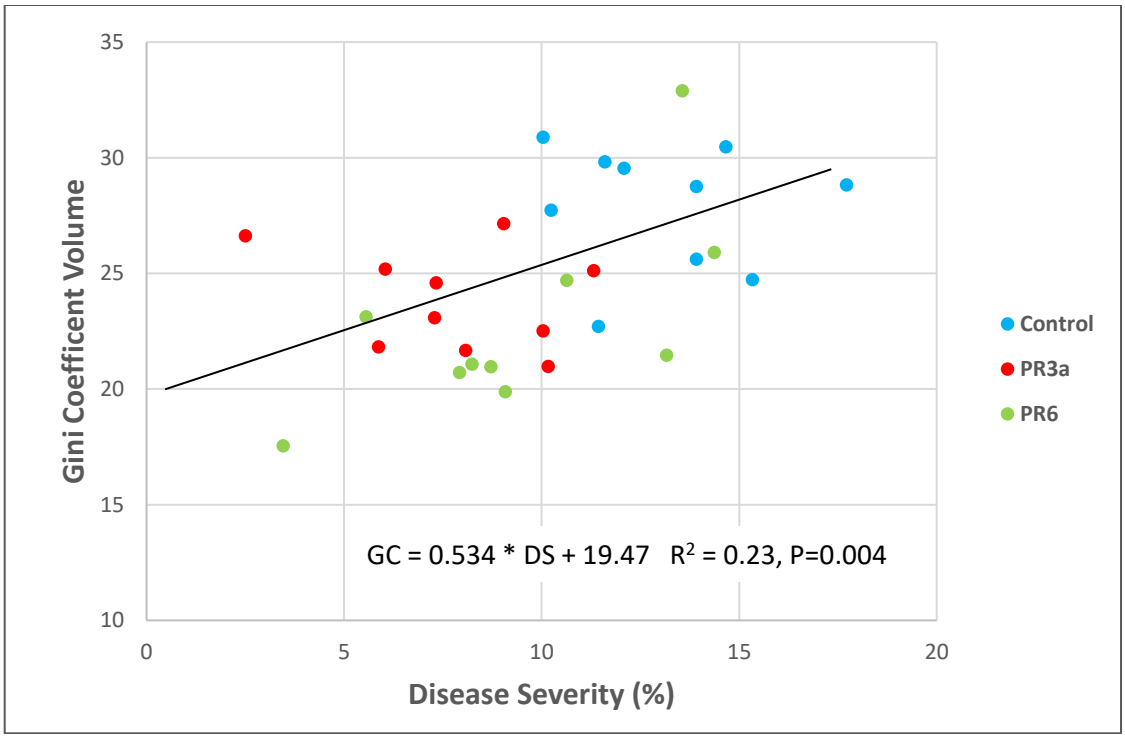

Figure 9: The relationship between Gini Coefficient (GC) for tree volume and *Dothistroma septosporum* disease severity (DS, %) in plots of the Kaingaroa 660_2 trial on 17 August 2022.

Airborne laser scanning (ALS) has been utilised to assess and monitor the structural complexity of pine forests on a large scale (Dash et al. 2016). ALS technology, in combination with structural indices like GC, may provide cost-efficient opportunities for extensive monitoring of the impact of silvicultural practices like nursery *Trichoderma* tree inoculation on forest structure. This knowledge will become increasingly important for refining silviculture and harvest management practices as forestry development occurs in new areas due to Government policy (ie. One Billion Trees by 2028) and the impact of climate change extends the environmental boundary trees are grown in.

2.5 Feasibility of treating established trees with *Trichoderma* root bioprotectants to mitigate disease problems (Milestone 3.4)

Practical and efficient methods for introducing beneficial *Trichoderma* inoculum into nursery stock have been developed and progressively more inoculated trees are being out-planted into forests. However, much of New Zealand's plantation forests do not contain inoculated trees. Three pilot trials in Bay of Plenty/Waikato were set-up to investigate the feasibility of directly inoculating established plantation trees with PR6 and PR3a mixtures to induce disease suppression and growth benefits (Whelan 2018b and 2020). In the Kawerau 2017 (subsequently harvested in mid-2021) and Kaingaroa 2019 trials, three *Trichoderma* treatments (spores sprayed onto the trunk, injected into the trunk or inserted into roots via infused dowels) promoted growth (height and/or DBH) at various measurements times, but there was no single treatment that consistently increased growth in all trials and measurements times. The root dowel treatment had the most consistent growth response, possibly due to the direct contact of spores allowing germination and development inside the tree roots. These application methods were generally unpractical for large-scale application of bioprotectants. The Kinleith 2019 trial involved hand-application of *Trichoderma* sprays on or around two-year-old plantation radiata pine trees, but treatments had no effect on root colonisation levels or tree growth fourteen and thirty months after application. This may have been due to an insufficient initial spore dose or water rate, or environmental effects that reduced the viability of the applied spores. Measurements in the Kinleith trial have been put on hold.

Application of *Trichodema* bioprotectants to forests for disease suppression or growth promotion will require substantial amounts of inoculum due to the large areas that are currently untreated. The most effective way of utilising the inoculum may be the development of formulations that contain encapsulated or microencapsulated spores and/or mycelium. Although the spores were generally tolerant of agrichemicals (Whelan, 2021), an encapsulated formulation may increase the viability of *Trichoderma* by providing improved protection against agrichemicals in the spray tank and environmental stressors once the bioprotectants have been applied. For disease control applications, precision spraying may also contribute to more efficient use of inoculum, particularly if sprays are targeted to zones that are more susceptible to infection (e.g. gullies). These bioprotectants may be best applied before canopy closure to maximise the chance of the spores reaching the ground as they are most effective as root colonisers.

2.6 Use of *Trichoderma* to boost new nursery areas being brought into production (Milestone 3.5)

In this research programme, nursery application of *Trichoderma* inoculants has been directly applied to the stock material, as part of a seed coat, a soil or potting mixture drench or a root dip. An alternative method for the introduction of *Trichoderma* in the nursery system is to apply it to the land before it is brought into production. The ability of *Trichoderma* bioprotectants to colonise new production areas and potentially improve the quality of stock planted into the treated area was determined in a pilot trial at Timberlands Ltd Te Ngae Nursery.

The trial was established in Block 9 (Te Ngae soil series; sandy pumice with some Rotomahana Mud) between 3 irrigation lines and comprised of four replicate plots of three *Trichoderma* bioprotectant treatments (PR6, PR3a and GM (isolates FCC320, FCC327 and LU633)) and one control (no *Trichoderma* spores or water). Each plot was approximately 8m wide and 25m long and contained 5 soil bed rows (Figure 10).

Soil samples (approximately 20 from 0 to 75mm depth) were taken at four times in each plot with a 30mm corer on:

- 18 December 2019 and 30 July 2020, before *Trichoderma* application,
- 3 February 2021, after *Trichoderma* application but before land preparation, and
- 16 May 2022 in the soil bed rows at harvest.

Figure 10: Cuttings in soil bed rows at harvest on 16 May 2021 in the Timberlands Te Ngae Nursery. Yellow lines indicate the borders of two adjoining plots.

The samples from each plot were bulked together, non-soil material (pieces of root, leaves) removed, and the soil passed through a 4mm screen. Ten grams of each soil sample were dried at 105°C for 24 hours to determine dry weight. An additional ten grams of each soil sample were added to 90 mL of 0.1% (w/v) solution of water agar (Dushefa Biochemie) and stirred for 10 min using a horizontal orbital shaker (INFORS HT Orbitron) at 200rpm. This solution was serially diluted (10^{-1} to 10^{-3}) and 0.1mL aliquots of the resulting solutions were placed on three replicate plates of *Trichoderma* selective culture media (Appendix A). After incubation at approximately 25°C for seven days in the dark, the colony forming units (CFU) were counted and the number of CFU g^{-1} dry soil was calculated (Figure 11). Soil *Trichoderma* data were analysed for significance by analysis of variance (ANOVA) and Fisher's unprotected least significant difference (LSD) test at the 5% level using Genstat, v21 (Genstat 2021).

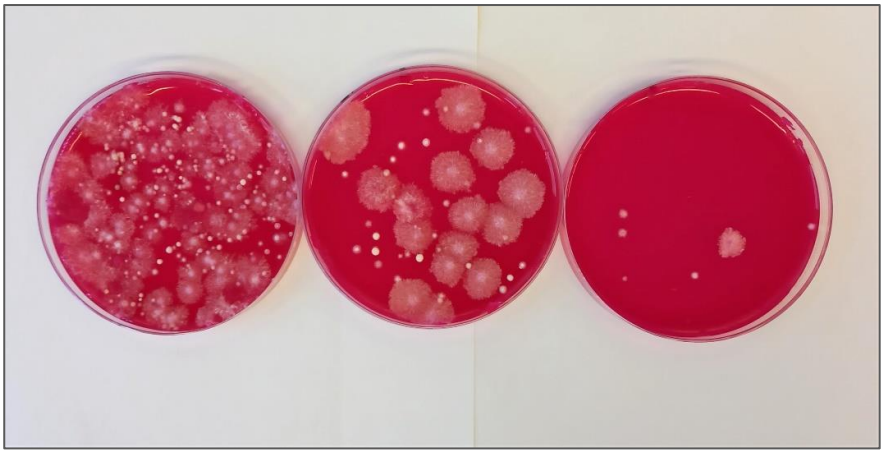

Figure 11: *Trichoderma* selective agar plates, with progressively diluted solutions (10^{-2}, 10^{-3} and 10^{-4}, left to right) used to determine *Trichoderma* colony counts in soil samples.

Spores are applied to fallow land (pasture with ryegrass and white clover) with a tractor-mounted sprayer at approximately 5.0E5 spores.ml^{-1} and a water rate of 300 litres.ha^{-1} (Figure 12) on 31 July 2020. The sprayer was cleaned before and between treatment applications and the spore suspensions were applied on a relatively calm day. The land was sprayed with Deal 510 RF (510g ai. glyphosate.l^{-1} at 3l.ha^{-1}) and Void (300g ai. clopyralid

.l-^{l} at 2l.ha^{-1}) in March 2021. Cultivation included ripping and discing; bed rows were formed and dibbled and treated with Oxy 500SC (500g ai. oxyfluorfen.l^{-1} at 2l.ha^{-1}) in May 2021. Cuttings were hand-planted into rows in May 2021 and Gesamil 500FW (500g ai. propazine.l^{-1} at 2l.ha^{-1}) and Ballistic (27.5g ai. deltamethrin.l^{-1} at 200ml.ha^{-1}) were applied prior to being covered with frost cloth (removal in October 2021). Between September 2021 and May 2022, ten herbicides (2x Hurricane®, 100g ai. haloxyfop-P.l^{-1} at 3l.ha^{-1}, 5 x Asset™, 500g ai. terbuthylazine.kg^{-1} at 2 or 3l.ha^{-1} and 3 x Oxy 500SC, 500g ai. oxyfluorten.l^{-1} at 1l.ha^{-1}), four insecticides (Ballistic at 200ml.ha^{-1}), three fungicides (2 x Foschek, 400g ai. phosphorus acid.l^{-1} at 6l.ha^{-1} and 1 x Blue Shield DF, 500g ai. copper hydroxide.kg^{-1} at 2kg.ha^{-1}) and five fertilisers or biostimulants (Seasol at 5l.ha^{-1}, Mycorrcin at 3l.ha^{-1}, 2 x Trace-it Mg and N at 4l.ha^{-1} and Wuxal® Microplant at 1.5l.ha^{-1}) . Canopy management included irrigation and lateral pruning, wrenching and topping of cuttings. The original plan was for one clone to be planted in the trial area but four (Clones 38, 50, 80 and 20/767, Forest Genetics Ltd, http://www.forest-genetics.com)) were established. Each clone was planted separately in at least two soil bed rows.

Figure 12: Application of *Trichoderma* spore suspensions in Timberlands Te Ngae Nursery on 31 July 2020.

Cuttings of each clone, planted in the original plot design, were harvested on 16 May 2022. In each plot, four randomly selected zones of 12 plants (2 rows x 6 plants) in central rows were sampled and survival, root collar diameter (RCD) and root, shoot and plant dry weight were measured. Root quality 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 13). The percentage of production cuttings in each plot was determined as a combination of the number of cuttings with a root score of 3 or 4 and an RCD greater than 6.9mm. *Trichoderma* root colonisation (%) was determined for 36 random root pieces from harvested plants in each plot. 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, 2018b).

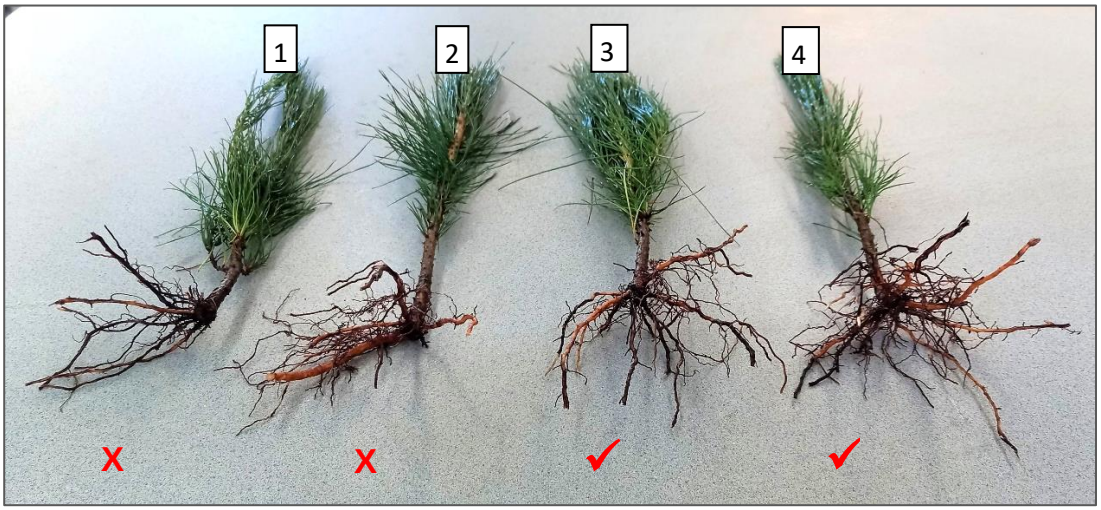

Figure 13: Root quality at harvest was assessed by the presence of strongly growing roots in each quadrant. Cuttings 1 and 2 had poor root scores of 1 and 2, whilst 3 and 4 had good root scores suitable for field planting.

A linear mixed-effects (LME) model approach (using the restricted maximum likelihood, REML algorithm) was used to analyse the data with the statistical software Genstat, v21 (Genstat 2021). This method of variance component analysis was chosen because of its robustness for unbalanced designs (Snedecor and Cochran, 1980). Tests of significance of main fixed effects (*Trichoderma*, clone) and interactions were based on the Wald test. All responses were assessed for normality and transformed using a binomial distribution where necessary. When data were transformed, back-transformed estimated means are presented. Means (predicted from the LME model) were compared for significance according to Fisher's 5% level unprotected LSD procedure. No statistically significant interactions were observed between *Trichoderma* treatment and clone for any of the measured parameters. Consequently, all results are discussed in terms of main effects.

The number of soil *Trichoderm*a colonies (ranging from 45 to 179 CFU g^{-1} dry soil) were not significantly different in each replicate on 18 December 2019. Soil *Trichoderma* levels did not change substantially after the *Trichoderma* spray was applied, until the 16 May 2022 sample had levels increased by up to 10-fold (Table 12). No significant differences were found in the *Trichoderma* treatments apart from on 3 February 2021, when GM was significantly (P<0.05) higher than the control (Table 12). In future studies, additional replicates may increase the likelihood of detecting treatment differences in colony numbers.

Table 12: The effect of *Trichoderma* treatments, applied as a spore suspension to fallow land ten months before the land was brought into production, on the mean number of *Trichoderma* colony forming units (CFU g^{-1} dry soil) in the soil at 31 July 2020, 3 February 2021 and at harvest on 16 May 2022.

Treatment	Date [a]		
	31 July 2020	**3 February 2021**	**16 May 2022**
PR6	84 (25) a	70 (11) ab	233 (26) a
PR3a	76 (33) a	77 (30) ab	538 (343) a
GM	43 (7) a	112 (21) b	446 (103) a
control	83 (25) a	27 (17) a	158 (13) a
LSD (5%)	66	66	600

[a] letters were assigned according to a Fisher's 5% level unprotected LSD procedure. For each date, values followed by different letters are significantly different at P<0.05. Standard error of means in brackets.

Trichoderma treatment significantly improved root *Trichoderma* levels (PR6 43%, PR3a 42% and General Mix 51%) compared to those in the uninoculated plots (29%, Table 13). In addition, these plants had significantly (P<0.05) higher survival rates and more cuttings with both RCD's >6.9mm and root scores of 3 or 4 (Table 13). The combination of these two variables resulted in significantly (P<0.05) more cuttings passing the production standard threshold for field planting (48 and 54% for General Mix and PR6 and PR3a treatments respectively), compared to the untreated control plots (30%, Table 14). However, there was no significant (P<0.05) difference between treatments for root, shoot and plant dry weights or root/shoot ratio (Table 13), possibly because the roots and shoots were trimmed during the year as part of standard nursery canopy practice, and this equalised any difference between treatments.

Although not planned, information was gained on the growth characteristics of the clones. The four clones had similar survival levels (Table 13) and three of the clones (38, 80 and 20/767) had similar production values (ranging from 38 to 50%, Table 14). However, the factors that contributed to these production values varied depending on the clonal type. For example, Clone 20/767 had the most cuttings with RCD > 6.9mm (89%) but fewer cuttings with root scores 3 or 4 (52%) compared to Clone 38 which had 46% and 70% of cuttings with RCD > 6.9mm and root scores 3 or 4 respectively (Table 14). Clone 80 had fewer cuttings with strongly grown roots (46%) and RCD's > 6.9mm (56%). In comparison, Clone 50 had significantly (P<0.05) more cuttings that passed the threshold with 69% of cuttings having strongly growing roots in three or four quadrants and 89% having stems greater than 6.9mm (Table 14).

The spraying of *Trichoderma* onto fallow land before being brought into production was an efficient and effective method for improving production outputs for stock planted in this area. Pre-production spraying of fallow land contributes to the suite of application methods developed in this research programme that improve nursery tree quality.

Table 13: The effect of *Trichoderma* treatments, applied as a spore suspension to fallow land ten months before the land was brought into production, on mean root *Trichoderma* (%) based on MRB plating data, survival of plants with roots (%), root, shoot and plant dry weight (g), and root/shoot ratio of radiata pine cuttings twelve months after setting into the inoculated soil.

Treatment	Root *Trichoderma* (%)	Survival of Plants with Roots (%)	Root Dry Weight (g) [a]	Shoot Dry Weight (g) [b]	Plant Dry Weight (g)	Root / Shoot Ratio
MAIN EFFECT MEANS: [c]						
a) *Trichoderma* Treatment:						
PR6	43.1 b	97.5 a	2.1 a	10.8 a	13.0 a	0.197 a
PR3a	41.8 b	98.1 a	2.1 a	10.1 a	12.2 a	0.220 a
General Mix	50.9 a	97.4 a	2.0 a	10.8 a	12.8 a	0.186 a
Control (untreated)	28.7 c	87.7 b	2.0 a	10.2a	12.2 a	0.197 a
LSD (5%)	6.1	8.1	0.2	2.0	2.1	0.062
b) Clone:						
38	61.1 a	96.9 a	1.8 b	8.3 a	10.0 a	0.210 a
80	55.7 a	90.9 a	2.1 a	10.6 a	12.8 a	0.209 a
50	32.0 b	95.3 a	1.9 ab	10.3 a	12.2 a	0.185 a
20/767	19.4 b	97.9 a	2.2 a	11.5 a	13.7 b	0.195 a
LSD (5%)	18.0	7.5	0.3	3.3	3.4	0.034

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

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

[c] letters were assigned according to approximate least significant differences (5% level) of REML means using Fisher's unprotected LSD procedure. For each main effect and variable, values followed by different letters are significantly different at P<0.05.

Table 14: The effect of *Trichoderma* treatments, applied as a spore suspension to pasture one year before the land was brought into production, on estimated mean root scores, percentage of plants with root scores 3 or 4, root collar diameter (RCD), percentage of plants with RCD of >6.9mm and production standard values (%) of radiata pine cuttings twelve months after setting into the inoculated soil.

Treatment	Root Score [a]	Plants with Root Score 3 or 4 (%)	Root Collar Diameter (mm)	Plants with RCD > 6.9mm (%)	Production Standard Values (%) [b]
MAIN EFFECT MEANS: [c]					
a) *Trichoderma* Treatment:					
PR6	3.0 a	70.8 a	7.88 a	73.1 a	53.9 a
PR3a	2.9 a	66.2 ab	7.89 a	66.7 b	53.9 a
General Mix	2.8 a	58.1 b	7.79 b	70.2 ab	48.2 a
Control (untreated)	2.1 b	41.4 c	7.37 b	50.4 c	30.0 b
LSD (5%)	0.4	11.9	0.50	4.9	13.9
b) Clone:					
38	2.99 a	70.1 a	6.85 c	45.8 d	39.3 b
80	2.32 c	45.8 c	7.59 b	56.3 c	37.8 b
50	2.92 ab	69.0 ab	7.74 b	69.3 b	58.6 a
20/767	2.52 bc	51.6 abc	8.76 a	89.1 a	50.3 b
LSD (5%)	0.423	19.2	0.53	3.8	20.5

[a] roots scored according to the presence of 1, 2, 3 or 4 quadrants of strongly growing roots.

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

[c] letters were assigned according to approximate least significant differences (5% level) of REML means using Fisher's unprotected LSD procedure. For each main effect and variable, values followed by different letters are significantly different at P<0.05.

2.7 Nursery and forest plantation trials in Douglas-fir (Milestone 5)

Douglas-fir (*Pseudotsuga menziesii)* is the second most widely planted forestry plantation crop in New Zealand and can be affected by plantation foliar diseases, especially Swiss needle cast (*Phaeocryptopus gaeumannii*; SNC). The potential for *Trichoderma* to control SNC will be determined in a Kaingaroa Forest plantation trial that was established on 18 August 2022.

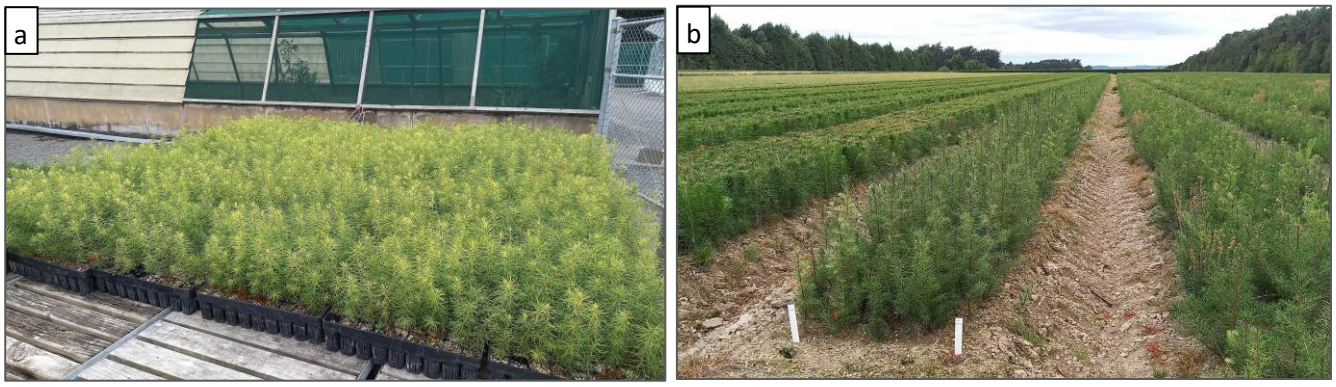

Figure 14: *Trichoderma* inoculated and uninoculated Douglas-fir seedlings in trays at Lincoln University (a) on 29 March 2021, and in the field at ArborGen Edendale Nursery (b, middle row) on 21 April 2022.

Approximately 3000 Douglas-fir (sourced from Ernslaw One Ettrick Seed Orchard) seeds were sown in trays according to established protocols (Whelan 2018b) at Lincoln University Nursery on 30 October 2020 (Figure 14a). Two seedlots were used:

- Tramway, of Washington provenance with a medium tolerance to SNC
- Fort Bragg, of California provenance with a low tolerance to SNC.

Four *Trichoderma* spore suspension treatments were pipetted (5ml of 5.0E6 spores/ml) onto seeds at sowing. Treatments included:

- PR6 mixture (isolates FCC55, FCC318, FCC327, FCC340)
- PR3a mixture (isolates FCC13, FCC14, FCC15 and FCC180)
- GM mixture (isolates FCC320, FCC327 and LU633) and
- Control – no *Trichoderma* treatment.

The seedlings were harvested on 13 April 2021, when approximately 15cm tall, and lined out in a soil bed at ArborGen Edendale Nursery, near Invercargill (Figure 14b). Seedlings was grown using standard nursery management and harvested on 10 August 2022. Root *Trichoderma* was measured on 29 March 2021 and 5 April 2022 (according to established protocols in Whelan 2018b) and confirmed sufficient levels for field planting (Table 15).

Table 15: Root colonisation (%) five months (29 March 2021) after seeding and *Trichoderma* application, and twelve months (5 April 2022) after lining out in a soil bed, based on MRB plating data.

Clone	Treatment	Root Colonisation (%)	
		29 March 2021	5 April 2022
Tramway	PR6	71.4	66.7
	PR3a	69.0	81.0
	GM	66.7	63.9
	Control (no *Trichoderma*)	12.2	40.5
Fort Bragg	PR6	64.3	58.8
	PR3a	73.8	88.1
	GM	40.5	82.9
	Control (no *Trichoderma*)	12.2	45.2

Seedlings were delivered to Rotorua using refrigerated road transport and a trial with eight treatments (see Table 15) and seven replicates was established using a randomised complete block design (Figure 15). Each plot contained 27 trees planted in a 3 x 9 grid pattern between the windrows. Trees were planted in the spot mounds at a stocking rate of approximately 800 stems per hectare. Measurement of tree height, disease and root colonisation will be performed in winter 2023.

Figure 15: The Douglas-fir plantation trial in Kaingaroa Forest, established on 18 August 2022.

3.0 COMMERCIALISATION OF *TRICHODERMA* ISOLATES

In this research programme, two *Trichoderma* mixtures PR6 and PR3a were found to have foliar disease suppression effects in young radiata pine plantation trials. In addition, these biocontrol agents also demonstrated growth promotion and increased tree uniformity in young radiata pine plantations and in radiata pine, Douglas-fir and cypress nursery trials. A business case for commercialisation of a forestry specific *Trichoderma* mixture is being developed; in the meantime Lincoln University continues to produce spores for distribution to interested nurseries.

4.0 PROJECT OUTPUTS (JANUARY 2022 TO DECEMBER 2022)

Whelan, H. 2022. Bioprotection for foliar diseases and disorders of radiata pine. Quarterly progress reports to NZ Forest Growers Research. March, June, September and December 2022.

Whelan H. 2022. *Trichoderma* bioinoculants for increased growth and reduced foliar diseases of Pinus radiata in New Zealand forests. New Zealand Plant Protection Conference, Christchurch. 9 August 2022.

Whelan H. 2022. Bioprotection for foliar disease and disorders of radiata pine: project overview. May 2020 to December 2021. NZ Forest Growers Research Technical Report BIO-T025. January 2022. 33p

Whelan H. 2022. Bioprotection for foliar diseases and disorders of radiata pine: project update. Research presentations to NZ Resilient Forests Technical Committee meetings, Rotorua or online: 5 May and 3 November 2022.

Whelan H. 2022. Separate industry reports for Ernslaw One Ltd, OneFortyOne, Timberlands Ltd, Manulife Forest Management (NZ) Ltd, Rayonier Matariki Forests, Juken NZ Ltd, Port Blakely Ltd, Wenita Forest Products Ltd and City Forests.

Whelan H. 2022. Can nursery application of *Trichoderma* inoculants improve stand uniformity? NZ Forest Growers Research Technical Report BIO-T026. December 2022. 16p

5.0 CONCLUSIONS

This research programme showed that nursery *Trichoderma* inoculation is an important management tool for production and disease control in radiata pine. In this fourteen-site dataset, plants were often larger when grown with the endomycorrhizal fungi *Trichoderma*, but the productivity response may have been dependent on the site's growth potential. In forests with high growth potential due to low-stress environments and balanced soil nutrients (ie. similar to the Gisborne Patunamu site) *Trichoderma* treatment may be less beneficial. However, many existing or future forestry sites in New Zealand will have low to medium growth potential with limiting soil nutrients and other environmental conditions, and beneficial growth responses to these *Trichoderma* strains would be expected. Both bioprotectants significantly reduced disease severity of *D. septosporum* and *Phytophthora pluvialis* during early stand establishment, and although they did not eliminate the presence of disease, offer an additional or alternative tool for disease management.

These studies provide the foundation for the development of effective fungal bioprotectant agents in New Zealand forestry. Not only were *Trichoderma* inoculants able to generate large gains in productivity (up to 40% in year four stand volume at one Northland site) but the uniformity of tree size was also increased (by approximately 10%). These bioprotectants are therefore recommended for both intensive, and low cost, extensive management regimes as practical and effective tools for foresters that want to reduce production or harvest costs and produce more timber of uniform size. The early onset of rapid production from *Trichoderma* is also important for the successful establishment of stands and for environmental, economic and social benefits with increased carbon sequestration. Use of these bioprotectants will lead to healthier forests with fast growth, reduced agrichemical use, and ultimately economic and sustainable gains for the forestry industry.

6.0 PROPOSED FUTURE RESEARCH

Priorities for research in 2023 most likely to lead to beneficial outcomes for the forestry industry were approved in December 2022. These included the monitoring of the 14 radiata pine validation trials and the newly established Douglas-fir trial to investigate the effect of *Trichoderma* on Swiss needle cast. Funding for investigating the business case for commercialisation of a specific *Trichoderma* mixture for forestry is also being sought.

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9.0 APPENDICES

APPENDIX A:

Malt Yeast Extract Agar with Rose Bengal (MRB) and Antibiotics Recipe:

- Malt extract 10 g
- Yeast extract 1 g
- Rose Bengal (50 mg/mL) 3 ml
- Terrachlor 75WP 0.2 g
- Agar 20 g
- Chloramphenicol stock solution (100 mg/ml) 1 ml
- Make up to 1 L with distilled water and autoclave at 121°C for 15 minutes.
- Add 10ml of sterile filtered 250mg/L streptomycin and 50mg/L chlortetracycline.

APPENDIX B:

Seasonal site-level variation in climate across the eight trials established in 2018.

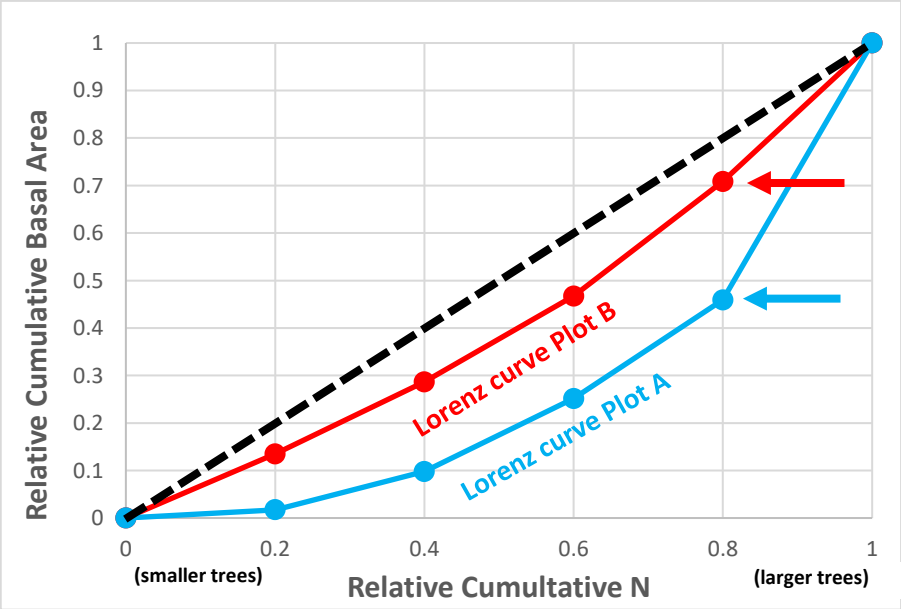
Climate Parameter	Period	Region and Site [a]							
		Gisborne		Northland		Bay of Plenty/Waikato		Nelson	
		Patunamu	Tauwhare-parae	Whatoro	Topuni	Kaing 209_4	Kaing 660_2	Berry-mans	Sherry
Mean air temperature (°C)	Dec to February	16.1 [d]	18.0 [c]	17.3 [d]	18.0 [b]	15.8 [c]	14.1 [c]	15.7 [c]	16.6 [d]
	March to May	12.3	14.0	15.1	15.5	11.0	9.9	11.3	12.0
	June to August	7.0	9.0	10.7	10.5	5.9	4.8	5.7	6.4
	September to November	10.8	13.0	12.8	13.6	10.5	8.7	10.7	11.5
	Annual	11.5	13.5	14.0	14.4	10.8	9.4	10.9	11.6
Mean monthly rainfall (mm)	Dec to February	129 [d]	58 [d]	109 [d]	99 [b]	108 [d]	128 [c]	97 [b]	81 [d]
	March to May	165	117	140	105	82	127	119	86
	June to August	166	121	221	130	101	149	116	100
	September to November	142	92	130	99	92	140	117	105
	Annual Total	1804	1163	1800	1301	1148	1631	1347	1115

[a] meteorological stations were within 8km of each trial site except for Tauwhareparae and Topuni that was 15km apart. Data for periods 1951-1980 ([b]), 1971-2000 ([c]) and 1981-2010 ([d]) according to National Institute of Water and Atmospheric Research (https://cliflo.niwa.co.nz/).

APPENDIX C:

An example of the calculation of the Gini coefficient for two plots with the same mean tree diameter at breast height (DBH) of 10.0cm but different levels of uniformity (Plot A has high, and Plot B has low uniformity of basal area respectively). The area under the Lorenz curve is calculated as the sum of trapezoids, each of which is calculated as $a_1 = (\Sigma ba_1 / BA + \Sigma ba_1\text{-}1 / BA) / 2 * (\Sigma n_1 / N - \Sigma n_1\text{-}1 / N)$. For example, the area for sample tree number 4 in Plot A is: $a_4 = (0.459 + 0.251) /2 * (0.8\text{-}0.6) = 0.0710$. The Gini coefficient for this plot (ie: the area under the Lorenz curve) is (0.5-0.265) / 0.5 = 0.47 or 47.0% and indicates a high uniformity of tree sizes in the plot.

Plot A with high uniformity of tree size:							
Sample Tree number	DBH (cm)	Σ n/N	Basal area (ba) (cm^2)	Σ ba	Σ ba/BA	Area under the Lorenz curve	Gini coefficient
1	5	0.2	8.552978775	8.552978775	0.017400058	0.001740006	
2	7.1	0.4	39.59188798	48.14486675	0.097945227	0.011534528	
3	10	0.6	75.4295759	123.5744427	0.251398076	0.03493433	
4	12.4	0.8	102.0702591	225.6447018	0.459048349	0.071044643	
5	15.5	1	265.9041776	491.5488794	1	0.145904835	
Σ			491.5488794			0.265158342	
mean	**10.0**						**0.47**
Plot B with low uniformity of tree size:							
1	7.8	0.2	54.10603378	54.10603378	0.134935558	0.013493556	
2	8.3	0.4	60.8211824	114.9272162	0.286618091	0.042155365	
3	9.9	0.6	72.3822336	187.3094498	0.46713284	0.075375093	
4	11.5	0.8	96.76882598	284.0782758	0.708465546	0.117559839	
5	12.5	1	116.8985639	400.9768397	1	0.170846555	
Σ			400.9768397			0.419430407	
mean	**10.0**						**0.16**



Lorenz curves for calculating Gini coefficients for Plots A and B where the relative cumulative basal area (Σ ba/BA) is plotted against relative cumulative N (Σ n/N). The blue arrow is the point for sample tree 4 in Plot A, where up to a DBH of 12.4 cm, 80% of the trees have a 45.9% share of the basal area of the stand. The red arrow is the point for sample tree 4 in Plot B, where up to a DBH of 11.5cm, 80% of the trees have a 70.8% share of the basal area of the stand. The dotted line represents the line of perfect equality, the area under the Lorenz curve for Plots A is 0.265 (GC = 0.47) and Plot B is 0.419 (GC = 16).