Bioprotection for foliar diseases and disorders of radiata pine

Project Overview
January 2023 to December 2023

Report prepared for New Zealand Forest Growers Research

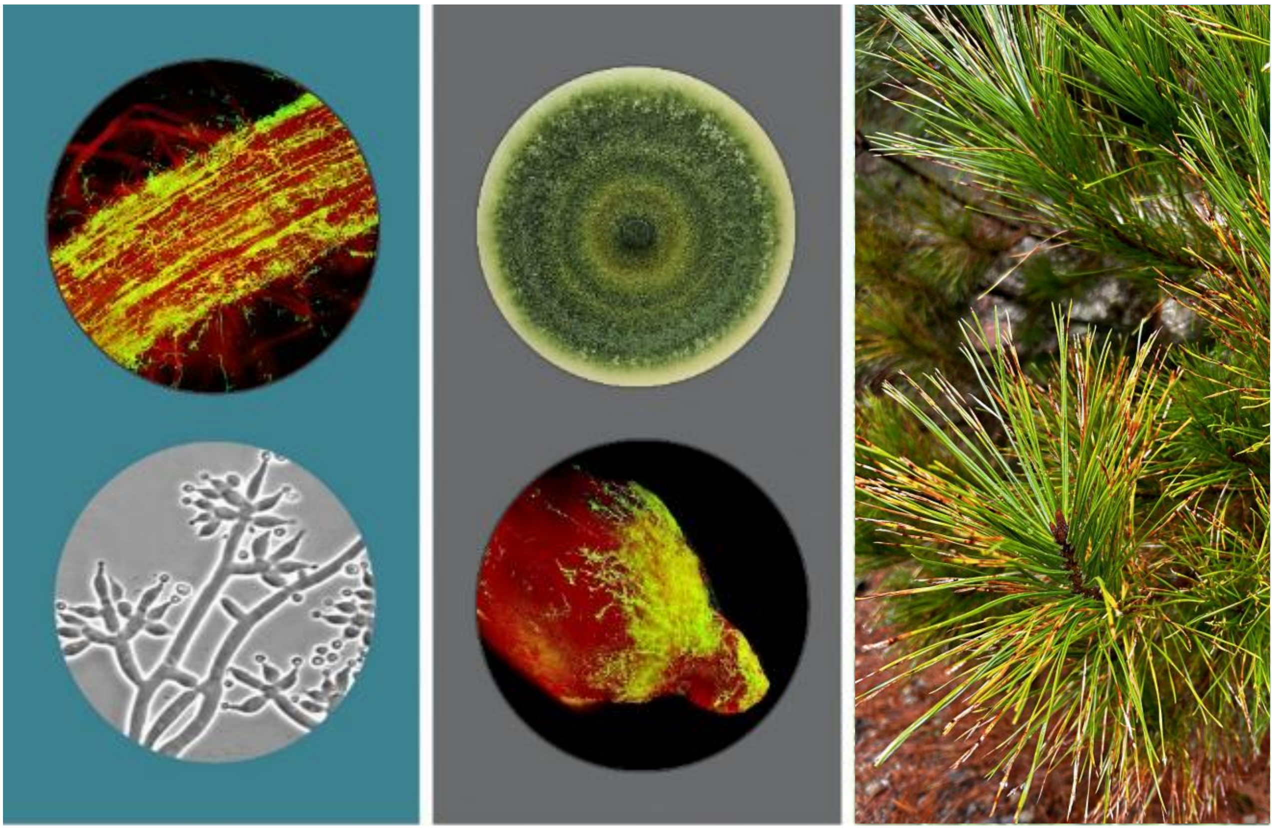

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TABLE OF CONTENTS

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 in a wide range of growing conditions in six forestry regions. *Trichoderma* significantly (P<0.01) increased stand volume by a mean of 13.2% in the eight 2018 trials, and tree height (P<0.05) by a mean of 9.0% in the five 2021 trials, compared to untreated controls (Table 1). Another Nelson trial with an additional *Trichoderma* treatment (PBI) also resulted in significantly (P<0.05) increased tree height by 13% in year two. These gains in young tree production from *Trichoderma* are important for the successful establishment of the stand and provide economic, environmental and social opportunities for rapid carbon sequestration. *Trichoderma* treatments also increased the size uniformity of tree stems by a mean of 9.6%, compared to the controls. These mixtures are therefore recommended as practical and effective tools for foresters who want to reduce production or harvest costs and produce timber of more uniform size. This technology is relevant to both intensive or low-cost, extensive management regimes and will ultimately lead to more efficient and sustainable use of forest resources.

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) [a]				2021 trials (year two) [a]	
	Survival (%) [b]	Height (m)	DBH (mm)	Stand Volume ($m^3.ha^{-1}$) [c]	Survival (%)	Height (cm)
PR6	94.3 ns	4.70 (7.1) ***	76.5 (7.0) **	14.5 (12.3) **	98.2 (ns)	59.8 (10.3) *
PR3a	95.0 *	4.69 (6.8) ***	77.0 (7.7) **	14.7 (14.1) **	97.1 (ns)	58.4 (7.8) *
Control	92.6	4.39	71.5	12.9	97.6	54.2

[a] Trials were established in Northland, Gisborne, Bay of Plenty/Waikato and Nelson in 2018 and Canterbury and Otago in 2021.

[b] significance of relationships P<0.05 (*), P<0.01 (**) and P<0.001 (***) and not significant (ns).

[c] stand volume was estimated by summing the under-bark stem volume of the trees within the plot (Kimberley and Beets 2007) and adjusting to a per-hectare basis based on the size of the plot.

Foliar disease epidemics of *Phytophthora pluvialis* (red needle cast, RNC) and *Dothistroma septosporum* (Dothistroma needle blight) developed in six trials during 2021 and 2022. The two *Trichoderma* inoculations suppressed foliar diseases by up to 63% (Whelan, 2023) but the epidemics were not severe (≤13% severity). The wet and warm conditions of 2023 encouraged further pathogen development in four of the trials (mean disease severity of 42% measured in August to November 2023) with *Trichoderma* treatments effective at reducing *P. pluvialis* by a mean of 28% (P<0.01) and *D. septosporum* by 17% (P<0.01) compared to the untreated plots. Although *Trichoderma* treatments did not eliminate the pathogen, the disease suppression offers an opportunity for delayed or fewer agrichemical sprays required to control the diseases.

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 (*Nothophaeocryptopus gaeumannii*, SNC). *Trichoderma* PR3a and PR6 treatments had significantly (P<0.001) increased tree height by 9.1% and 7.5% respectively compared to the control twelve months after planting. *Trichoderma* treatments had a greater increase (significant at P<0.01) in height in Fort Bragg, compared to the Tramway trees, with a 15.4% (PR3a) and 11.9% (PR6) increase when compared to the control. Disease was not obvious on 7 August 2023, however, the control trees had significantly (P<0.001) more damage to the stem tips and yellowing or browning of the needles, compared to the *Trichoderma* treatments. Levels of yellow or brown needles were low (4%) but a disease assessment would be beneficial in approximately twelve months to determine the cause and subsequent levels of needle discolouration.

Promotion of *Trichoderma* inoculants in the NZ forestry industry:
In 2023, *Trichoderma* inoculum was supplied to fourteen New Zealand forestry nurseries for the treatment of approximately 80 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 the commercialisation of a forestry specific mixture. Three nurseries now inoculate all their radiata pine seed with *Trichoderma*. Additional inoculum may be offered to the industry in 2024 depending on available funding.

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 native endomycorrhizal fungi *Trichoderma. Trichoderma* fungi can induce a broad-spectrum activity response from their host plants, enhancing the speed and strength of the plant's response to diseases. *Trichoderma* has also been shown to stimulate growth of plants. The products of this research project will be *Trichoderma* inoculated forestry tree species, with enhanced growth and improved foliar health, potential protection against biosecurity incursions, as well as reduced chemical application in nurseries and plantations.

This 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 (see section 2.2). The project will validate and investigate the commercialisation of 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. Project tasks completed for the period December 2018 to December 2022 were detailed in Whelan 2018a and b, 2019a and b, 2020, 2021, 2022a and b, and 2023 a and b. This report summarises research results from January 2023 to December 2023.

2.0 BIOPROTECTION PROJECT MILESTONES

2.1 Production of *Trichoderma* inoculum (Milestone 1)

Trichoderma inoculum was supplied to the following companies in 2023: Appletons Tree Nursery, ArborGen (Te Teko, Kaikohe, Puha, Tokoroa, Nelson, Edendale and Awatere Seed Nurseries), Leithfield Nursery, PF Olsen Ltd Waiuku and Seddon Nurseries, Rangiora Nursery, Murrays Nurseries and Kauri Park Nursery, for coating of approximately 80 million seeds or drench and root applications to cuttings or ramets. Distribution of spores will contribute to information gain for the business case for the commercialisation of a specific forestry mixture. Appletons Tree Nursery treated all their radiata pine seeds in September 2023 for whole estate plantings of OneFortyOne in 2024. Similarly, Leithfield Nursery and PF Olsen Waiuku treated all their radiata pine seed.

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 (Whelan, 2022a). 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 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. The internal 25 trees (5 trees x 5 rows) were measured for growth and disease variables at various dates.

Across the eight sites, *Trichoderma* treatments significantly ($P<0.05$) increased stem volume (by 12.5% for PR6 and 16.1% for PR3a) and stand volume (12.3% for PR6 and 14.1% for PR3a), compared to the control (Table 2). The growth benefits were due to an increase in tree height (mean of 7%) and trunk basal area (mean of 12.2%), and also survival (by 2.4%) in the PR3a treatment (Table 2). These gains in young tree production from *Trichoderma* are important for the successful establishment of the stand and provide economic, environmental and social opportunities for rapid carbon sequestration. *Trichoderma* treatments also increased the size uniformity of tree stems by a mean of 9.6%, compared to the controls (Whelan, 2022a and b). These mixtures are therefore recommended as practical and effective tools for foresters who want to reduce production or harvest costs and produce timber of more uniform size. This technology is relevant to both intensive or low-cost, extensive management regimes and will ultimately lead to more efficient and sustainable use of forest resources.

Table 2: 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 brackets), in the 2018 trials approximately four years after planting.

Treatment	Survival (%) [a]	Height (m)	DBH (mm) [b]	Basal Area (m^2) [c]	Stem Volume (m^3) [c]	Stand Volume ($m^3.ha^{-1}$) [c]
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	0.15	3.3	0.000431	0.00155	1.14
Significance	P<0.05	P<0.001	P<0.01	P<0.01	P<0.05	P<0.01

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

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

[c] tree basal area (m^2) and under-bark stem volume (v) of an individual tree (m^3) were calculated using the following formula: v=h*ba*(B1*(h-1.4)**(-B2)+ B3
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 calculated 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 Assessed at Year Five:

Disease severity was scored in 5% increments (see Appendices A and B) using a combined total of current and previous infections including needles that had been cast.

Figure 1: The XPKANG 209/4 trial on 11 August 2023 five years after planting.

Figure 2: The XPKANG 660/2 trial on 10 August 2023 five years after planting.

Bay of Plenty/Waikato Kaingaroa Forest Trials:

- Dothistroma needle blight (*D. septosporum*) developed in the XPKANG 209/4 and XPKANG 660/2 trials in the first and second years after planting (Table 3).

- In the XPKANG 209/4 trial, the incidence and severity of *D. septosporum* increased progressively from August 2019 to August 2023, apart from a slight reduction in August 2022, possibly due to a reduced spore load caused by an operational copper spray in the surrounding stand. By August 2023, all trees had disease symptoms with a very high mean severity level of 44%, due to the wet and warm conditions of summer 2022/23 (Figure 1).

- Between August 2019 and August 2022 in the XPKANG 209/4 trial, *Trichoderma* PR6 and PR3a treatments significantly (P≤0.003) reduced disease severity by a mean of 45% (August 2019), 35% (July 2020) and 48% (August 2022), compared to the control (Table 3). In August 2023, *Trichoderma* treatments also significantly (P<0.001) reduced disease severity by a mean of 16%, compared to the control. Both *Trichoderma* treatments were similarly effective at reducing disease at each date but did not eliminate disease at any time (Table 3).

- Similar to the XPKANG 209/4 trial, Dothistroma needle blight disease in the KPKANG 660/2 trial increased progressively, and by August 2023 all trees had disease symptoms with a very high mean severity level of 51% (Table 4, Figure 2).

- *Trichoderma* PR6 and PR3a treatments significantly (P≤0.001) reduced disease severity by a mean of 34% (August 2022) and 18% (August 2023), compared to the control (Table 4). Both *Trichoderma* treatments were similarly effective at reducing disease in August 2022 and 2023 but did not eliminate disease at any time (Table 4).

- The smaller percentage reduction in the effectiveness of the *Trichoderma* treatments in the 2023 assessments, compared to earlier dates, may have been due to the influence of high inoculum levels in the untreated control plots on the treated trees in neighbouring plots (OEPP/EPPO Bulletin 2012). A copper spray was planned for application over both Kaingaroa trials in spring 2023 to reduce the inoculum load and its effects on the future growth potential.

Table 3: Effect of *Trichoderma* treatments on *D. septosporum* disease incidence (%) and severity (%) in the XPKANG 209/4 trial at four measurement dates.

Treatment	Disease							
	Incidence (%) [a]				Severity (%) [b]			
	21 Aug 2019	29 Jul 2020	16 Aug 2022	11 Aug 2023	21 Aug 2019	29 Jul 2020	16 Aug 2022	11 Aug 2023
PR6	47.4 (4.4) b	94.4 (2.9) a	73.4 (3.6) b	100.0 a	1.6 (0.3) c	5.8 (0.7) b	3.8 (0.6) b	38.7 (1.4) b
PR3a	56.2 (3.4) ab	96.2 (1.3) a	74.5 (2.5) b	100.0 a	2.8 (0.4) b	7.5 (0.8) b	3.9 (0.6) b	41.4 (2.1) b
Control	67.1 (4.6) a	98.8 (0.9) a	88.1 (2.3) a	100.0 a	4.0 (0.6) a	10.2 (1.0) a	7.3 (1.3) a	47.6 (2.7) a
LSD (5%)	12.7	5.2	6.0	0.0	1.1	2.1	2.1	3.6
Significance	P=0.02	NS	P<0.001	NS	P=0.001	P=0.001	P=0.003	P<0.001

[a] Letters were assigned according to a Fisher's 5% level unprotected LSD procedure. Two means with the same letter within one column are not significantly (NS) different at P=0.05. The standard error of the mean is in brackets.

[b] Disease severity (%) was assessed as the percentage of needles infected with current and previous infection, including needles that had been cast.

Table 4: Effect of *Trichoderma* treatments on *D. septosporum* disease incidence (%) and severity (%) in the XPKANG 660/2 trial at four measurement dates.

Treatment	Disease							
	Incidence (%) [a]				Severity (%) [b]			
	21 Aug 2019	28 Jul 2020	16 Aug 2022	10 Aug 2023	21 Aug 2019	28 Jul 2020	16 Aug 2022	10 Aug 2023
PR6	0.0 a	30.4 (2.2) ab	93.1 (2.2) a	100.0 a	0.0 a	3.3 (0.6) a	9.5 (1.1) b	48.7 (2.3) b
PR3a	0.0 a	30.0 (4.0) b	94.5 (2.1) a	100.0 a	0.0 a	3.0 (0.5) a	7.8 (0.8) b	45.7 (1.7) b
Control	0.0 a	40.0 (2.2) a	99.6 (0.4) b	100.0 a	0.0 a	4.4 (0.7) a	13.1 (0.8) a	57.2 (1.5) a
LSD (5%)	0.0	9.9	4.8	0.0	0.0	1.5	2.6	4.4
Significance	NS	P=0.05	P=0.03	NS	NS	NS	P=0.001	P<0.001

[a] Letters were assigned according to a Fisher's 5% level unprotected LSD procedure. Two means with the same letter within one column are not significantly (NS) different at P=0.05. The standard error of the mean is in brackets.

[b] Disease severity (%) was assessed as the percentage of needles infected with current and previous infection, including needles that had been cast.

Northland Whatoro Trial:

- Red needle cast *(P. pluvialis)* was the main disease present on 19 October 2023 (Figure 3), with incidence of RNC at very high levels (mean of 96%, Table 5). *Trichoderma* PR6 (94.1%) treatment had significantly (P<0.05) fewer trees infected, compared to the control (99.2%, Table 5).

- Disease severity was also at very high levels in the trial (mean of 34%). The *Trichoderma* PR6 and PR3a treatments significantly (P<0.01) reduced severity by 33% (28.0%) and 27% (30.4%) respectively, compared to the control (41.7%, Table 5).

Figure 3: Red needle cast in trees in the Northland Whatoro trial five years after establishment on 19 October 2023.

Table 5: Effect of *Trichoderma* treatments on *D. septosporum* (7 Jul 2022) and *P. pluvialis* (10 Aug 2023) disease incidence (%) and severity (%) in the Northland Whatoro trial.

Treatment	Disease			
	Incidence (%) [a]		Severity (%) [b]	
	7 Jul 2022	19 Oct 2023	7 Jul 2022	19 Oct 2023
PR6	14.1 (4.1) a	94.1 a	1.3 (0.6) a	28.0 (3.3) b
PR3a	9.2 (2.6) a	96.5 ab	0.8 (0.5) a	30.4 (3.0) b
Control	16.0 (4.8) a	99.2 b	1.2 (0.4) a	41.7 (3.7) a
LSD (5%)	9.8	4.6	0.6	7.3
Significance	NS	P<0.05	NS	P<0.01

[a] Letters were assigned according to a Fisher's 5% level unprotected LSD procedure. Two means with the same letter within one column are not significantly (NS) different at P=0.05. The standard error of the mean is in brackets.
[b] Disease severity (%) was assessed as the percentage of needles infected with current and previous infection, including needles that had been cast.

Gisborne Patunamu Trial:

- Red needle cast *(P. pluvialis)* was the main disease present on 24 November 2023 (Figure 4) with disease severity at very high levels (mean of 38%, Table 6).

- *Trichoderma* PR3a and PR6 treatments significantly (P<0.001) reduced severity by 27% (33.8%) and 25% (34.6%) respectively, compared to the control (45.9%, Table 6).

Figure 4: Red needle cast in trees in the Gisborne Patunamu trial five years after establishment on 24 November 2023.

Table 6: Effect of *Trichoderma* treatments on *P. pluvialis* disease incidence (%) and severity (%) in the Gisborne Patunamu trial on 24 November 2023.

Treatment	**Disease**			
	Incidence (%) [a]		**Severity (%)** [b]	
	8 Sept 2022	24 Nov 2023	8 Sept 2022	24 Nov 2023
PR6	23.6 (6.4) a	100.0 a	0.7 (0.3) a	34.6 (2.4) a
PR3a	22.2 (6.3) a	100.0 a	0.7 (0.4) a	33.8 (1.8) a
Control	48.4 (8.4) b	100.0 a	1.9 (0.4) b	45.9 (1.5) b
LSD (5%)	16.8	-	1.0	6.3
Significance	P<0.01	NS	P<0.05	P<0.001

[a] Letters were assigned according to a Fisher's 5% level unprotected LSD procedure. Two means with the same letter within one column are not significantly (NS) different at P=0.05. The standard error of the mean is in brackets.

[b] Disease severity (%) was assessed as the percentage of needles infected with current and previous infection, including needles that had been cast.

2.2.1.2 Plantation validation trials established in 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 (Whelan, 2022a). Five other trials were established in Canterbury and Otago in 2021 to test PR6 and PR3a mixtures in cooler, and sometimes drier, locations than that of the earlier sites (Table 7 and Figure 5). 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. Height was measured in the central 24, 25 or 30 trees in each plot and data was analysed for significance by analysis of variance (ANOVA) and the Fisher's unprotected least significant difference (LSD) test at the 5% level using Genstat, v22 (Genstat 2022).

Tree survival was high in all trials (mean of 96.2%), 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 91.2% (Table 8). Across the 2021 trials, *Trichoderma* treatments did not affect survival in the trials at year two (Table 8). However, *Trichoderma* had a large effect on tree height and was significantly ($P \leq 0.05$) greater in four of the five sites compared to the control (Table 8). Kakahu had the largest increase in height of all trials (20% in the PR6 treatment), whilst Hillend had no significant increase (an ex-pasture site with potentially high fertility soils) in the *Trichoderma* treatments. Over all sites, *Trichoderma* significantly ($P=0.015$) increased height by 10.3% and 7.9% in the PR6 and PR3a treatments compared to the control (Table 8). Root colonisation in the inoculated trees was approximately double that of the controls (Table 9).

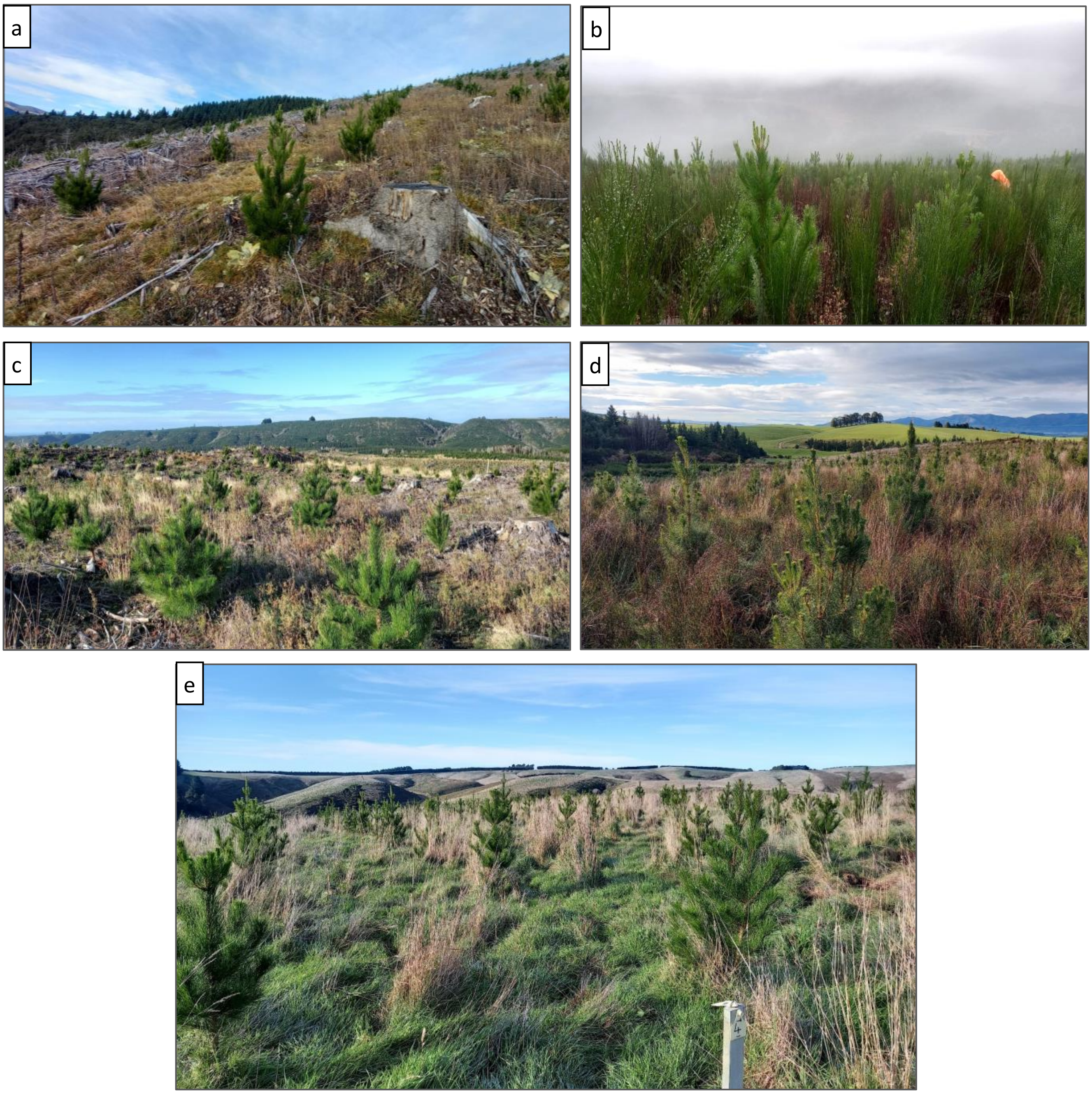

Figure 5: Trials in the Okuku (a) and Kakahu (b) Forests, Canterbury, and Dusky (c) and Akatore Forests (d) and Hillend region (e), Otago, at the year two assessment in June 2023.

Table 7: Establishment details and updated comments in the 2021 radiata pine trials.

Region	Company and Forest / Trial Name	Location	Altitude (m asl)	Planting Date	No Replicates	Planting Stocking Rate and Spacing
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 two assessment. Deer had recently eaten some tips (5%) and may 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. The growth of 18% 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 (22%), but also the PR3a (16%) and control (12%) treatments. The wet summer of 2022/2023 caused strong growth of broom; this weed was controlled with a release spray in November 2023. Future risks include regenerating pine seedlings and gorse and broom growth, and a regen pull is being considered.				
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 (130 meters asl), rolling-top site 12km west of Milton, South Otago. The trees have grown strongly since establishment and were healthy with low weed pressure at the year two assessment. 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)
		The 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. Survival in June 2022 was relatively low (92.5%) due to wet soils and *Hylastes ater* (black pine bark beetle) or grass grub (*Costelytra zealandica)* damage at planting. A systemic herbicide spray containing triclopyr and clopyralid was applied in January 2023 to control dense broom. In June 2023, twisted and curled shoots, shortened needles, elongation of stems and clubbed shoots were observed in 5 to 10% of trees, possibly because of the active ingredient triclopyr. Animal grazing damage (cattle and deer) was also observed in 8% of trees. Data from trees with animal damage to the main shoots was removed from the statistical analysis of tree height. No disease was present in June 2023. Future risk to the trial includes animal grazing.				
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 two assessment, there was no disease and minimal (4%) animal grazing damage. The growth of 12% of trial trees was severely reduced by compacted soil caused by machinery and log movement during the previous crop harvest. Trees affected were mainly in the PR3a (22%), but less so in the PR6 (8%) and control (4%) treatments. There was no animal damage or disease present in June 2023.				

Table 8: Effect of *Trichoderma* treatments on tree survival (%) and height (cm), including percentage difference compared to the untreated controls, in the 2021 trials approximately two years after planting.

Region and site	Treatment	Survival (%) [a]	Height (cm) [b]
Canterbury Kakahu [c]	PR6	96.3 a	192.8 (20.2) a
	PR3a	96.7 a	180.5 (12.5) a
	Control	96.7 a	160.4 b
	LSD (5%)	**4.1**	**18.1**
	Significance	**NS**	**P<0.01**
Canterbury Okuku	PR6	99.4 a	115.5 (5.0) ab
	PR3a	98.9 a	121.4 (10.3) b
	Control	98.9 a	110.1 a
	LSD (5%)	**2.2**	**10.6**
	Significance	**NS**	**P=0.05**
Otago Hillend	PR6	98.8 a	162.5 (7.2) a
	PR3a	98.8 a	163.5 (7.9) a
	Control	99.2 a	151.6 a
	LSD (5%)	**2.9 NS**	**16.7**
	Significance	**NS**	**NS**
Otago Akatore [d]	PR6	96.0	120.0 (7.3) a
	PR3a	92.7	112.7 (0.8) b
	Control	97.6	111.8 b
	LSD (5%)	**6.4**	**7.0**
	Significance	**NS**	**P<0.05**
Otago Dusky	PR6	94.0 a	150.3 (9.1) b
	PR3a	90.0 a	146.4 (6.2) ab
	Control	89.6 a	137.8 a
	LSD (5%)	**6.6**	**11.4**
	Significance	**NS**	**P<0.05**
Mean	PR6	96.9 a	148.2 (10.3) a
	PR3a	95.4 a	144.9 (7.9) a
	Control	96.4 a	134.3 b
	LSD (5%)	**2.3**	**8.7**
	Significance	**NS**	**P=0.015**

[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 the Kakahu trial, tree height was impacted by compacted soil conditions (18% of total trees). Height data for severely stunted trees (defined as short stature, light green or yellow foliage, short upper needles and cast lower needles) 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).

[d] in the Akatore trial, tree height was impacted by compacted soil conditions. Height data for severely stunted trees (defined as above) was removed from the dataset before ANOVA analysis was performed.

Table 9: *Trichoderma* colonisation (%) of radiata roots in the 2021 trials, sampled two years after planting, based on MRB plating data.

Region and Site	***Trichoderma* root colonisation (%) [a]**		
	PR6	**PR3a**	**Control**
South Otago, Hillend	74	98	56
South Otago, Dusky	50	44	38
South Canterbury, Kakahu	100	100	79
North Canterbury, Okuku	97	94	53
South Otago, Akatore	50	50	29
Mean [b]	74 (11) a	77 (12) a	51 (9) b
LSD (5%)	**14**		
Significance	**P<0.05**		

[a] root pieces for each treatment were bulked together at each site. *Trichoderma* was then isolated from approx. 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 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 (*Nothophaeocryptopus gaeumannii,* formerly *Phaeocryptopus gaeumannii,* SNC). The potential for *Trichoderma* to control SNC and enhance tree growth will be determined in a Kaingaroa Forest plantation trial established on 18 August 2022.

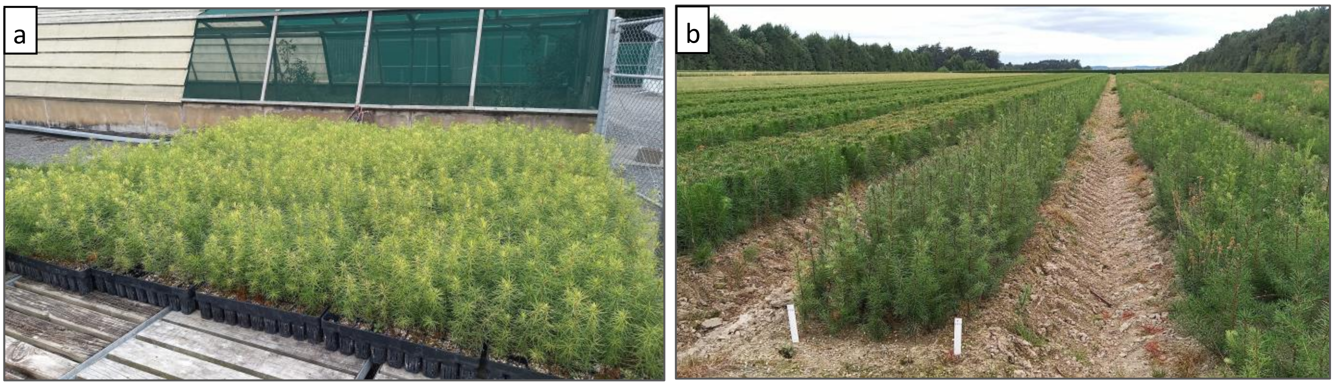

Figure 6: *Trichoderma* inoculated and uninoculated Douglas-fir seedlings in trays at Lincoln University (a) on 29 March 2021, and in the ArborGen Edendale Nursery (b, middle row) on 21 April 2022.

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 6a). Three *Trichoderma* spore suspension treatments were pipetted (5ml of $5.0E^6$ spores/ml) onto seeds at sowing, including:

- PR6 mixture (isolates FCC55, FCC318, FCC327, FCC340)
- PR3a mixture (isolates FCC13, FCC14, FCC15 and FCC180)
- GM mixture (isolates FCC320, FCC327 and LU633), and
- an untreated control.

Two seedlots were used:

- Tramway (Blk1-4, 8&9) of Washington State, USA provenance with a medium tolerance to SNC, and
- Fort Bragg (Blk 7), of California, USA provenance with a low tolerance to SNC.

The seedlings were harvested, lined out in soil at ArborGen Edendale Nursery, near Invercargill on 13 April 2021, and grown using standard nursery management (Figure 6b). 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 (Whelan, 2022a). Trees were harvested on 10 August 2022 and delivered to Rotorua using refrigerated road transport.

The trial was established in Kaingaroa Forest on a relatively warm, flat, elevated (180 metres asl) site east-south-east of Rotorua and approximately 15km north of Murupara (-38.368079, 176.737774) on 18 August 2022. The trial comprised a 2 x 4 factorial in a randomised complete block design (RCBD) with seven replicate blocks. Each plot contained approximately 27 trees planted into spot-mounded soil in a 9 x 3 grid pattern with a 3.5 x 3.5m tree spacing (approximately 817 trees per hectare). Each plot was bounded by two windrows of slash material and approximately 24 internal trees were sampled. 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, v22 (Genstat 2022).

Figure 7: The Douglas-fir plantation trial in Kaingaroa Forest measured one year after planting on 7 August 2023.

During the first year of growth, weed pressure was relatively low (mainly fleabane, grass and broadleaf species). Blackberry plants that covered the site at planting had fully desiccated in most places (Figure 7), however, broom had grown in the plots on the SW side of the trial beside the skid site.

Deer grazing, including damage to the main stem was found in 11% of trees on 7 August 2023. No statistically significant difference (P<0.05) was found in the percentage of trees affected by animal grazing in the treatments. In addition, bending ("shepherds crook") and death of some main and side stem tips were observed (Figure 8b and c) possibly due to winter frost injury. On 15 June 2023, a frost event of -4.2℃ (2m above the ground) at a meteorological station 4.5km SW from the trial was measured (A. Manig, Timberlands *pers. comm.*). Herbicide spray damage and fungal pathogens were considered as a cause of stem tip bending and death, including *Sphaeropsis sapinea* (Diplodia tip blight) and *Phomopsis lokoyae* (Phomopsis canker). However, some symptoms of Diplodia tip blight were not present (resinous cankers, black pycnidia and short needles on the dead shoots) and the pathogen is typically expressed under drought stress conditions. No Phomopsis stem cankers were observed. Tree stem tip death was visually assessed using scores of 0 (no damaged), 1 (approximately 1 to 20%), 2 (approximately 21 to 40%), 3 (approximately 41 to 60%), 4 (approximately 61 to 80%) and 5 (approximately 81 to 100%) tips damaged.

Yellow and brown foliage (Figure 8a and b) was present on 22% of trees on 7 August 2023. The severity of yellow and brown foliage was assessed in 5% increments of needles affected.

Trial Assessed at Year One (7 August 2023):

- Survival was very high (> 99%) with no significant (P<0.05) difference between the treatments (Table 11).

- PR3a and PR6 treatments had significantly (P<0.001) increased tree height by 9.1% and 7.5% respectively compared to the control (Table 11). GM treatment had a height similar to that of the control. Fort Bragg had significantly (P<0.05) increased tree height (by 1.3cm) compared to Tramway. *Trichoderma* treatments had a greater increase in height in the Fort Bragg, compared to the Tramway trees, with a 15.4% (PR3a), 11.9% (PR6) and 6.5% (GM) increase when compared to the control (Table 11).

Figure 8: Trees with yellow and brown foliage (a, b), frost-damaged stem tips (b, c), and no damage (d) in the Kaingaroa Forest Douglas-fir trial on 7 August 2023.

- Disease was not obvious on 7 August 2023, however, the control trees had significantly ($P<0.001$) more damage to the stem tips and yellowing or browning of the needles (Table 11) compared to the *Trichoderma* treatments. Levels of yellow or brown needles were low (4%) but a disease assessment would be beneficial in approximately twelve months to determine the cause and subsequent levels of needle discolouration.

- *Trichoderma* root colonisation (based on MRB plating data) sampled three years after application in the nursery, was at medium to high levels in the *Trichoderma* treatments (mean of 51%), and lower in the controls (mean of 33%, Table 10). Therefore, *Trichoderma* applied to the trees in the nursery generally persisted and may have dominated the natural strains in the plantation.

Table 10: Root colonisation (%) three years after *Trichoderma* application at seed sowing, based on MRB plating data.

Provenance	*Trichoderma* Treatment	Root colonisation (%) [a]
Tramway	PR6	58.6
	PR3a	72.2
	GM	39.3
	Control (no *Trichoderma*)	40.0
Fort Bragg	PR6	43.3
	PR3a	44.4
	GM	46.7
	Control (no *Trichoderma*)	26.7

[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).

Table 11: The effect of *Trichoderma* treatments and provenance on height (cm), survival (%), stem tip damage score and yellow and brown foliage (%) of Douglas-fir trees one year after planting in the KPANG 1250/7 trial on 7 August 2023.

Treatment	Height (cm) [a]	Survival %	Stem Tip Damage Score[b]	Yellow and Brown Foliage (%)
Main Effects Means:				
***Trichoderma*:**				
PR6	54.3 (0.6) a	99.7 (0.3) a	0.45 (0.09) b	1.9 (0.3) b
PR3a	55.1 (0.9) a	100.0 a	0.36 (0.08) b	1.8 (0.7) b
GM	51.8 (0.8) b	100.0 a	0.32 (0.07) b	0.9 (0.3) b
Control	50.5 (0.7) b	99.7 (0.3) a	0.88 (0.12) a	4.2 (0.6) a
LSD (5%) **Significance**	1.7 P<0.001	0.5 NS	0.21 P<0.001	1.4 P<0.001
Provenance:				
Fort Bragg	53.6 (0.7) a	99.9 (0.1) a	0.45 (0.08) a	2.2 (0.4) a
Tramway	52.3 (0.5) b	99.9 (0.1) a	0.55 (0.07) a	2.3 (0.4) a
LSD (5%) **Significance**	1.2 P<0.05	0.8 NS	0.15 NS	1.0 NS
***Trichoderma* x Provenance Interaction Means:**				
Fort Bragg:				
PR6	55.3 (0.3)	1.00	0.30 (0.09)	2.2 (0.4)
PR3a	57.0 (1.1)	1.00	0.40 (0.08)	2.4 (1.3)
GM	52.6 (1.0)	1.00	0.19 (0.07)	0.8 (0.4)
Control	49.4 (1.3)	0.99 (0.5)	0.92 (0.18)	3.3 (0.8)
Tramway:				
PR6	53.2 (1.0)	0.99 (0.5)	0.60 (0.14)	1.7 (0.4)
PR3a	53.2 (1.0)	1.00	0.33 (0.14)	1.3 (0.4)
GM	51.1 (1.1)	1.00	0.46 (0.11)	1.1 (0.4)
Control	51.5 (0.6)	1.00	0.83 (0.17)	5.1 (1.0)
LSD (5%) **Significance**	2.4 P<0.01	0.4 NS	0.30 NS	1.9 NS

[a] Letters were assigned according to a Fisher's 5% level unprotected LSD procedure. Two means with the same letter within one variable are not significantly (NS) different at P=0.05. The standard error of the mean is in brackets.

[b] Stem tip damage possibly caused by frost was scored between 0 (no damage) and 5 (between 81 to 100% tips damaged).

3.0 COMMERCIALISATION OF *TRICHODERMA* ISOLATES

In this research programme, two *Trichoderma* mixtures PR6 and PR3a were found to suppress foliar disease and promote tree growth and stem size uniformity in numerous young radiata pine plantation trials. The benefits of these agents are currently being promoted to the industry to build the business case for commercialisation of a forestry specific *Trichoderma* mixture. No FGR funding has been allocated to this research programme for 2024. Lincoln University has distributed inoculum to interested nurseries to gain experience using the product. Inoculum may be offered to the industry in 2024 depending on sourcing funding.

4.0 PROJECT OUTPUTS (JANUARY 2023 TO DECEMBER 2023)

Whelan, H. 2023. Bioprotection for foliar diseases and disorders of radiata pine. Quarterly progress reports to NZ Forest Growers Research. March, June, September and December 2023.

Whelan H. 2023. Tolerance of *Trichoderma* bioinoculants to Forestry Agrichemicals. New Zealand Plant Protection Conference, Rotorua. 8-10 August 2023.

Whelan H. 2023. Bioprotection for foliar disease and disorders of radiata pine: project overview. January 2022 to December 2022. NZ Forest Growers Research Technical Report BIO-T027. January 2023. 41p.

Whelan H. 2023. Bioprotection for foliar diseases and disorders of radiata pine: project update. Research presentations to NZ Resilient Forests Technical Committee meetings: 9 Feb and 1 November 2023.

Whelan H. 2023. Separate industry reports for Ernslaw One Ltd, 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. 2023. Can *Trichoderma* increase nutrient uptake in planted radiata? NZ Forest Growers Research Technical Report BIO-TO28. 20p.

5.0 CONCLUSIONS

This research programme showed that nursery *Trichoderma* inoculation is an important management tool for production and disease control in radiata pine and other forestry species. Both bioprotectants significantly reduced disease severity of *D. septosporum* and *Phytophthora pluvialis* during early stand establishment (by up to 63%). These did not eliminate the presence of disease but could be an additional or alternative disease management tool to the use of agrichemicals that is efficient and cost-effective to apply. The disease suppression by *Trichoderma* also offers an opportunity for delayed or fewer agrichemical sprays required to control disease.

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.

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Whelan H. 2023a. Bioprotection for foliar disease and disorders of radiata pine: project overview. January 2022 to December 2022. NZ Forest Growers Research Technical Report BIO-T027. January 2023. 41p.

Whelan, H. 2023b. Can *Trichoderma* increase nutrient uptake in planted radiata? NZ Forest Growers Research Technical Report BIO-TO28. January 2023. 20p.

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- Keven Delegat, Puketawa Station, and
- John Cannon, Tauwhareparae Farms

8.0 APPENDICES

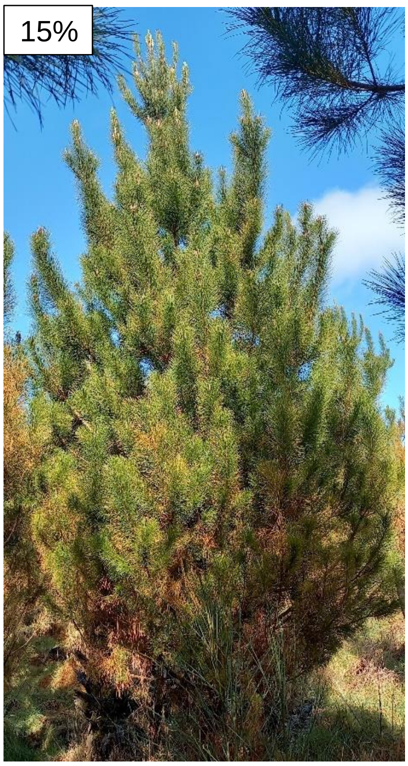
APPENDIX A:

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 sulphate and 50mg/L chlortetracycline.

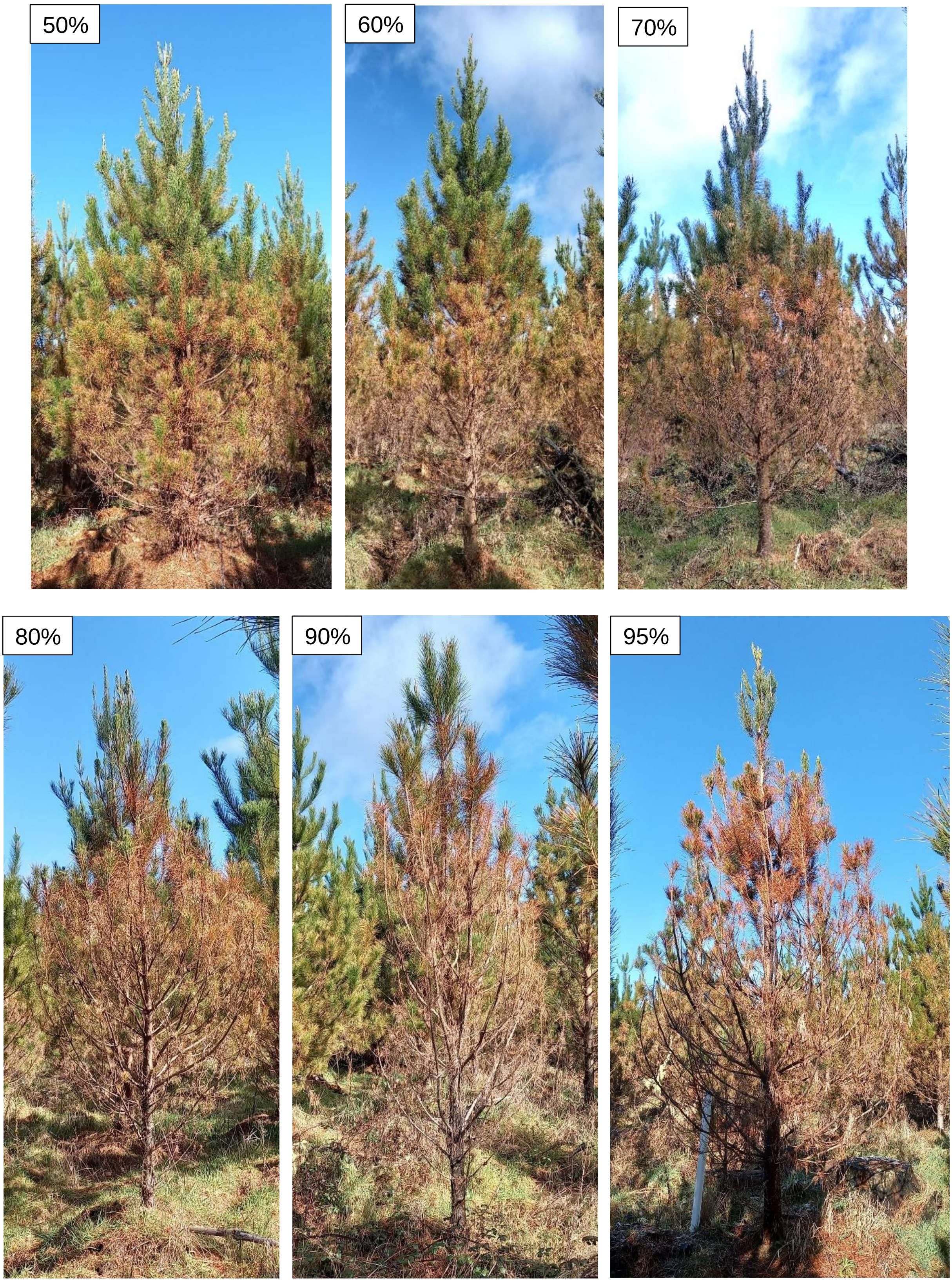
APPENDIX B:

Infection levels (5 to 40%) of Dothistroma needle blight (*Dothistroma septosporum)* on five-year-old radiata pine trees in the XPKANG 209/4 trial on 7 August 2023.



APPENDIX C:

Infection levels (50 to 95%) of Dothistroma needle blight (*Dothistroma septosporum)* on five-year-old radiata pine trees in the XPKANG 209/4 trial on 7 August 2023.



APPENDIX D:

Infection levels (<1 to 50%) of *Phytophthora pluvialis* on five-year-old radiata pine trees in the Northland Whatoro trial on 19 October 2023.

APPENDIX E:

Infection levels (60 to 95%) of *Phytophthora pluvialis* on five-year-old radiata pine trees in the Northland Whatoro trial on 19 October 2023.