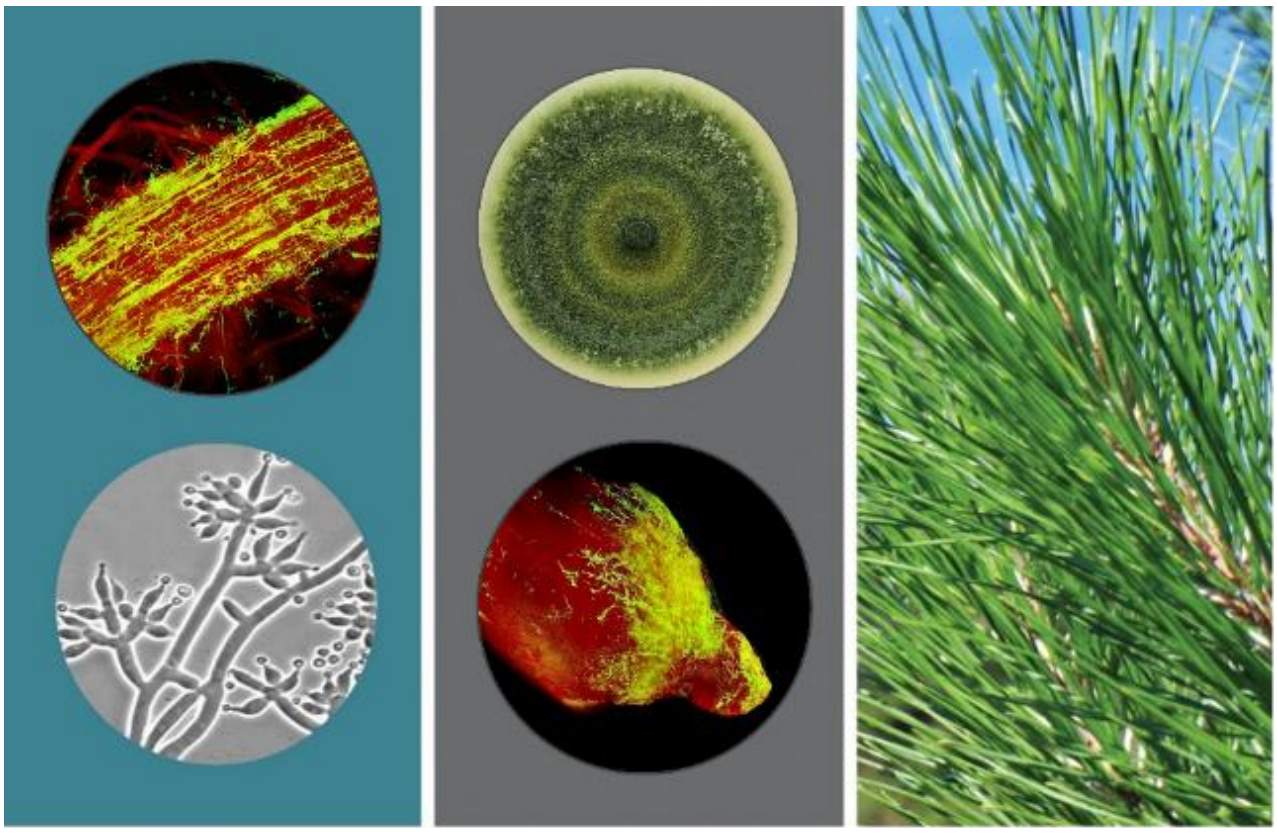
Bio-Protection
Bioprotection science for New Zealand

Bioprotection for foliar diseases and disorders of radiata pine

Project Overview
May 2020 to December 2021

Report prepared for New Zealand Forest Growers Research



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FOREST GROWERS RESEARCH

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EXECUTIVE SUMMARY

Foliar diseases and disorders are the most significant cause of economic loss for the New Zealand Forestry industry. 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 a beneficial microbe, *Trichoderma*. *Trichoderma* fungi can induce a broad-spectrum activity response from their host plants, enhancing the speed and strength of the plant's response to diseases. *Trichoderma* has also been shown to stimulate growth of plants. The products of this research project will be *Trichoderma* inoculated forestry tree species, with enhanced growth and improved foliar health, potential protection against biosecurity incursions, as well as reduced chemical application in nurseries and plantations.

This report summarises research results between May 2020 to December 2021 and other tasks that have recently been concluded:

- In 2020 and 2021*, Trichoderma* inoculum was supplied to twelve New Zealand forestry nurseries for 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 commercialisation of a forestry specific mixture. Two nurseries now inoculate all their radiata pine seed with *Trichoderma*.

- Following laboratory, greenhouse and plantation screening programmes, two *Trichoderma* mixtures (PR6 and PR3a) were tested against an untreated control in eight large-scale plantation trials in four important forestry regions established in 2018. Data measured in these trials two years after establishment found:
  i) mean tree survival was significantly ($P<0.05$) higher in the PR3a treatment (96.7%) compared to the control (94.6%)
  ii) *Trichoderma* treatments significantly ($P<0.05$) increased mean tree height by 5.9% and 6.1% for PR6 (160.2cm) and PR3a (160.5cm) respectively, compared to the control (151.3cm)
  iii) mean trunk diameter at knee-height, measured in four trials, was significantly ($P<0.05$) increased by 8.6% and 10.4% in the PR3a and PR6 treatments respectively, compared to the control
  iv) *Trichoderma* treatments significantly ($P<0.05$) reduced *Dothistroma septosporum* disease severity in a Kaingaroa Forest trial by 30% (PR3a) and 45% (PR6) compared to the control.

  The positive results in the 2018 validation trials led to six new trials with the PR6 and PR3a mixtures being established in 2020 and 2021 in cooler, and sometimes drier locations of Nelson, Canterbury and Otago.

- The sensitivity of the eight *Trichoderma* isolates that comprise the PR6 and PR3a mixtures was determined in the presence of fifty-one agrichemicals commonly used in New Zealand forestry in 2020. Agrichemicals had a range of effects on isolate spore germination, colony mycelial growth and sporulation when there was direct contact in laboratory studies, ranging from nil to full inhibition. However, the sensitivity of *Trichoderma* PR6 mixture (measured as the amount of *Trichoderma* in the root) when established in seedling radiata pine roots, to a single application of agrichemicals at recommended rates, was minimal or nil. In the plant

assays, there may have been sufficient physical distance between the site of agrichemical application and the developing or established root *Trichoderma* to avoid harm to the fungi. In addition, in some experiments root colonisation in seedlings was enhanced in the first month of growth when resident competitive fungal species may have been suppressed by the agrichemical, allowing the applied *Trichoderma* to dominate. Recommendations included:

i) using seed coat techniques to safely apply *Trichoderma*, fungicides (captan, iprodione, metalaxyl-M, and thiram) and bird-repellent, methiocarb
ii) herbicides (except glyphosate, propazine and terbuthylazine in Assett), insecticides (including *Bacillus thuringiensis*), fertilisers (except boron - disodium octaborate tetrahydrate), adjuvants and biostimulants could be tank mixed with PR6 and PR3a isolates because spore viability was not affected
iii) avoid tank mixing of PR6 or PR3a isolates and some fungicides (in particular, captan, chlorothalonil, copper hydroxide, fluazinam, prochloraz and thiram) because of potential fungicidal effects on spore germination
iv) soil- or foliar-applied fungicides and herbicides (at standard rates) can be applied to seeds or plants with inoculant or established *Trichoderma*
v) both mixtures, PR6 and PR3a, were recommended for use as growth promotants and biocontrol agents in nurseries that use agrichemicals
vi) future research should further investigate the complex interaction between agrichemicals (combination, timing and rates) and *Trichoderma* bioprotectant agents, particularly in commercial nursery soil beds, the role of co-formulants and the synergy between them, the characteristics of individual isolates in an inoculant mixture in the presence of agrichemicals, and the potential of encapsulation of spores and/or mycelium to increase the viability of *Trichoderma* products.

- Most New Zealand's plantation forests have not been inoculated with *Trichoderma* bioprotectants. Three pilot trials in 2017 and 2019, involving application of a *Trichoderma* mixture (PR6) within, onto or around established plantation trees were set-up. In the Kawerau 2017 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 diameter at breast height (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. The application methods used were generally unpractical for large-scale application of bioprotectants. An additional pilot trial (Kinleith 2019) using foliar and ground sprays on, or around two-year-old plantation radiata pine trees was established but *Trichoderma* treatments had no effect on root colonisation levels or tree growth. This may have been due to an insufficient spore dose or environmental effects that reduced the viability of the applied spores.

1.0 INTRODUCTION

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

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

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

2.0 BIOPROTECTION PROJECT MILESTONES

The project tasks completed for the period July 2012 to July 2019, August 2019 to April 2020 and May 2020 to December 2021 were detailed in Hill (2016) and Whelan (2019a), Whelan (2020), and this report respectively.

2.1 Milestone 1 – Production of *Trichoderma* inoculum

Trichoderma inoculum was supplied in 2020 and/or 2021 to the following companies: Appletons Tree Nursery, ArborGen Edendale and Te Teko Nurseries, Leithfield Nursery, Murrays Nurseries, PF Olsen Ltd Waiuku and Seddon Nurseries, Proseed New Zealand Ltd, Rangiora Nursery, Rotorua Forest Nursery, Southern Cypresses and Timberlands Ltd Te Ngae Nursery, for coating of approximately 80 million seed 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 2019 and 2020 and stock was used for the whole estate plantings of OneFortyOne. In addition, PF Olsen Waiuku treated all their radiata pine seed, as they have done consistently for the past twenty years.

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

Selected *Trichoderma* isolates were found to be fast, abundant and persistent colonisers of containerised radiata pine, Douglas-fir and cypresses seedlings and cuttings grown under greenhouse or nursery conditions (Whelan and Hill 2017, Whelan 2019b). These attributes are one of the components for the successful development of a bioprotectant product.

2.2.1 Colonisation and persistence of *Trichoderma* inoculants in nursery and field and conditions

The persistence of applied *Trichoderma* bioprotectants in nursery and field conditions, is important for long-term growth benefits and protection from disease in forests. In six radiata pine plantation trials, molecular techniques using species- and strain-specific polymerase chain reaction (PCR) primers confirmed the persistence of an applied isolate *T. atroviride* LU633, in the majority (94%) of root pieces tested, 3.5 years after tree establishment. In three of the trials measured with high concentrations of root LU633, one recorded significantly ($P<0.05$) increased tree height and trunk diameter 3.5 years after tree establishment. The persistence of this isolate in these trees should be confirmed in the future to determine its lifespan. Molecular techniques for identification of isolates used in the validation trials (section 2.3.2) have not been developed due to funding unavailability.

2.2.2 Impact of agrichemicals on colonisation and persistence of *Trichoderma* inoculants

Agrichemicals are an important component in the management of diseases, weeds and insects in many New Zealand forestry nurseries and forests (Rolando et al. 2017 and 2019). Recently there has been emphasis in New Zealand forest production to minimise the environmental impact of agrichemicals and to find more sustainable ways to operate (Hall 2019). Integrated management strategies promote the use of agrichemicals in a more sustainable manner by combining them with biological and cultural methods. The successful integration of biocontrol organisms into radiata pine production systems requires knowledge of their compatibility with commonly used pesticides and other products.

Sensitivity of eight isolates to one application (at standard rates) of fifty-one agrichemicals (14 fungicides, 2 fumigants, 14 herbicides, 6 insecticides, 4 fertilisers, 5 adjuvants, 2 adherents, 3 biostimulants and 1 bird repellent) commonly used in the New Zealand Forestry industry were determined (Table 1, Whelan 2021b). Isolates tested were those that comprised the PR6 (FCC55, 327 and 340 (*T. harzianum*) and FCC318 (*T. atrobrunneum*)) and PR3a (FCC13 (*T. asperellum*), FCC14 and FCC15 (*T. atroviride*) and FCC180 (*T. crassum*)) mixtures, used in the 2018, 2020 and 2021 validation plantation trials (Whelan 2019a and 2020, section 2.3.2). Sensitivity of each isolate to agrichemicals was determined by measuring spore germination, colony mycelial growth (Figure 1) and sporulation in in-vitro laboratory studies. Sensitivity of the PR6 mixture (applied as part of a seed coat recipe or a soil drench) to individual agrichemicals applied at various times (before (Figure 2), at (Figure 3a) or soon after seeding, and 3.5 months after seedling emergence (Figure 3b)) was determined by measuring root *Trichoderma* levels during seedling growth in eight containerised tray greenhouse studies in radiata pine.

Agrichemicals had a range of effects on isolate spore germination, colony mycelial growth and sporulation, with most inhibiting development by 50% or less (Table 1). However, 16% of agrichemicals tested (dazomet and metam fumigants, prochloraz, thiram and cuprous oxide fungicides and glyphosate and terbuthylazine in Assett but not AGPRO Terbuthylazine 500 herbicides) fully inhibited spore germination or mycelial growth of the *Trichoderma* isolates (Table 1).

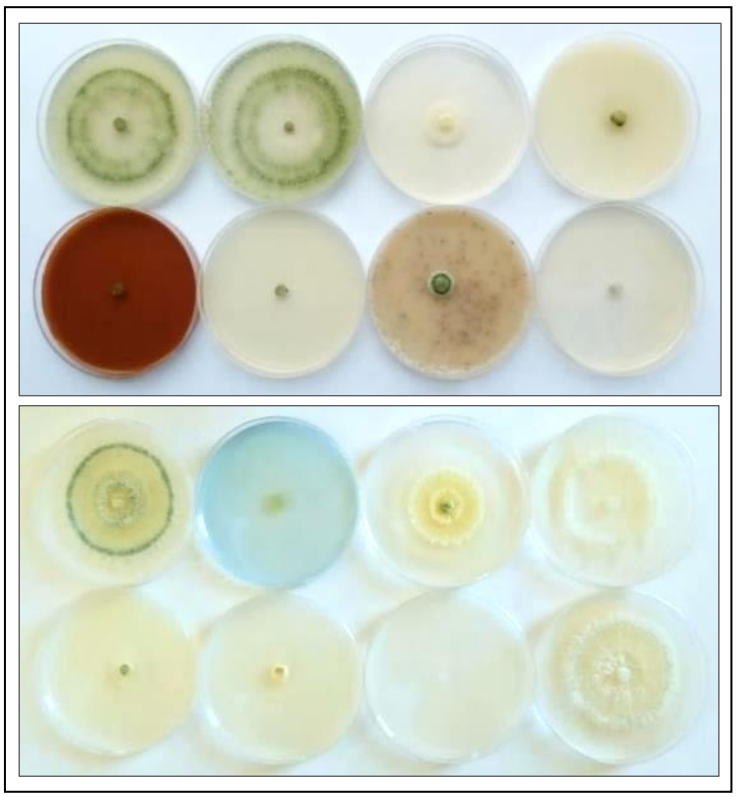

Figure 1: Mycelial growth of *Trichoderma* isolate FCC327 on malt yeast extract agar (top left to top right) with no agrichemical, metalaxyl-M (Apron), thiram and captan, (row 2 left to right) cuprous oxide, metam, metalaxyl-M/mancozeb (high rate - 56g/l) and dazomet, (row 3 left to right) iprodione, copper hydroxide, metalaxyl-M (Ridomil Gold SL) and phosphorous acid, and (bottom left to right), pyrimethanil, chlorothalonil, prochloraz and methiocarb, 68 hours after inoculation with mycelium agar plugs.

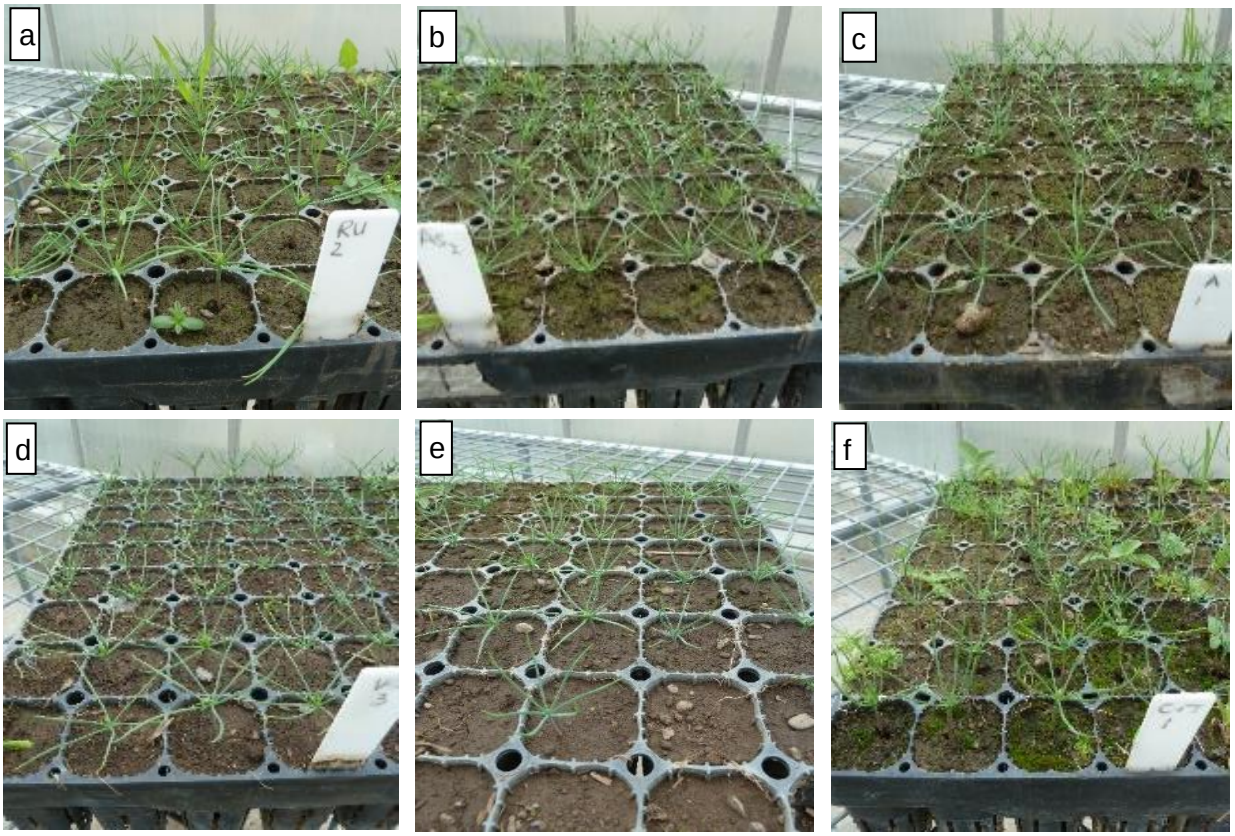

Figure 2: *Trichoderma* inoculated *P. radiata* seedlings one week after emergence from soil treated with herbicides a) glyphosate, b) terbuthylazine, c) atrazine and d) hexazinone 78 days before seeding and e) simazine 8 days before seeding. Image f) is *Trichoderma* inoculated seedlings with no herbicide treatment.

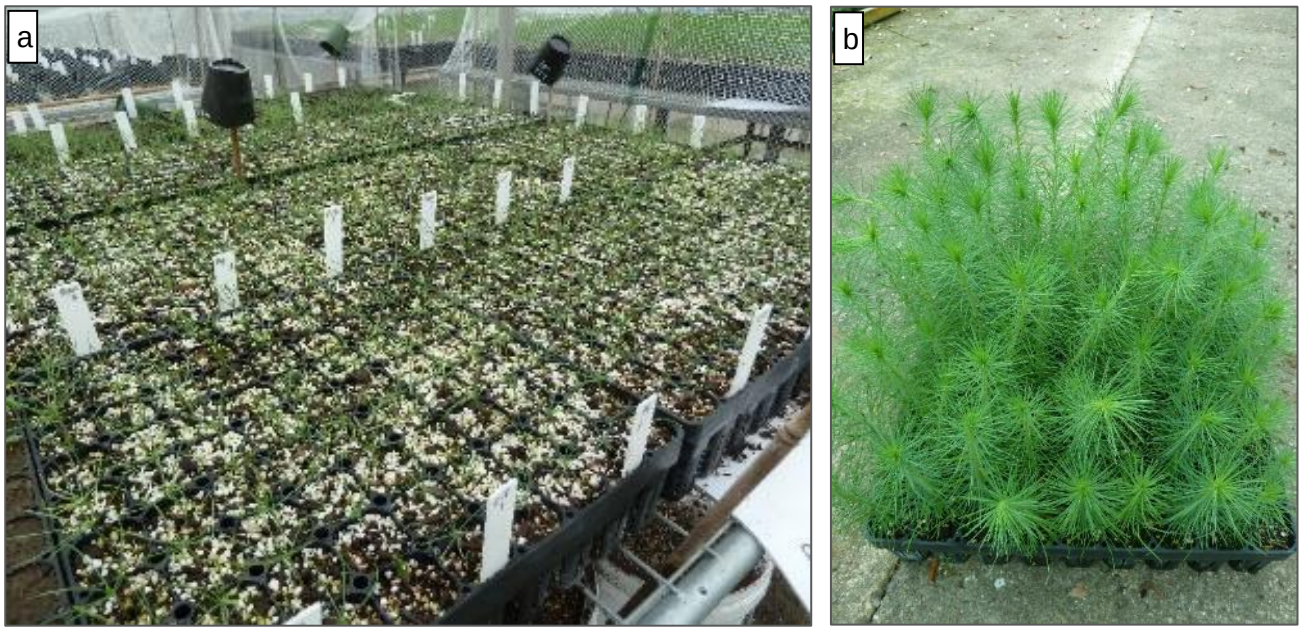

Figure 3: *P. radiata* seedlings (a) 27 days after seeding of agrichemical- and *Trichoderma*-coated seed in potting mixture, and (b) 134 days after seeding immediately prior to aerial fungicide and herbicide applications.

The general recommendation is to avoid mixing fungal biocontrol agents with most fungicide and herbicide products in spray tanks because of potential loss of spore viability (e.g. the Bioworks 2020 guideline). The tolerance of the eight isolates to agrichemicals, as defined in this study, provided a guideline for spray tank mixing, if chosen in nursery and forest operations.

Recommendations for spray tank mixing included:

- PR6 and PR3a mixtures can be mixed with insecticides (including *Bacillus thuringiensis*), fertilisers (except boron), adjuvants, biostimulants and most herbicides (atrazine, clopyralid, haloxyfop-P, hexazinone, hexazinone/terbuthylazine, oxyfluorfen, picloram, simazine, terbuthylazine (AGPRO Terbuthylazine 500) and triclopyr) because spore viability was not affected. However, avoid mixing with glyphosate, propazine and terbuthylazine (in Assett) herbicides.
- PR6 and PR3a mixtures can be mixed with some fungicides (cuprous oxide, iprodione, metalaxyl-M, metalaxyl-M/mancozeb, phosphorous acid, pyrimethanil) but avoid captan, chlorothalonil, copper hydroxide, fluazinam, prochloraz and thiram because of potential fungicidal effects on spore germination.

In this study, *Trichoderma* PR6 established in the radiata pine roots (via spores applied by seed coat or soil drench inoculation) was highly tolerant to the single application of agrichemicals at recommended nursery rates. In the plant assays, there may have been sufficient physical distance between the site of agrichemical application and the developing or established root *Trichoderma* to avoid harm to the fungi. Even in agrichemicals with systemic mode of action in the plant (e.g. Sportak EW, Ridomil Gold SL), including those that inhibit fungal mycelial growth (e.g. Scala, Foschek), there may still have been insufficient contact to cause harm to the fungus. In addition, the growing media may have provided a buffer, even when the agrichemical moved down the profile, and product degradation before seeding may also have reduced the potential negative impacts on *Trichoderma* development.

Trichoderma established in plants may be tolerant to more frequent and/or higher rates of agrichemical applications, than those tested in this study. During BPRC studies high root

colonisation levels in inoculated radiata pine were found at harvest in commercial nurseries that used frequent or high-rate fungicide and herbicide applications during the production cycle. Examples of *Trichoderma* root levels include:

- 49% and 32% - Waiuku Nursery (Whelan 2019a)
- 74% and 45% - Rangiora Nursery (Whelan 2021)
- 40% and 46% - Appletons Tree Nursery (Whelan 2021) and
- 98% and 69% - Leithfield Nursery (Whelan unpublished data)

for stock treated with PR6 and PR3a respectively.

Seed coating was recommended as the most practical, efficient, low cost and socially acceptable method for application of *Trichoderma* in nursery and plantation systems (Whelan 2021b). This study determined that *Trichoderma* spores could be safely applied with four fungicides (captan, iprodione, metalaxyl-M, and thiram), a bird-repellent (methiocarb) and two adherents (paint and polyvinyl acetate (PVA glue)), as part of a seed coat recipe. In addition, the fungicide treatments enhanced root colonisation in the first month of seedling growth, when resident competitive fungal species may have been suppressed by the agrichemical allowing the applied *Trichoderma* to dominate.

The isolates in the PR6 mixture had significantly ($P<0.05$) increased mean spore germination (87.7%) and mycelial growth inhibition (36.3%) compared to the PR3a mixture (87.4% and 39.4% for mean spore germination and mycelial growth inhibition respectively), when analysed using all agrichemical data. However, the difference between mixtures was not considered biologically important and both mixtures were therefore recommended for use as bioprotection agents in nurseries that use agrichemicals.

Future studies of the impact of agrichemicals on *Trichoderma* bioprotection agents could include:

- testing at higher rates, or additional or multiple applications of fungicides or herbicides, particularly in commercial nursery soil beds, to confirm the tolerance of *Trichoderma* applied to seeds or seedlings.
- the characteristics of individual isolates in an inoculant mixture; it is possible that one or two isolates may dominate when colonising roots and therefore respond differently to applied agrichemicals.
- the role of co-formulants, and the synergy between them, in agrichemical formulations and their impact on *Trichoderma* isolates (e.g. the large difference in spore germination 24 hours after agrichemical and spore contact found in the Assett (0%) and AGPRO Terbuthylazine 500 (100%) treatments with the same level of terbuthylazine active ingredient). An understanding of this role in biological systems could lead to safer products with lower toxicities.
- understanding the interaction between bacterial and *Trichoderma* biocontrol organisms, particularly with recent emphasis on improved sustainability and lower agrichemical usage in nurseries.
- development of *Trichoderma* formulations which contain encapsulated or microencapsulated spores and/or mycelium to provide better protection against agrichemicals in spray tanks and reduce the impact of environmental stressors.

Table 1: Summary of the effect of fifty-one agrichemicals at recommended rates on spore germination and mycelial growth of eight *Trichoderma* isolates and colonisation of one *Trichoderma* mixture, PR6, in *P. radiata* seedling roots.

Agrichemical Group	Assay	Enhancement or inhibition (%) effect of agrichemicals on *Trichoderma* [a] (mean for eight isolates in spore germination or mycelial growth, or PR6 mixture application in in vivo experiments)				
		Enhanced	Nil or minimal (0 to 10%)	Fungistatic Activity [b]		Fungicidal Activity [b] (≥99%)
				Some 11-50%	Severe 51-98%	
Insecticides	Spore germination	na	*Bacillus thuringiensis*, chlorpyrifos, cypermethrin, deltamethrin, lambda-cyhalothrin, tau-fluvalinate	-	-	-
	Mycelial growth	lambda-cyhalothrin, tau-fluvalinate	chlorpyrifos, deltamethrin	*Bacillus thuringiensis*, cypermethrin	-	-
	Root colonisation	-	*Bacillus thuringiensis* (1)	-	-	-
Adjuvants, fertilisers and biostimulants	Spore germination	na	Nitrophoska Extra, SuperHume, Trace-It Magnesium, methiocarb, AGPRO Crop Oil, AgriSea Foliar, AgZyme, Cropmaster DAP, Synoil, AGPRO Green Organosilicone, AGPRO Organosilicone, Penatra	-	disodium octaborate tetrahydrate (0.3%)	-
	Mycelial growth	Nitrophoska Extra, Trace-It Magnesium, AGPRO Crop Oil, Synoil	SuperHume, methiocarb, AgriSea Foliar, AgZyme	Cropmaster DAP, AGPRO Green Organosilicone, AGPRO Organosilicone, Penatra	disodium octaborate tetrahydrate (0.3%)	
	Root colonisation	-	disodium octaborate tetrahydrate (0.3%) (1), methiocarb (2)	-	-	-
Fungicide and fumigants	Spore germination	na	Iprodione, metalaxyl-M, metalaxyl-M/mancozeb, phosphorous acid, pyrimethanil	cuprous oxide	captan, chlorothalonil, copper hydroxide, fluazinam [c]	dazomet, metam, prochloraz, thiram,
	Mycelial growth	metalaxyl-M (Apron)	metalaxyl-M/mancozeb (low rate)	fluazinam, iprodione, metalaxyl-M (Ridomil Gold SL), phosphorous acid	captan, chlorothalonil, copper hydroxide, metalaxyl-M/mancozeb (high rate), pyrimethanil, thiram	cuprous oxide, dazomet, metam, prochloraz
	Root colonisation	Captan (2), iprodione/metalaxyl-M (2), metalaxyl-M (Apron) (2), metalaxyl-M/mancozeb (4), thiram (2; in early seedling growth)	captan (3), chlorothalonil (1), copper hydroxide (1), cuprous oxide (1), dazomet (3), fluazinam (1), iprodione (1), metalaxyl-M/mancozeb (4), metam (3), phosphorous acid (1), prochloraz (1), pyrimethanil (1), thiram (3; in older seedlings)	-	-	-

Herbicides	Spore germination	na	atrazine, clopyralid, haloxyfop-P, hexazinone, hexazinone/terbuthylazine, oxyfluorfen, picloram, simazine, terbuthylazine (AGPRO Terbuthylazine 500), triclopyr	-	propazine	glyphosate, terbuthylazine (Assett)
	Mycelial growth	picloram	clopyralid, hexazinone/terbuthylazine, oxyfluorfen	haloxyfop-P, hexazinone, simazine, terbuthylazine (AGPRO Terbuthylazine 500), triclopyr	atrazine, glyphosate	propazine, terbuthylazine (Assett)
	Root colonisation	simazine (3), propazine (4) (in young seedlings)	atrazine (3), clopyralid (1), haloxyfop-P (1), hexazinone (3), hexazinone/terbuthylazine (1), oxyfluorfen (4), propazine (1), simazine (1), terbuthylazine (Assett) (1,3), triclopyr (1)	glyphosate (3), terbuthylazine (AGPRO Terbuthylazine 500) (1)	-	-

na = not applicable because spore germination for all isolates with no agrichemical addition was 100%.

[a] Agrichemicals tested (with application rates detailed in Whelan 2019b) were:

Insecticides: Bactur WDG (*Bacillus thuringiensis sp. kurstaki*), Lorsban 50EC (chlorpyrifos), Ripcord (cypermethrin), Ballistic (deltamethrin), Karate Zeon (lambda-cyhalothrin) and Mavrik Aquaflo (tau-fluvalinate).

Adjuvants, fertilisers and biostimulants: AGPRO Sprayable Boron (disodium octaborate tetrahydrate), Nitrophoska Extra (macro- and micro-nutrients), grochem Trace-It Magnesium (magnesium, nitrogen), Mesurol 200SC (methiocarb), AGPRO Crop Oil (mineral oil), AgriSea Foliar, Ag Concepts AgZyme and Ag Concepts SuperHume (multiple components), Ravensdown Cropmaster DAP (nitrogen, phosphate and sulphur), Orion Agriscience Synoil (paraffinic oil and polyol fatty acid esters), Penatra, AGPRO Green Organosilicone Batch 10187#5 and AGPRO Organosilicone (polyether modified polysiloxane).

Fungicide and fumigants: Fruitfed Captan 80WG (captan), Cavalry 720SC (chlorothalonil), Kocide Opti (copper hydroxide), Nordox 75WG (cuprous oxide), Gem (fluazinam), Rapid 500 (iprodione), Rancona Dimension (ipconazole/metalaxyl-M), Apron (metalaxyl-M), Ridomil Gold SL (metalaxyl-M), Ridomil Gold MZ WG (metalaxyl-M/ mancozeb), Foschek (2kg/ha phosphorous acid), Sportak EW (prochloraz), Scala (pyrimethanil), Thiram (40F thiram), Basamid Granular (dazomet), Fumasol (metam).

Herbicides: Atrazine 900 WG (atrazine), Void (clopyralid), Deal 510 RF (glyphosate), Hurricane and AGPRO Haloxyfop 100 (haloxyfop-P), AGPRO Valzine 500 (hexazinone and terbuthylazine), Viper 90DF (hexazinone), Goal Advanced (oxyfluorfen AGPRO), Picloram 200 (picloram), Gesamil 500FW (propazine), AGPRO Simazine 500 (simazine), Assett and AGPRO Terbuthylazine 500 (terbuthylazine) and AGPRO Triclop (600 triclopyr).

Agrichemicals applied to (1) foliage of seedlings 3.5 months-old, (2) the seed as a seed coat, (3) soil before seeding and (4) soil before seedling emergence

[b] fungistatic and fungicidal were defined as agrichemicals that inhibited (11-98%) or killed (≥99%) respectively.

[c] agrichemicals that delayed spore germination at 24 hours, but levels were ≥ 95% at 48 hours.

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

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

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

Trichoderma bioprotectants had positive effects on the growth and production values of two hard-to-root radiata pine clones in 2017 (Whelan 2018) and 2018 trials (Whelan 2019a) at Te Ngae Nursery, Rotorua. In the 2018 trial, application of five *Trichoderma* bioprotectants mixtures (by soil bed drench at setting) to clonal cuttings significantly ($P<0.05$) increased production values by up to 75% (average of 32%), compared to an untreated control (control minus fungicide; 20%). Production values were derived using two variables: plants with strongly growing roots in 3 or 4 quadrants, and plants with root collar diameters (RCD) > 6.9mm. *Trichoderma* significantly ($P<0.05$) increased the percentage of plants with strong roots in 3 or 4 quarters and with RCD > 6.9mm by 55% and 38% respectively, compared to the control minus fungicide treatment. In addition, one or more *Trichoderma* treatments significantly ($P<0.05$) increased survival and root, shoot and plant dry weight by up to 12%, 10%, 11% and 10% respectively, compared to the control minus fungicide treatment. *Trichoderma* increased survival, growth and production values by a similar amount when compared to another control treatment, control plus fungicide, which represented nursery standard practice. Therefore, cutting survival, growth and production values of hard-to-root clones could be increased by reducing or eliminating fungicides from standard nursery management practice and replacing with one *Trichoderma* application at setting. For reference, the percentage of production level cuttings in the Control + fungicide treatment was similar to that found for these two clones in the overall nursery 2019 production figures. In response to these results, Te Ngae Nursery applied *Trichoderma* soil drench to 13 hectares of cuttings in December 2019 and spores to approximately 8 million seeds in winter 2021.

2.3.2 Forestry plantation validation trials for the two most effective treatments

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

2.3.2.1 Plantation validation trials established in 2018

The two mixtures PR6 and PR3a were applied to seed in a commercial containerised nursery and tested against an untreated control in eight large-scale plantation trials in four important forestry regions in 2018 (Table 2, Figure 4, Whelan 2019a).

Trial tree establishment levels were high, with mean survival of 96.7% (Whelan 2020) and 95.6% (Figure 5a) in year one and two respectively. In year two, survival was significantly ($P<0.05$) higher in the PR3a treatment (96.7%) compared to the control (94.6%, Figure 5a). The impact of *Trichoderma* PR3a on tree survival was particularly evident in the Nelson trials with a difference of 4.5% (significant at $P<0.05$) and 6.2% (not significant at $P<0.05$) between PR3a treatment and the

controls (Table 3). Improved survival with PR3a treatment was also found in a new validation trial in Nelson established in 2020 (see section 2.3.2.2.1) and indicates that this mixture may aid in survival of young plants in cold, frosty sites.

Trichoderma treatments had significantly (P<0.05) increased tree height by 4.5% to 10.8%, compared to the control in six of the eight 2018 trials in year two (Table 3). The largest increases in height due to *Trichoderma* were in the Northland (10.1% and 10.8%), followed by the Bay of Plenty/Waikato (9.6% and 9.7%) and Gisborne (4.5% and 9.6%) trials. In the Nelson Golden Downs trials, the PR6 and PR3a mixtures had no effect on height compared to the controls (Table 3); similar to that found in the year one measurements (Whelan 2020). In the third Golden Downs validation trial (section 2.3.2.2.1) PR6 and PR3a were also ineffective, however, a new *Trichoderma* mixture PBI significantly (P<0.05) increased height by 21% compared to the control.

When analysed over all 2018 trials, *Trichoderma* treatments significantly (P<0.05) increased tree height by 6.2% (average of 160.4cm) compared to the control (151cm; Figure 5b). This result was similar to the significant height increases (mean of 6.7%) found in the *Trichoderma* treatments in the first year of measurements (Whelan 2020).

Trichoderma treatments had significantly (P<0.05) increased tree trunk diameter (either at groundline or 600mm/knee height) ranging from 9.3% to 20.8%, compared to the control, in three of the 2018 trials two years after establishment (Table 3). When analysed over the four trials where trunk diameter was measured, *Trichoderma* treatments significantly (P<0.05) increased trunk diameter by 8.6% and 10.4% in the PR3a and PR6 treatments respectively, compared to the control (Figure 5c).

Trichoderma was found in the tree roots of each trial treatment, with the Gisborne trials appearing to have high natural levels at the time of measurements (Table 4). Generally, *Trichoderma* treatments that generated growth responses had higher levels of root colonisation one and two years after planting, compared to the controls. Therefore, the inoculated isolates appeared to have a more dominant role in the growth response compared to the naturally occurring strains. When analysed over all trials within each year, root colonisation in the *Trichoderma* treatments was 62% (not significant) and 95% (significantly, P<0.05) higher compared to the controls in year one and two respectively (Table 4).

At the year two assessment*, Dothistroma septosporum* was present in five trials (both Bay of Plenty/Waikato and Nelson trials and Gisborne Patunamu) at relatively low levels (<4% mean disease severity) apart from medium levels in the Kaingaroa xPKANG 209/4 trial (Table 5). In the xPKANG 209/4 trial, PR6 and PR3a *Trichoderma* treatments reduced disease severity by 43% and 27% respectively, compared to the control (Figure 5d).

Table 2: Establishment details and trial comments for the 2018 validation *P. radiata* trials.

Region	Company	Forest / Trial Name	Location	Altitude (m asl)	Planting Date	No Replicates	Planting Density and spacing
Bay of Plenty / Waikato	Timberlands	Kaingaroa xPKANG 209/4	-38.559711 176.445696	180	24/07/18	10	925 (4m x 2.7m)
			Spot-mounded, flat, free-draining soils. Fleabane was present at the Year two assessment but caused limited competition. *D. septosporum* was present at moderate levels.				
		Kaingaroa xPKANG 660/2	-38.868971 176.280092	240	23/07/18	10	925 (4m x 2.7m)
			Spot-mounded, flat free-draining soils. Fleabane was present at the Year two assessment but caused limited competition. Blackberry present in the NW side of the trial has not regrown by July 2020. *D. septosporum* was present at low levels.				
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 two assessment gorse, cutty grass (*Gahnia lactera*), pampas grass and rushes had established strongly, particularly in the lower half of the trial. No disease or insect damage were present in Year one or two.				
		Whatoro	-35.708955 173.676602	330-350	08/08/18	10	833 (4m x 3m)
			10km from coast, flat. At the two-year assessment, annual grasses and broadleaf weeds were prevalent with pampas grass beginning to establish. Trees were beginning to outgrow the inkweed (*Phytolacca octandra*) present in three replicates. Socketing observed in the one-year-old trees had generally corrected by August 2020. However, a recent wind event had caused severe leaning of many trees in the four elevated replicates. Needle browning was present due to greenhouse thrip (*Heliothrips haemorrhoidalis*) and *D. septosporum*.				
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. No weed competition observed at the Year two assessment (although fleabane and annual grasses were prevalent). Blackberry, boxthorn, hawthorn and pampas grass were present at low levels. Pigs and goats had damaged approximately 3% of plants. Dothistroma needle blight and insect damage were at low levels (<1%).				
	PFOlsen	Tauwhareparae	-38.198800 178.099317	400-425	11/08/18	10	1000 (3.3m x 3m)
			Variable slope (flat to 10°) and aspect (south, NE and west). At the Year one assessment, 9% of plants had yellow stunted growth or death due to wet soil. At Year two, most of the trees with yellow foliage had recovered but were shorter than the remainder of the trees. Leaning of trees was observed in 7% of plants. Soil slippage was found in five plots, but most trees were measured. Annual grasses and broadleaf weeds were prevalent. No disease or insect damage were present in Year one or two.				
Nelson	OneFortyOne	Golden Downs / Sherry	-41.448233 172.651675	310-380	03/09/18	8	800 (5m x 2.5m)
			West slope (7-10° bottom half and 17-32° top half of trial); replicates placed across the slope. Cold, snow-prone site but more sheltered from winds compared to Berrymans. The bottom 3 replicates had low survival (average 87%) due to frost damage. AGPRO Valzine 500 release spot-spray in November 2018. Ex-Douglas-fir. Weed competition was low at the Year two assessment, apart from a narrow strip of broom in the middle of the trial. *D. septosporum* was present at low levels.				
		Golden Downs / Berrymans	-41.458333 172.90833	450	05/09/18	7	800 (5m x 2.5m)
			South 8° slope with internal gullies; plots placed to avoid these. A range of slash levels but plots arranged to have similar levels in each replicate. Very cold, frosty, wind-exposed site. The bottom 3 replicates had low survival (average 83%) due to frost damage. Lower growth potential compared to Sherry. Weed competition was low at the Year two assessment, although gorse seedlings were beginning to grow. A possible nutrient deficiency (yellow needle tips) was observed in 6% of trees. *D. septosporum* was present at low levels.				

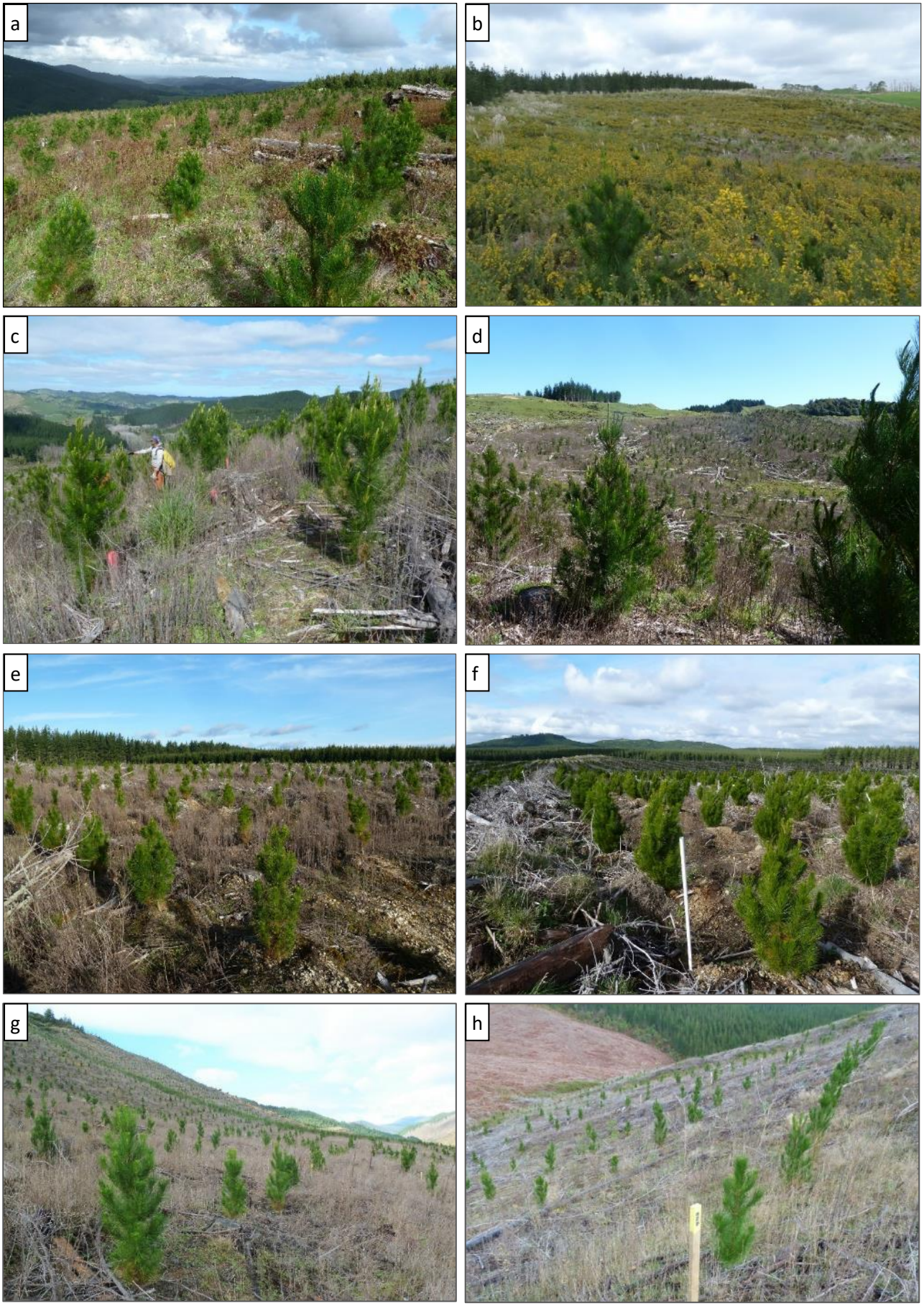

Figure 4: Validation trials a) Whatoro and b) Topuni, Northland, c) Patanamu and d) Tauwhareparae, Gisborne, e) Kaingaroa xPKANG 209/4 and f) xPKANG 660/2, Bay of Plenty/Waikato and g) Sherry and h) Berrymans, Nelson at the Year two assessment in winter 2020.

Table 3: Effect of *Trichoderma* treatments on tree survival (%), height (cm) and trunk diameter (mm) including % difference compared to the untreated controls, in the 2018 validation trials approximately two years after planting.

Region and Site	Treatment	Survival [a]	Height		Trunk diameter [b]	
		%	(cm)	% difference [c]	(mm)	% difference [c]
Bay of Plenty / Waikato xPKANG 209/4	PR6	99.6 (0.4) a	129.1 (1.5) a	9.6	35.2 (0.5) a	9.3
	PR3a	97.2 (0.9) b	122.1 (1.7) b	3.7	32.7 (0.5) b	1.6
	Control	97.2 (1.0) b	117.8 (2.1) b	-	32.2 (0.7) b	-
	LSD (5%)	**2.3**	**4.7**	-	**1.8**	-
Bay of Plenty / Waikato xPKANG 660/2	PR6	99.6 (0.4) a	142.8 (2.0) a	9.7	-	-
	PR3a	99.6 (0.9) a	138.5 (1.9) b	6.4	-	-
	Control	99.6 (0.4) a	130.2 (2.0) c	-	-	-
	LSD (5%)	**1.3**	**4.3**	-	-	-
Northland Topuni	PR6	95.0 (1.2) a	147.2 (5.0) ab	6.0	-	-
	PR3a	97.9 (1.7) a	152.9 (4.9) a	10.1	-	-
	Control	94.5 (2.0) a	138.9 (4.5) b	-	-	-
	LSD (5%)	**5.6**	**13.9**	-	-	-
Northland Whatoro	PR6	97.2 (0.8) a	188.0 (6.2) a	10.8	36.6 (2.2) a	20.8
	PR3a	96.8 (1.0) a	184.2 (4.5) ab	8.6	34.5 (1.4) ab	13.9
	Control	96.0 (0.8) a	169.6 (5.8) b	-	30.3 (1.6) b	-
	LSD (5%)	**2.9**	**17.1**	-	**4.8**	-
Gisborne Patunamu	PR6	95 (1.6) a	195.0 (3.4) a	4.5	36.4 (1.2) a	5.8
	PR3a	96.8 (1.5) a	190.2 (2.5) ab	1.9	35.9 (0.8) a	4.4
	Control	97.3 (2.0) a	186.6 (3.7) b	-	34.4 (1.5) a	-
	LSD (5%)	**4.9**	**6.1**	-	**2.4**	-
Gisborne Tauwhareparae	PR6	94.3 (1.4) a	225.3 (6.6) ab	4.5	42.1 (1.7) ab	7.4
	PR3a	95.2 (2.4) a	236.4 (4.4) a	9.6	44.8 (1.2) a	14.3
	Control	92.2 (2.7) a	215.6 (9.1) b	-	39.2 (2.2) b	-
	LSD (5%)	**7.0**	**18.4**	-	**4.5**	-
Nelson Sherry	PR6	93.5 (2.1) ab	157.3 (4.7) a	2.1	-	-
	PR3a	97.0 (2.0) a	159.3 (5.6) a	3.4	-	-
	Control	92.5 (3.8) b	154.1 (4.8) a	-	-	-
	LSD (5%)	**4.5**	**10.2**	-	-	-
Nelson Berrymans	PR6	91.9 (4.8) a	96.9 (4.5) a	-0.7	-	-
	PR3a	93.1 (2.7) a	100.1 (5.0) a	2.6	-	-
	Control	86.9 (4.0) a	97.6 (5.8) a	-	-	-
	LSD (5%)	**8.2**	**6.6**	-	-	-

[a] Letters were assigned according to a 5%, 1% or 0.1% level unprotected LSD procedure. Two means with the same letter within one year are not significantly (NS) different at P<0.05. Standard error of means in brackets.

[b] Trunk diameter was measured at 600mm (knee-height), apart from a ground-line measurement in the Kaingaroa xPKANG 209/4 trial.

[c] *Trichoderma* treatment measurements expressed as a percentage difference compared to the control within each trial.

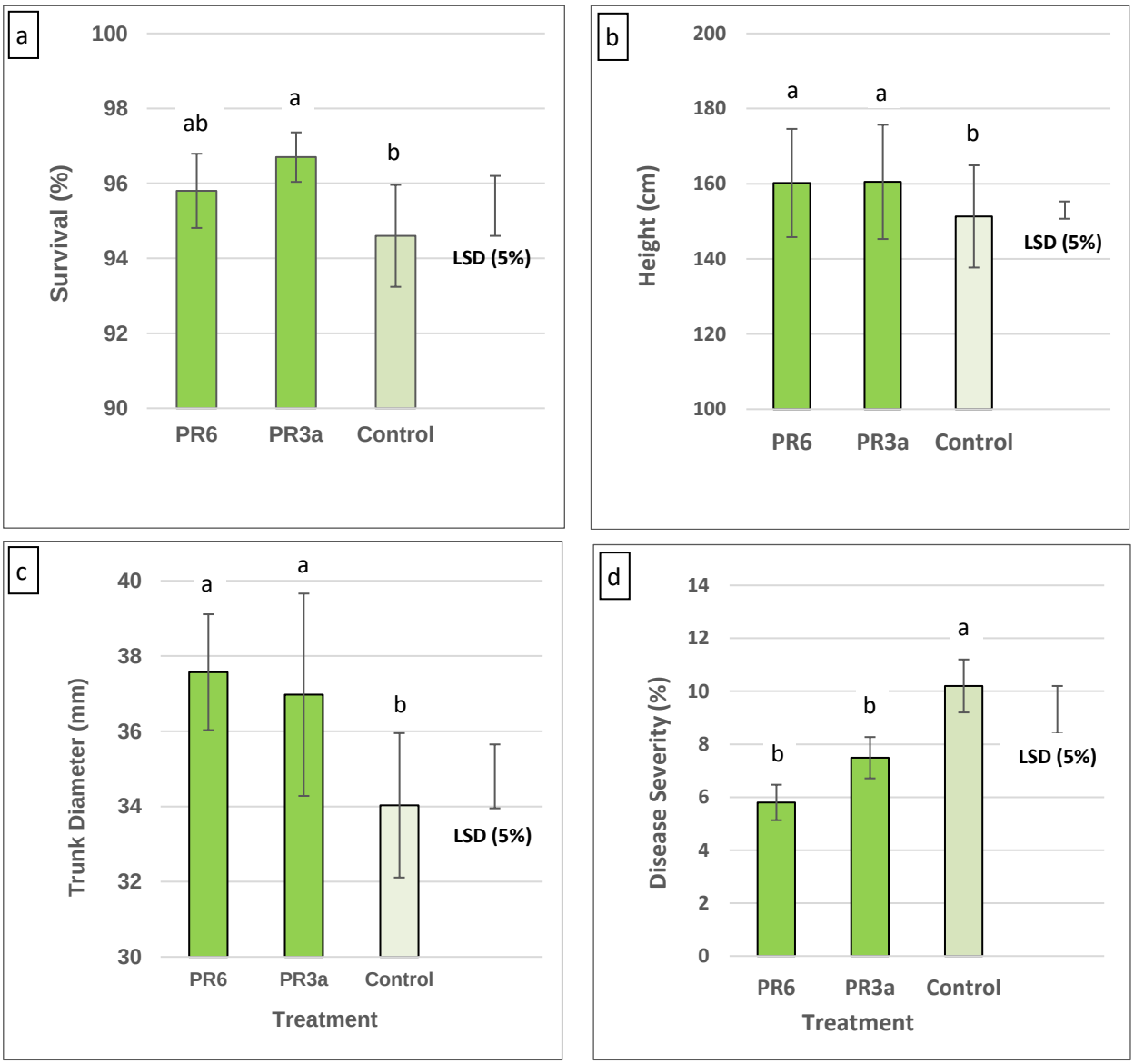

Figure 5: Effect of *Trichoderma* treatments on mean a) tree survival (%), b) height (cm) and c) trunk diameter (mm) for the 2018 validation plantation trials, and d) *Dothistroma septosporum* disease severity (%) in the Bay of Plenty/Waikato xPKANG 209/4 trial, measured approximately two years after planting. Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. Bars = standard error of mean or the LSD at 5% level.

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

Region	Site	*Trichoderma* root colonisation [a]					
		2019 (Year One)			2020 (Year Two)		
		PR6	PR3a	Control	PR6	PR3a	Control
Gisborne	Patunamu	64.3	81.0	51.1	52.8	43.3	21.4
	Tauwhareparae	59.5	92.9	72.2	66.7	61.1	56.7
Bay of Plenty / Waikato	xPKANG 209/4	88.6	36.7	22.2	43.8	29.2	20.4
	xPKANG 660/2	43.5	38.9	13.5	37.8	33.4	12.3
Northland	Topuni	47.5	41.7	16.7	30.0	44.4	33.3
	Whatoro	34.6	70.1	32.3	70.8	33.3	12.5
Nelson	Sherry	30.0	26.7	4.2	33.3	41.7	11.1
	Berrymans	14.0	20.0	6.7	42.3	26.9	26.0
Mean (all trials) [b]		47.8 (8.2) a	51.0 (9.5) a	28.1 (8.7) a	47.2 (5.3) a	39.2 (3.9) a	24.2 (5.4) b
LSD (5%)		**26.5**			**14.4**		

[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 2018).

[b] Letters were assigned according to a 5% level unprotected LSD procedure. Two means with the same letter within one year are not significantly different at P<0.05. Standard error of means in brackets.

Table 5: Effect of *Trichoderma* treatments on disease severity (%) in four of the 2018 validation trials two years after planting.

Region [a]	Site	Disease Severity (%)			
		PR6	PR3a	Control	LSD (5%)
Bay of Plenty / Waikato	xPKANG 209/4	5.8 (0.7) b	7.5 (0.8) b	10.2 (1.0) a	2.1
	xPKANG 660/2	3.3 (0.6) a	3.0 (0.5) a	4.4 (0.7) a	1.5
Nelson	Sherry	1.1 (0.4) b	1.1 (0.3) b	2.6 (0.6) a	0.8
	Berrymans	2.6 (0.8) ab	1.9 (0.5) b	3.7 (0.7) a	1.0
Mean [b]		3.2 (1.0) b	3.4 (1.4) b	5.2 (1.7) a	1.0

[a] Northland Whatoro trial had moderate needle browning (mean severity of 9% in all trees) caused by Dothistroma needle blight and greenhouse thrip (*Heliothrips haemorrhoidalis),* but results were not presented because symptoms were similar.

[b] Letters were assigned according to a 5% level unprotected LSD procedure. Two means with the same letter within one year are not significantly (NS) different at P<0.05. Standard error of means in brackets.

2.3.2.2 Plantation validation trials established in 2020 and 2021

A 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 (section 2.3.2.2.1, Figure 6a and Table 6). Inoculated bare root seedlings (1/0) from Appletons Tree Nursery (Seedlot 19/505) were supplied. Five additional validation trials were established in Canterbury and Otago in 2021 to test the PR6 and PR3a mixtures in cooler, and sometimes drier, locations than that of the 2018 and 2020 trials (Figure 6b to f and Table 6). 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. Trials were 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.

Figure 6: Kings Ridge validation trial in Golden Downs Forest, Nelson at the Year one assessment on 4 October 2021 (a), and newly established trials in Kakahu (b) and Okuku Forests (c), Canterbury, and Dusky Forest (d) Hillend region (e) and Akatore Forest (f), Otago in winter 2021.

Table 6: Establishment details for the 2020 and 2021 validation *P. radiata* trials.

Region	Company	Forest / Trial Name	Location	Altitude (m asl)	Planting Date	No Replicates	Planting Density 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 sprayed. Plots were overlayed over the windrows. Replicates were generally arranged across the slope to minimise variation in soil types and altitude. Average tree height was similar irrespective of the altitude, however, survival decreased down the slope. Weed competition was relatively low at the Year one assessment, however an aerial release spray (1.5L/ha Versatill Powerflo, 0.3L/ha Triumph BK and 0.5L/ha Silmaxx) was applied on 21 Oct 2021 to control the strongly developing broom plants. No disease was present in October 2021.				
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 sprayed with sparse grass and broadleaf weeds at planting. Ex-Douglas-fir site with regenerating seedlings that will require removal. Each plot had a central windrow with 4 trees on either side, and a windrow on each downhill boundary.				
Canterbury	Port Blakely Ltd	Kakahu	-44.128841, 171.067654	320-370	13/07/21	10	800 (5m x 2.5m)
			Trial between Fairlie and Geraldine. Rolling top site with a range of aspects (northerly, easterly- and south-easterly). Potential wallaby damage in the future. A clean, pre-plant sprayed site, although broom seedlings beginning to develop. Plots were placed between windrows.				
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. Flat/rolling top site with grazed short pasture. An herbicide planned to be applied in summer.				
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. Windrows were burnt pre-planting so potential nutrient differences in the future. Large numbers of broom seedlings were emerging at planting. Plots were arranged over the windrows. A pre-plant spray was applied although pasture had regrown by planting. An aerial herbicide of Release KT was applied in November 2021.				
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. Exposed to wind, occasional snow and some frosts. The rolling top, cut-over site was pre-plant sprayed and clean at planting. Plots were placed between windrows. Potential possum damage in the future.				

2.3.2.2.1 OneFortyOne Nelson Kings Ridge 2020 Trial Assessed at Year One (4 October 2021)

Trichoderma PBI mixture had significantly ($P<0.05$) increased tree height by 21%, compared to the control (Figure 7a). Tree height in the PR3a and PR6 mixtures was similar to the control and reinforced the results found in the two 2018 Nelson trials (refer to Table 3). PBI may be a more beneficial mixture than PR6 and PR3a for early tree growth in the Golden Downs region. Survival in the PR3a treatment was 100% and was significantly ($P<0.05$) higher compared to the control

and PR6 treatments (92.4%; Figure 7b). Survival in the PBI treatment was also high (96.5%) but was not significantly different from the other treatments. The bottom row of plots had relatively low survival (average 90%) possibly due to frost damage.

Further measurement of growth, disease and root colonisation will be made in winter 2022 in the fourteen validation trials.

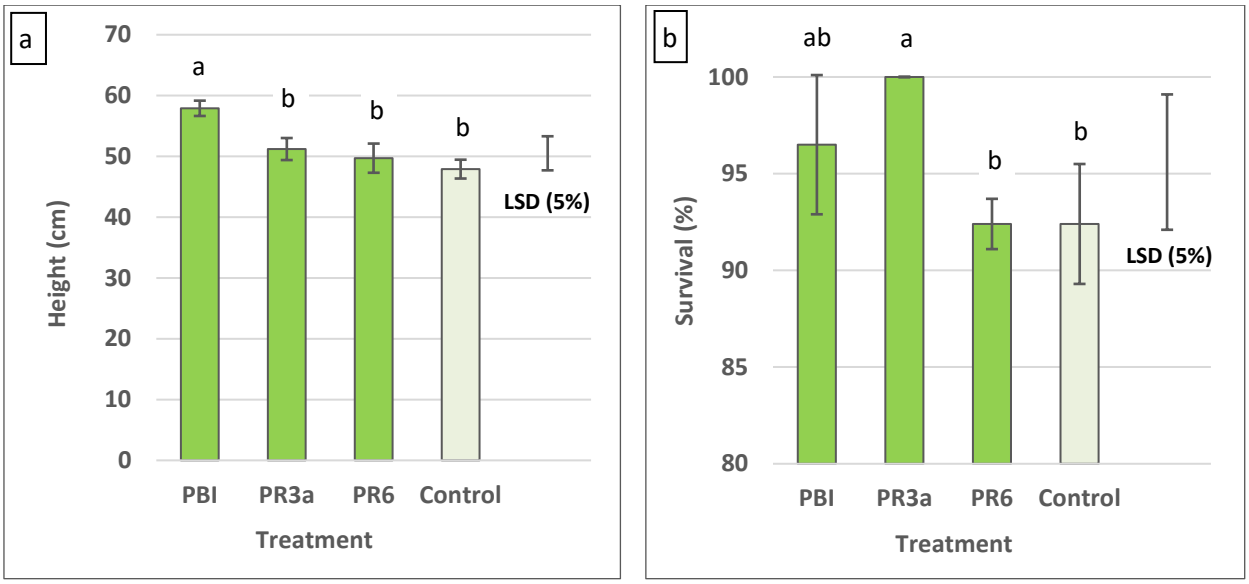

Figure 7: Effect of *Trichoderma* treatments on tree a) height and b) survival one year after planting in the Kings Ridge 2020 trial. Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. Bars = standard error of mean or the LSD at 5% level.

2.3.3 Forestry plantation trials 2012 to 2015:

Between 2012 and 2015, 24 forestry plantation trials were established in radiata pine growing areas throughout the North Island and in Nelson (Hill 2016). The most effective 24 isolates identified in nursery screening trials and disease assays were selected and tested individually or as mixtures in these trials. The final survival, height and health score measurements for all but the Ernslaw One Ltd. Whanganui Harakeke trial were summarised in Whelan (2019a) and Whelan (2020). The last measurement in the Ernslaw One Ltd. Whanganui Harakeke trial was taken on 11 November 2020 (section 2.3.3.1). No more growth measurements will be taken in this series of trials, apart from root samples for persistence of the LU633 isolate, if required.

2.3.3.1 Ernslaw One Ltd. Harakeke 2014 trial (Year 7.5):

Some trees were thinned on the morning of the assessment (Figure 8). Tree height and diameter for the fallen trees were measured using the vertex hypsometer and diameter tape, allowing for the height of the stump.

Figure 8: The Ernslaw One Ltd. Harakeke trial on 11 November 2020.

- All four *Trichoderma* treatments had significantly (P<0.05) increased height by 6.6% (PR2) to 10.5% (PR1), compared to the untreated control (Figure 9a).
- PBI and PR1 treatments had significantly (P<0.05) DBH by 14.2% and 12.4% respectively, compared to the control (Figure 9b). Trunk diameter at breast height was also increased by 12.1% and 11.0% in the PR2 and PR3 treatments respectively but these were not significantly different from the control.
- Tree survival ranged from 92.5% (PR2 and PR3) to 100% (PBI) but was not significantly different from the control (92.5%) due to the large amount of variability.

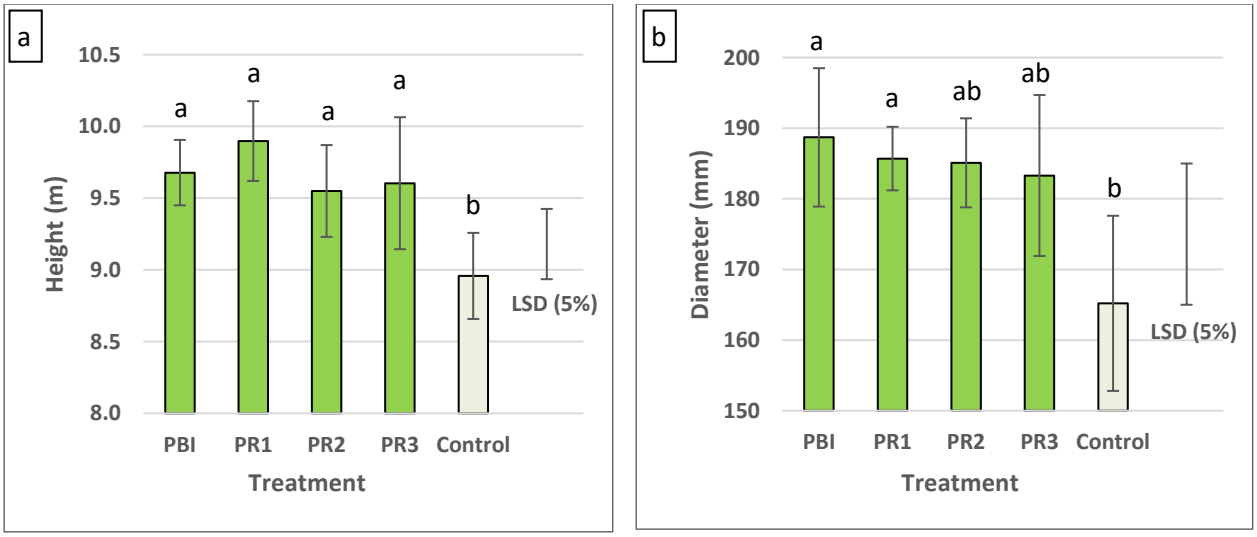

Figure 9: Effect of *Trichoderma* treatments on tree a) height and b) trunk diameter at breast height approximately 7.5 years after planting, in the Ernslaw One Ltd. Harakeke 2014 trial. PBI included LU132, LU140, LU584 and LU633 isolates, PR1 included FCC318, FCC319, FCC320, FCC322 and FCC340, PR2 included FCC362, FCC368, FCC49 and FCC55), PR3 included FCC180, FCC327, FCC275 and FCC161 and untreated control was not treated with *Trichoderma*. Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at P<0.05. Bars = standard error of mean or the LSD at 5% level.

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

In New Zealand, promising results have been obtained in *Trichoderma* plantation trials, with the most effective *Trichoderma* treatments increasing tree height by up to 20% (Hill and Whelan 2016, Hill et al. 2015, Whelan 2019a, Whelan 2020) or trunk diameter by 21% (Table 3) in one- to 7½-year-old stands. Application of *Trichoderma* inoculum was by seed coat in the nursery (this being a practical, effective, low-cost and socially acceptable method for application), however, most of New Zealand's plantation forests are not inoculated with *Trichoderma* bioprotectants.

Three pilot trials in Bay of Plenty/Waikato were set-up to investigate the feasibility of directly inoculating established plantation trees with *Trichoderma* bioprotectants to induce disease suppression and growth benefits. Trial establishment and design were detailed in Whelan (2018) and Whelan (2020).

2.3.4.1 Kawerau (Tarawera Forest) 2017 Trial:

Five *Trichoderma* treatments of PR6 mixture (spore-infused root dowels and spore suspensions applied as a trunk injection, trunk spray and soil drench) were applied to single 23-year-old trees and compared to an untreated control in five replicate blocks. The presence of natural soil *Trichoderma* fungi, before treatment application in November 2017, was estimated at 6%, by sampling small diameter, shallow roots from the five trees allocated treatments in replicate five and another three trees within or near replicate five (Table 7). Root colonisation levels in the control trees remained relatively low in the subsequent three measurement dates (Table 7).

Table 7: *Trichoderma* colonisation (%) of *P. radiata* roots in trees measured prior to, 11, 24 and 38 months after treatment application in the 2017 Kawerau trial.

Treatment	***Trichoderma* root colonisation (%)**			
	30 Nov 2017 (before treatments applied) [a]	**30 Oct 2018 (11 months after treatment) [a]**	**7 November 2019 (24 months after treatment) [b]**	**3 February 2021 (38 months after treatment) [b]**
Treated Trees:				
Trunk Injection	0	18	22.4 (3.7) b	27.0 (2.7) b
Root Dowel	0	25	28.3 (3.6) ab	47.7 (6.7) a
Trunk Spray	4	15	25.8 (5.2) ab	44.0 (5.8) a
Soil Drench	8	25	40.3 (11.4) a	40.7 (2.4) ab
mean of treated trees	**4**	**21**	**28.0**	**39.9**
Untreated Trees:				
Control	4	5	22.5 (3.8) b	26.0 (5.6) b
neighbouring tree	[8]	[5]	[8]	[10]
neighbouring tree	[20]	[13]	[16]	[23]
neighbouring tree	[0]	[13]	[8]	[13]
LSD (5%)	**-**	**-**	**17.0**	**14.7**

[a] root colonisation measured only in replicate five and the surrounding area (approximately 15 random root pieces per tree were sampled, then 40 random root pieces selected for testing).

[b] root colonisation measured in all trees (approximately 10 root pieces per tree, then 24 or 30 random root pieces selected for testing). Letters were assigned according to a 5% level unprotected LSD procedure. Two means with no letters in common differ significantly at $P<0.05$. Standard error of means in brackets.

Trunk diameter at breast height was determined to be influenced by the initial DBH (*ie*: larger trees grew more than smaller trees irrespective of the treatment applied). Therefore, initial DBH was used as a covariate to calculate the increment DBH's in ANCOVA analysis. DBH increased in all five treatment eleven, 24- and 38- months after treatment application (Table 8). The root dowel treatment resulted in 92% and 31% increase (P<0.05) in DBH increment at eleven- and 38- months after treatment compared to the control. These trees also had significantly (P<0.05) higher root colonisation levels at 38-month measurement date (Table 7). However, other treatments with significantly higher root colonisation (soil drench and trunk spray) did not have corresponding increases in growth.

At trial establishment, red needle cast (*Phytophthora pluvialis)* was present at very high levels (mean of 70% disease severity) but no disease was present at the three measurements dates. The growth response in the root dowel treatment may have been due to increased disease resistance, quicker recovery of canopy green tissue, and/or growth enhancement due to the presence of *Trichoderma*. The trial was concluded with stand harvest in mid-2021.

Table 8: Adjusted DBH (mm) increment 11, 24 and 38 months after treatment application in the Kawerau 2017 trial.

Treatment	**Adjusted DBH (mm) Increment [a]**		
	11-month period from Nov 2017 to October 2018	**24-month period from Nov 2017 to Nov 2019**	**38-month period from Nov 2017 to Nov 2019**
Root Dowel	10.2 a	22.7 a	48.0 a
Trunk Injection	7.8 ab	18.9 ab	38.7 ab
Trunk Spray	6.4 ab	16.2 b	36.7 b
Soil Drench	6.1 b	16.8 b	37.1 b
Control	5.3 b	17.6 ab	36.6 b
LSD (5%)	**4.1**	**5.5**	**10.0**

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

2.3.4.2 Kaingaroa Forest 2019 Trial:

The Kaingaroa 2019 trial had similar treatments to Kawerau 2017 except there were two untreated control treatments per replicate, more buffer trees between treatments, six replicate blocks and the trees were younger (11-years old). Root colonisation levels were initially high at trial establishment (average of 40%) but reduced in all treatments twelve and 24 months after treatment application (Table 9). Soil drench and trunk spray treatments had approximately three or four times the colonisation levels of the control twelve months after treatment application (P<0.05, Table 9). By 24 months, root colonisation in the trunk spray was 155% of the control (P<0.05).

Height increment, twelve months after treatment application, was significantly (P<0.05) greater (by 29%) in the trunk injection treatment, compared to the control (Table 10). This growth promotion continued for the next 12 months but was not significantly different to the control. *Trichoderma* treatments had no effect on DBH in the first 12 months, but trunk spray and root dowel treatments were significantly (P<0.05) greater at 24 months (Table 10). In this trial, there was a weak correlation between root colonisation and tree growth (e.g. the large DBH increment and root colonisation levels at 24 months for trunk spray treatment) but it could not be used to explain all the results. *D. septosporum* presence was low (1% disease severity) in both measurement dates, therefore, the effect of *Trichoderma* on disease expression was not determined.

Table 9: *Trichoderma* colonisation (%) of radiata pine tree roots measured prior to, 12- and 24-months after treatment application in the Kaingaroa 2019 trial [a].

Treatment	*Trichoderma* root colonisation (%)		
	21 Feb 2019 (before treatments were applied) [b]	14 Feb 2020 (12 months after treatment) [b]	5 Feb 2021 (24 months after treatment) [b]
Root Dowel	47.7 (4.7) a	16.7 (6.2) abc	26.4 (5.0) ab
Trunk Injection	35.5 (9.4) a	7.6 (5.2) bc	27.4 (14.6) ab
Trunk Spray	40.7 (8.2) a	17.2 (6.3) ab	37.8 (13.6) a
Soil Drench	43.0 (5.9) a	24.4 (8.6) a	28.5 (7.4) ab
Control	35.1 (3.6) a	5.7 (1.5) c	14.8 (2.7) b
LSD (5%) for comparison of double-replicated Control with any other treatment	**15.7**	**12.9**	**20.5**
LSD (5%) for comparison of any two non-Control treatments	**13.4**	**11.0**	**17.7**

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

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

Table 10: Height (m) and DBH (mm) increment 12- and 24- months after treatment application in the Kaingaroa 2019 trial.

Treatment	Height (m) Increment [a]		DBH (mm) Increment [a]	
	12-month period from Feb 2019 to Feb 2020	24-month period from Feb 2019 to Feb 2021	12-month period from Feb 2019 to Feb 2020	24-month period from Feb 2019 to Feb 2021
Root Dowel	1.82 (0.07) ab	2.87 (0.20) a	16.4 (1.4) a	34.2(2.8) a
Trunk Injection	1.97 (0.26) a	2.85 (0.16) a	15.6 (0.9) a	31.1 (1.3) abc
Trunk Spray	1.62 (0.16) ab	2.74 (0.22) a	16.8 (1.6) a	33.0 (2.4) ab
Soil Drench	1.67 (0.07) ab	2.83 (0.09) a	13.8 (1.4) a	28.0 (3.0) bc
Control	1.53 (0.08) b	2.63 (0.11) a	14.1 (1.6) a	27.7 (2.0) c
LSD (5%) for comparison of double-replicated Control with any other treatment	**0.43**	**0.50**	**4.1**	**6.0**
LSD (5%) for comparison of any two non-Control treatments	**0.42**	**0.44**	**3.5**	**5.2**

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

In the Kawerau 2017 and Kaingaroa 2019 trials, all four *Trichoderma* treatments promoted growth at various times, but there was no single treatment that consistently increased growth. The root dowel treatment had the most consistent growth response, possibly due to the direct placement of spores allowing germination and development inside the tree roots. The difficulty of sampling and measuring *Trichoderma* in sufficient amounts in large, inoculated trees, may have led to the weak correlations between root colonisation levels and tree growth in the *Trichoderma* treatments. Spatial variation of *Trichoderma* in the roots may also have contributed (Liang et al. 2021). Sampling of more roots deeper in the soil profile may be required to represent *Trichoderma* levels more accurately in large trees but this is time-consuming, invasive and potentially damaging to the tree.

2.3.4.3 Kinleith Forest 2019 Trial:

Application methods used in the Kawerau 2017, Kaingaroa 2019 and nursery trials are not practical for large-scale application of bioprotectants. Conventional crop management techniques, for example, aerial release herbicide or fungicide disease control sprays, may offer a practical opportunity for bioprotectants to be incorporated. However, effective application methods, inoculum application rates and the ability of the PR6 and PR3a isolates to colonise needles would need to be determined.

A proof-of-concept trial was established in May 2018 at Lincoln University Nursery to determine the ability of seven *Trichoderma* mixtures to colonise radiata pine needles (and subsequently roots) after application to the foliage of six-month-old seedlings. The trial was a RCBD with three replicates of seedlings in 48-cell plastic containers. Spore suspensions (20ml of 1.0 x 10^{7} spores/ml plus 0.1% Pulse penetrant per tray) were sprayed as a fine mist with a hand-atomiser onto the top third portion of seedlings, with the bottom third portion covered with tightly layered facial tissues to avoid the liquid reaching the potting mixture. Trays were covered with plastic bags to maximise humidity around the plants, bags removed after 48-hours, then trays were placed in large flat trays to allow for bottom watering. Needles and roots were sampled from seedlings in the internal rows of the trays ten and 17 days after spraying. Samples were surface sterilised with Virkon (0.1%) for 10 minutes, placed onto malt yeast extract and rose bengal agar plates and *Trichoderma* colonies were counted according to established protocols (Whelan 2018).

Table 11: *Trichoderma* colonisation (%) of needles and roots of six-month old *P. radiata* seedlings sprayed 10 and 127 days after spraying with seven *Trichoderma* treatments in Lincoln University Nursery**.**

Treatment	***Trichoderma* root colonisation (%) [a]**			
	needle		**root**	
	10 days	**17 days**	**10 days**	**17 days**
Mixture A	10.5 (1.6) a	3.1 (1.7) a	23.6 (2.9) a	16.4 (3.3) a
FCC327	0.0 b	1.7 (0.1) ab	20.7 (2.6) a	10.3 (2.1) bc
GenMix	1.9 (1.1) b	1.2 (1.2) ab	9.8 (2.5) b	11.4 (1.4) ab
PR3a	0.0 b	0.0 b	7.1 (1.0) bc	5.5 (1.6) cd
PR6	0.0 b	2.8 (1.7) a	6.1 (0.6) bc	3.6 (0.2) d
ModArb	1.8 (1.1) b	1.8 (1.1) ab	5.4 (2.0) bcd	4.0 (0.8) d
PBI	0.0 b	0.0 b	2.7 (1.4) cd	5.8 (1.2) cd
Control	0.0 b	0.0 b	1.0 (1.0) d	1.1 (0.5) cd
LSD (5%)	**2.1**	**2.5**	**4.9**	**5.2**

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

Five *Trichoderma* treatments (Mixture A, FCC327, GenMix, PR6 and ModArb) had colonised needles seventeen days after spraying, although only two, Mixture A and PR6 were significantly (P<0.05) higher than the unsprayed control (Table 11). Although some *Trichoderma* were successful at colonising the needle tissue, the levels were low, even with experimental conditions designed to encourage colonisation (high spore dose, presence of a penetrant and high humidity and temperature in the first 48 hours for optimal germination of spores). This suggests that these isolates are not well adapted to survive and thrive in needle tissue if sprayed onto the surface. However, five treatments (Mixture A, FCC327, GenMix, PR3a and PR6) had significantly (P<0.05) greater colonisation of roots (levels ranged from 6.1 to 23.6%) suggesting some spores may have traversed inside the plant and commenced development in the roots. This result supports the original selection process that these isolates were strong endophytic root colonisers. Therefore,

aerial application of *Trichoderma* spores may be a feasible method for inoculating *Trichoderma* in roots of established trees. Further experiments should be considered to confirm the ability of these isolates to move from the foliage to the roots.

An additional pilot trial in Kinleith Forest using foliar and ground sprays was established in 2019 to determine if *Trichoderma* could be inoculated into established trees in an efficient and effective manner and generate a growth and disease suppression effect (Figure 10). The trial was a RCBD with five replicates and 20 two-year-old trees per plot (2 adjoining rows of 10 trees each row).

In the Kinleith 2019 trial, *Trichoderma* treatments were sprayed onto or around the trees with a 20-litre knapsack on 11 December 2019 and included:

1. PR6 mixture, applied to foliage (40ml per tree)
2. PR3a mixture, applied to foliage (40ml per tree)
3. PR6 mixture, applied to the ground (80ml per tree in an approximately 1.0m radius around the truck)
4. PR3a mixture, applied to the ground (80ml per tree in an approximately 1.0m radius around the truck)
5. PR6 mixture, applied to the foliage and ground (40ml foliage + 80 ml ground per tree)
6. PR3a mixture, applied to the foliage and ground (40ml foliage + 80 ml ground per tree)
7. an untreated control.

Figure 10: Trees in the Kinleith 2019 trial on 2 February 2021, fourteen months after *Trichoderma* treatment.

Trichoderma spore application rates in the trial (@ 5.0 x 10^{11} spores per hectare) were based on recommended rates for New Zealand *Trichoderma* products Kiwivax (a soil drench for the reduction of PSA (*Pseudomonas syringae pv. actinidiae*) disease in kiwifruit) and Plantmate (a foliar spray for beneficial growth of plants). Water rates for the foliar and ground treatments were originally calculated at recommended rates for forestry aerial herbicide application at 150 litres per hectare (equal to 15ml per tree with foliage covering 1m^2 ground area, or 47ml per tree with a spray radius of 1.0m (3.1m^2) ground area). However, it was decided by the applicator to apply higher water rates of 40ml per tree to allow better coverage of the foliage and 80ml around the base of the trunk to encourage spray flow through the weeds to the ground. The *Trichoderma* spore concentrations per litre were therefore diluted by the increased water rates, but the application rate remained at 5.0 x 10^{11} spores per hectare.

Trichoderma treatments, applied to the foliage, the ground around the trunk or a combination of both, had no significant effect on root colonisation (Table 12), tree height or DBH (Table 13) fourteen months after application. This negative result was most likely caused by an insufficient inoculum dose to cause colonisation of needles or roots, due to either spore degradation by adverse environmental conditions (e.g. insufficient humidity to allow spore germination) or a low initial spore application rate. This trial also had substantially lower water rates per hectare compared to the soil drench or trunk spray treatments in the Kawerau 2017 and Kaingaroa 2019 trials which may have reduced the ability of the spores to be absorbed and develop in the topsoil. The very high natural population of *Trichoderma* in the soil may also have dominated the applied isolates. Additional trials in forests may be needed to determine whether aerial or ground based applications of bioprotectants can successfully inoculate trees.

Table 12: *Trichoderma* colonisation (%) of *P. radiata* tree roots measured prior to and 14-months after treatment application in the Kinleith 2019 trial**.**

Treatments	***Trichoderma* root colonisation (%) [ab]**					
	11 December 2019			**2 February 2021**		
	Foliar	**Ground**	**Foliar + Ground**	**Foliar**	**Ground**	**Foliar + Ground**
PR6 Mixture	44.1 (4.4) a	50.9 (13.6) a	36.8 (10.3) a	71.5 (11.9) a	67.8 (6.1) a	69.8 (4.6) a
PR3a Mixture	47.0 (10.5) a	30.8 (10.6) a	38.7 (5.6) a	77.0 (5.2) a	60.4 (5.5) a	73.6 (10.8) a
Control	26.0 a			60.0 a		
LSD (5%)	**28.1**			**22.2**		

[a] approximately 3 small diameter shallow root pieces from 3 (2019) or 4-6 (2021) trees were sampled, then 24-30 random root pieces were selected for testing.

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

Table 13: Height increment (m) and DBH (mm) 14 months after treatment application in the Kinleith 2019 trial.

Treatments	**Growth Parameters [a]**					
	Height (m) Increment			**DBH (mm)**		
	Foliar	**Ground**	**Foliar + Ground**	**Foliar**	**Ground**	**Foliar + Ground**
PR6 Mixture	3.06 (0.08) a	3.02 (0.09) a	2.97 (0.11) a	71.5 (3.0) a	72.0 (3.4) a	69.6 (6.0) a
PR3a Mixture	3.15 (0.08) a	3.10 (0.07) a	3.13 (0.11) a	74.3 (3.7) a	72.6 (7.0) a	72.4 (2.2) a
Control	3.15 a			72.9 a		
LSD (5%)	**0.29**			**9.3**		

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

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 uninoculated. The most effective way of utilising the inoculum may be development of formulations that contain encapsulated or microencapsulated spores and/or mycelium. These formulation types may increase the viability of *Trichoderma* by providing 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.

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

The ability of *Trichoderma* bioprotectant sprays to colonise new production areas and subsequently improve stock production is being determined in a Timberlands Ltd Te Ngae Nursery trial. Three bioprotectants were sprayed with a tractor-based sprayer onto pasture (Figure 11), the area then cultivated and cuttings were planted in winter 2020. Cuttings will be harvested and growth parameters measured in May 2022.

Figure 11: Application of *Trichoderma* treatments on 31 July 2020 in Timberlands Te Ngae Nursery.

2.4 Milestone 4 – Nursery and forest plantation trials in Cypresses

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

A containerised cypress seedling trial with six *Trichoderma* treatments (applied as a potting mixture drench) was established at Southern Cypresses Nursery in 2017 (Whelan 2018). *Trichoderma* significantly ($P<0.05$) increased RCD and height by 7% and plant dry weight by 12% in one or more treatments, compared to an untreated control. *Trichoderma* had the biggest impact on root dry weight (up to a 27% increase) and resulted in up to a 26% increased root/shoot ratio. Therefore, application of *Trichoderma* to cypress seedlings in nurseries may result in trees with improved establishment in the field, due to increased nutrient and water uptake and less socketing and windthrow, compared to untreated trees.

Due to funding limitations, the following projects have been stopped:

1. *Trichoderma* inoculated plants were distributed to members of the New Zealand Cypress Development Group in 2018 and 2019 and canker infection was to be monitored over time.
2. A pot trial with *Trichoderma* inoculated and uninoculated plants were to be infected with *Seiridium cardinale* in 2022 when the trees were large enough.

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

Douglas-fir (*Pseudotsuga menziesii)* is the second most widely planted forestry plantation crop in New Zealand and can be affected by nursery and plantation foliar diseases, including Swiss needle cast (*Phaeocryptopus gaeumannii).*

Two containerised Douglas-fir seedling trials were established at Lincoln University Nursery in 2017 (Whelan 2018) and 2018 (Whelan 2020). Seedling roots were found to be highly compatible to six *Trichoderma* mixtures, applied as a potting mixture drench, with *Trichoderma* present in 30% to 97% of root pieces sampled twelve months after application. Seedling survival of an Oregon provenance seedlot was significantly ($P<0.05$) increased by 10% (96%) in one *Trichoderma* treatment, compared to the untreated control (87%). *Trichoderma* was also highly beneficial to Oregon seedling growth, with significantly ($P<0.05$) increased RCD, height, root, shoot and plant dry weight by up to 14, 25, 45, 38 and 42% respectively, compared to the control. These results suggest that *Trichoderma* could reduce costs in nurseries by increasing production values and improving establishment of seedlings due to larger initial plant size once planted in the field. The PR6 mixture, selected for the radiata pine validation plantation trials, had very high levels of root colonisation (>80%), survival and growth in the Oregon seedlings. This mixture may be suitable for containerised Douglas-fir nursery production, but further study is required in other genotypes and production systems (e.g. soil beds).

The potential for *Trichoderma* to control Swiss needle cast will be determined in one or two plantation trials in winter 2022. Approximately 3000 *Trichoderma* inoculated and untreated Douglas-fir plants were lined out in an industry nursery on 13 April 2021 for use in these plantation trials.

3.0 COMMERCIALISATION OF *TRICHODERMA* ISOLATES

The two *Trichoderma* mixtures PR6 and PR3a were found to have growth promotion effects in young radiata pine plantations and in radiata pine, Douglas-fir and cypress nursery trials. The business case for commercialisation of a forestry specific *Trichoderma* mixture will be considered by Agrimm Technologies Ltd, Lincoln, Canterbury and other interested stakeholders in 2022.

4.0 PROJECT OUTPUTS (MAY 2020 TO DECEMBER 2021)

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

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5.0 CONCLUSIONS

Research at BPRC continues to improve our understanding of the health and growth benefits of *Trichoderma* bioprotectants in forestry nurseries and plantations. Results from most of the plantation trials established in 2018 confirmed that two *Trichoderma* mixtures (PR6 and PR3a), selected from previous national trials, could significantly increase tree height at one and two years of age. In addition, both mixtures significantly reduced disease severity of *D. septosporum* in the two-year-old trees in one trial, although did not eliminate the presence of disease. The two mixtures being evaluated in the plantation 2018 trials have also performed well in the nursery trials. This improved the likelihood of successful adoption in both production systems. Six additional trials in cold and/or dry sites in the South Island will further evaluate these two mixtures as bioprotection agents.

To be contenders for commercial consideration in nurseries that use agrichemicals, the isolates need to be either tolerant, or have formulations that enable low sensitivity to agrichemicals. The tolerance of *Trichoderma* PR6 isolates established in roots of containerised radiata pine seedlings, to a single application of forestry agrichemicals at recommended rates, was found to be high. In addition, in some experiments (e.g. when spores were applied with fungicides as part of a seed coat recipe) root colonisation was enhanced in the first month of growth when resident competitive fungal species may have been suppressed by the fungicides, allowing the applied *Trichoderma* to dominate. This result was important as seed coating is the most practical, efficient, low cost and socially acceptable method for application of *Trichoderma* in nursery and plantation systems. Also, this indicates that the PR6 mixture, when established in plant roots, is suitable for use in nursery systems that use agrichemicals. In laboratory studies with direct contact of agrichemicals and PR6 and PR3a mixture isolates, there was a range of effects (from nil to full inhibition) on spore germination, colony mycelial growth and sporulation. Therefore, consideration should be made of agrichemicals and bioprotectants combinations in spray tanks to maximise the viability of the spores.

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

6.0 PROPOSED FUTURE RESEARCH

Priorities for research in 2022 most likely to lead to beneficial outcomes for the forestry industry were approved in December 2021. Additional tasks include determining the effect of inoculant *Trichoderma* on the nutritional status of tree foliage and the effect on variation in tree size and whether *Trichoderma* could be used as a management tool to improve stand uniformity. Funding for investigating the business case for commercialisation of a specific *Trichoderma* mixture for forestry is also being sought.

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