Bio-Protection
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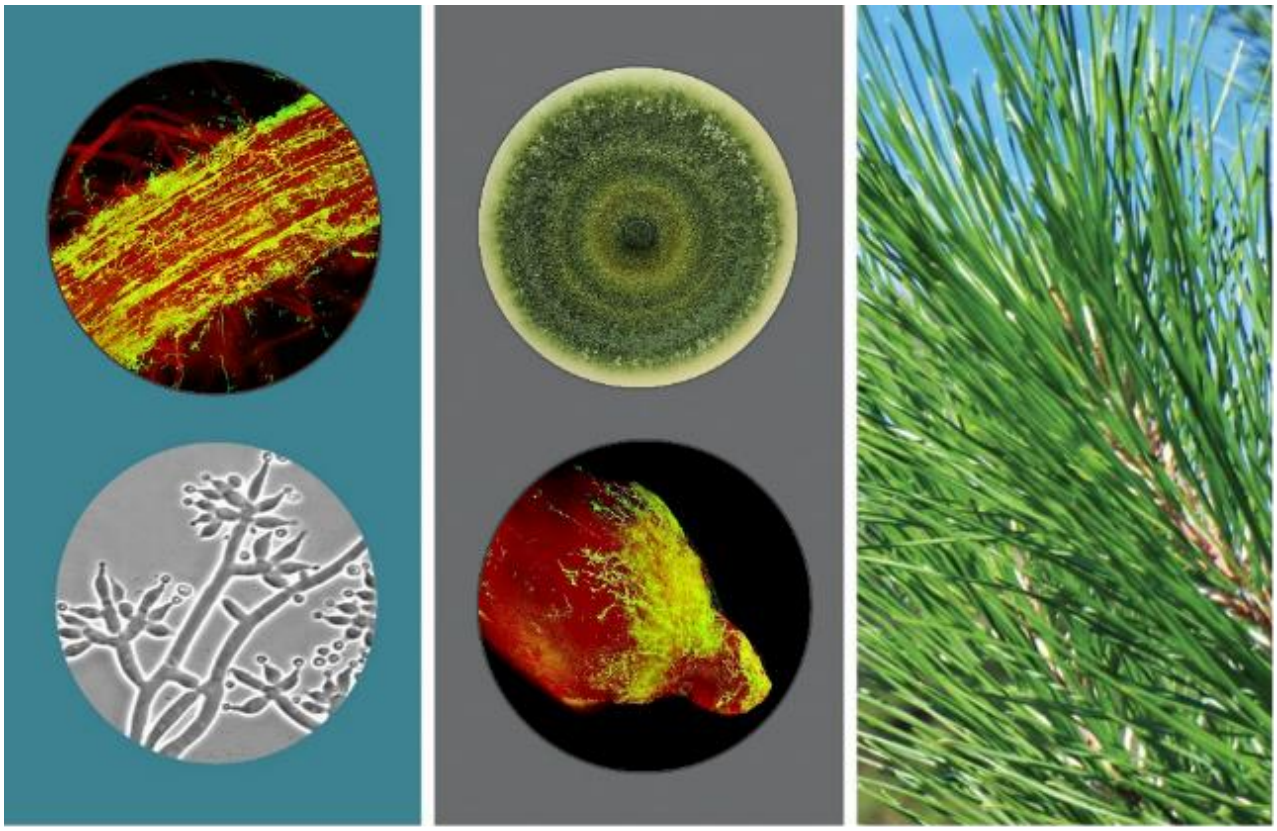
Tolerance of *Trichoderma* isolates to Forestry Agrichemicals

Report prepared for New Zealand Forest Growers Research



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FOREST GROWERS RESEARCH

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1.0 EXECUTIVE SUMMARY

Two *Trichoderma* isolate mixtures PR6 and PR3a show promise to enhance tree growth and suppress foliar disease in New Zealand's most important forest species, radiata pine. Availability of one or both mixtures may lead to increased productivity, sustainability, and economic gain in the New Zealand forest industry. The successful integration of these biocontrol organisms into radiata pine production systems requires knowledge of their compatibility with commonly used pesticides and other products.

The sensitivity of the eight isolates that comprise the PR6 and PR3a mixtures was determined in the presence 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 New Zealand forestry. Sensitivity of these isolates to agrichemicals was determined by *in-vitro* laboratory studies (spore germination, colony mycelial growth and sporulation) and *in-vivo* greenhouse studies using containerised radiata pine seedlings.

Agrichemicals had a range of effects on isolate development in laboratory studies (Table 1). However, the sensitivity of *Trichoderma* PR6 established in radiata pine roots, to the single application of agrichemicals at recommended rates, was minimal or nil (Table 1). In some experiments, root colonisation in seedlings was enhanced in the first month of growth when resident competitive fungal species may have been suppressed allowing the applied *Trichoderma* to dominate.

Recommendations from this study include:

- avoid tank mixing of PR6 or PR3a isolates and some fungicides (in particular, captan, chlorothalonil, copper hydroxide, fluazinam, prochloraz and thiram) or boron (disodium octaborate tetrahydrate) because of potential fungicidal effects on spore germination.
- herbicides (except glyphosate, propazine and terbuthylazine in Assett) could be tank mixed with PR6 and PR3a isolates because spore viability was not affected.
- soil- or foliar-applied fungicides and herbicides could be applied to seeds or plants with inoculant or established *Trichoderma.*
- use seed coat technology to safely apply *Trichoderma,* fungicides (captan, iprodione, metalaxyl-M, and thiram) and bird-repellent, methiocarb. Seed coating is also the most practical, efficient, low cost and socially acceptable method for application of *Trichoderma* in the nursery and plantation.
- tank mixing of PR6 or PR3a isolates and insecticides, including *Bacillus thuringiensis* was appropriate. A single application of *Bacillus thuringiensis* applied to seedling foliage was also safe to inoculated *Trichoderma* in the roots.
- care is required when using biocontrol agents in the first two months after application of soil sterilant products if the active ingredients have not fully dissipated.
- although the PR6 mixture isolates were statistically (significant at $P<0.05$) more tolerant than those in PR3a to the agrichemical tested, the difference between mixtures was not large. Both mixtures are therefore recommended for use as growth promotant and biocontrol agents in nurseries that use agrichemicals.

Further studies could involve:

- testing more, or multiple applications of fungicides or herbicides, particularly in commercial nursery seed beds, to confirm that root *Trichoderma* establishment or

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 radiata pine seedling roots.

Agrichemical Group	Assay	Enhancement or inhibition (%) effect of agrichemicals on *Trichoderma* (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 [a]		Fungicidal Activity [a] (≥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 [b]	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	metalaxyl-M, metalaxyl-M/mancozeb, phosphorous acid, pyrimethanil		captan, chlorothalonil, copper hydroxide, cuprous oxide, fluazinam, iprodione [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] fungistatic and fungicidal were defined as agrichemicals that inhibited (11-98%) or killed (≥99%) respectively.

[b] for brevity, adjuvants, fertilisers and biostimulants were generally referred to as the product brand name, rather than the active ingredients.

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

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

persistence is not affected.

- the role of individual isolates in an inoculant, and their potential characteristics (*eg.* dominance) when colonising roots and therefore their different sensitivities to agrichemicals could be important.
- knowledge of the role of co-formulants, and the synergy between them, in agrichemical formulations, could lead to safer products with lower toxicities when applied to biocontrol agents.
- understanding interactions between pesticides (combinations, timing and rates) and biocontrol organisms may lead to lower chemical usage in nurseries.
- development of *Trichoderma* formulations that contain encapsulated or microencapsulated spores and/or mycelium to provide better protection against agrichemicals in spray tanks and increase the viability of *Trichoderma*.

2.0 INTRODUCTION

Foliar diseases and disorders are the most significant cause of economic loss for the New Zealand forestry industry and cost 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, and other forestry species. Availability of *Trichoderma* biocontrol agents may contribute to healthier forests with fast growth, potential protection against biosecurity incursions, improved industry sustainability and ultimately economic gains for the New Zealand forestry industry.

Results from eight plantation trials in four important NZ forestry regions, demonstrated that two *Trichoderma* mixtures (PR6 and PR3a) significantly increased tree height (mean of 6%) and trunk diameter (mean of 10%) at two years of age, compared to untreated trees (Whelan 2020b). In addition, *Trichoderma* PR6 mixture significantly reduced disease severity of *Dothistroma septosporum* by 45%, compared to untreated trees, in one trial. Benefits of one or both of these two *Trichoderma* mixtures to nursery production were also demonstrated in recent trials, including a 49% significant increase in radiata pine cuttings suitable for plantation planting, a significant increase in plant survival (by 7%) and dry weight (by 42%) of Douglas-fir seedlings and a significant increase (17%) in root dry weight of cypress plants (Whelan, 2020a). These mixtures may be good contenders as biocontrol agents for potential commercialisation for use in the New Zealand forestry industry.

Agrichemicals are an important component in the management of diseases, weeds and insects in many New Zealand forestry nurseries and forests (Reglinski *et. al*., 2009, Rolando *et al.,* 2011). Recently there has been emphasise in New Zealand forest production to minimise environmental impact 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 require knowledge of their compatibility with commonly used pesticides and other products.

In this report, the sensitivity of the *Trichoderma* PR6 and PR3a isolates to 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 was determined. Sensitivity of these isolates to agrichemicals was determined by in-vitro laboratory studies (spore germination, colony mycelial growth and sporulation) and in-vivo greenhouse studies using containerised radiata pine seedlings.

3.0 METHODS

The sensitivity of eight *Trichoderma* isolates to 51 agrichemicals was determined using *in vitro* (spore germination, poison food and sporulation) and *in-vivo* methods**.**

Isolates tested were used in the 2018 New Zealand validation plantation trials (Whelan, 2019 and 2020a,b) and included:

- FCC55 *(T. harzianum)*
- FCC318 *(T. atrobrunneum)*
- FCC327 *(T. harzianum)* and
- FCC340 *(T. harzianum),* that comprise the PR6 mixture, and

- FCC13 *(T. asperellum)*
- FCC14 *(T. atroviride)*
- FCC15 *(T. atroviride)* and
- FCC180 *(T. crassum),* that comprise the PR3a mixture.

Agrichemicals tested were 14 fungicides, 2 fumigants, 14 herbicides, 6 insecticides, 4 fertilisers, 5 adjuvants, 2 adherents, 3 biostimulants and 1 bird repellent. Their product name, registration status in forestry, chemical group, rate of active or principle ingredient in the product, rate of product application, mode of action, recommended timing and frequency of spraying, diseases, weeds or pests controlled and rate used in experimentation are listed in Table 2. Agrichemicals included those currently recommended for forestry nursery and plantation use (NZ Novachem Agrichemical Manual 2020/2021), those used "off-label" at Timberlands Te Ngae and PF Olsens Waiuku Nurseries, and those made available by various New Zealand chemical companies for experimental use in this study (see section 6.0). For brevity, adjuvants, fertilisers and biostimulants are generally referred to as the product brand name, rather than the active ingredients.

Due to the large number of isolate x agrichemical combinations (x408), laboratory testing was conducted and analysed in four groups: insecticide, adjuvant/fertiliser/biostimulant, fungicide/fumigant and herbicide agrichemicals.

3.1 Spore Germination

Agrichemical stock solutions at twice the recommended rate (Table 2) were prepared by dissolving the agrichemical in sterilised distilled water in Schott bottles. Stock spore suspensions (2×10^6 spores/ml) of each isolate were mixed in cold sterilised potato dextrose broth (24g/l) in Schott bottles and chilled at 2°C until required. Aliquots (0.5ml) of the two stock solutions were pipetted to 3 replicate 1.7ml microcentrifuge tubes, wrapped in foil and placed in a rotating oven (Stuart Hybridisation oven/shaker SI30H) at 25°C. Tubes were set-up at staggered times during the day to allow for completion of spore counting at the appropriate time. Percentage spore germination, defined as the number of spores with germ tubes ≥ 2 times the diameter of the spore out of 100 spores, was determined at 24 and 48 hours using an Olympus BX51 microscope. Tubes with spore suspensions diluted with 0.5ml sterile distilled water but no agrichemicals served as a control.

3.2 Mycelial Growth

The poison plate assays were carried out in petri dishes (90mm diameter) following *Dhingra and Sinclair's* (1985) method. Agrichemical solutions at recommended rates (Table 2) were prepared by dissolving the required quantities of agrichemicals in sterilised molten malt yeast extract (MYE; Appendix A) cooled to 55°C. Approximately 20ml of the agrichemical-amended medium was poured into sterilized plastic petri dishes and allowed to set. Three replicate agrichemical-amended dishes were inoculated with single, inverted 6mm diameter agar plugs (from the margin of 5-days old stock cultures) placed in the centre of the petri dishes. Petri dishes were wrapped with gladwrap and placed in a climate-controlled growth cabinet (Contherm Biocell 1000, Contherm Scientific Ltd, New Zealand) set to 25°C and 12 hours light (1350Lm) and dark conditions. Petri dishes containing isolate plugs and no amended agrichemical were used as negative controls. Experiments were carried out in a randomised complete block design (RCBD). After 68 hours, the colony diameter was measured in two perpendicular directions with a ruler and mean radial growth rate per day (mm.day-1) was calculated allowing for the initial radius of the plug. The growth inhibition percent of treatments compared to negative control was calculated by the following formula: Inhibition (%) = [(X-Y)/X] x 100: where X and Y are the radial growth rate (mm/day) of isolate in the control and agrichemical-amended petri dishes, respectively (Sundar *et. al.* 1995).

3.3 Sporulation of *Trichoderma* cultures

Sporulation of the *Trichoderma* cultures was observed (present or absent) 68 and 130 hours after placing the inoculated petri dishes in the growth cabinet.

3.4 *Trichoderma* root colonisation studies

Agrichemicals that resulted in greater than approximately 40% inhibition of mean spore germination and/or mycelial growth were selected for further testing in greenhouse studies. These studies were designed to replicate nursery or field agrichemical use, within the time available for this study.

Stratified, unsterilised *Pinus radiata* seeds (2011BF, sourced from PF Olsen Seeds, Seddon, New Zealand) were sown in plastic trays (BCC Sweden) at various times between June and October 2020 (refer to Table 3). Each tray had 63 or 81 cells with a volume of 100ml per cell (39mm diameter and 85mm depth) and contained either unsterilised potting mix (ratio of: 60L composted bark, 30L peat, 30L perlite medium grade, 360g Osmocote Exact (16-3.9-10, 3-4 month), 480g gypsum, 180g dolomite and 120g Hydroflo wetting agent) or unsterilised sieved soil (Wakanui silt loam). Seeds were inoculated with the *Trichoderma* PR6 mixture spore suspension by seed coat (1×10^6 spores/seed; method in section 3.4.4) or overhead drench (approximately 5ml of 1×10^6 spores/ml per cell; method in Whelan and Hill, 2017). Greenhouse experiments with *Trichoderma* applied as individual isolates was not attempted due to the large amount of resources that would have been required. Control treatments were trays with *Trichoderma* inoculated seeds but no agrichemicals (Control + *Trichoderma*) or no *Trichoderma* or agrichemicals (Control – *Trichoderma*).

Agrichemical solutions at recommended rates (see Table 2) were applied once (refer to Table 3 for dates) to full, half or a third of three replicate trays (depending on the experiment) by seed coat or drench using 500ml atomiser sprayers (for application to bare growing media) or a 5l Solo garden sprayer (for application over seedlings). Individual atomiser sprayers were used and the 5l sprayer

was triple washed between agrichemical applications and followed the order of fertiliser, insecticide, fungicide, and herbicide, where appropriate. The amount of spray required per treatment (at a standardised water rate of 300l per ha for herbicides and recommended water rates for fungicides, fertilisers and the insecticide) was calculated on an area basis and confirmed by weight of spray before and after treatment application for the atomisers. Foliar sprays were applied to the seedlings to the wet but no run-off stage for the fungicide/insecticide/fertiliser treatments and to the run-off stage for the herbicide treatments. The spray treatments applied to bare growing media were to full coverage. Two stiff plastic sheets were used to separate the sprayed section from the unsprayed section during spraying of each tray. No phytotoxicity symptoms were observed apart from in the triclopyr treatment (Experiment 8) where seedling tips were stunted and necrosed within one week of spraying with new tips produced in late December 2020.

3.4.1 Experiment 1: Fumigants

Moist soil (200mm depth) was placed into three 35 litre plastic bins (0.22m^2 soil surface) with drainage holes on 3 July 2020. Dazomet (Basamid Granular at 8.8g, being a rate of 40g/m^2) was mixed into the soil of one bin and the bin was sealed with a polythene sheet. The sheet was removed after 10 days, soil watered, then the sheet replaced until 14 days later when the sheet was removed, and soil mixed again. Metam (Fumasol at 8.8ml mixed in 8.8ml tap water, being a rate of 40ml/m^2) was pipetted into pencil holes 50mm apart in the soil and the bin was sealed with a polythene sheet. The sheet was removed, soil mixed, and sheet replaced 7, 14 and 21 days after fumigant application. In addition, the soil was watered at day 14. One bin with no fumigant addition was covered with a polythene sheet, watered at day 14 and served as the control. At five weeks after fumigant application soil from each bin was transferred into trays, placed in a greenhouse, and planted with *Trichoderma* coated seeds on 9 August 2020 (see section 3.4.4). Seedlings emerged on 25 August 2020 and were harvested 13 and 60 days later, on 7 September and 24 October 2020.

3.4.2 Experiment 2: Fungicide soil drench before sowing or before seedling emergence

Captan and thiram were applied on 17 July 2020 to trays containing soil, seven days before seeding and *Trichoderma* drench application (24 July 2020), whilst metalaxyl-M/mancozeb and sufficient water to wash it to the bottom of the cell) was applied on 4 August 2020, 19 days after seeding and *Trichoderma* drench application (16 July 2020). Seedlings emerged on 13 August 2020 and were harvested 11 (24 August 2020) and 75 (27 October 2020) days after captan and thiram application. Seedlings emerged on 6 August 2020 and were harvested 11 (17 August 2020) and 82 (27 October 2020) days after metalaxyl-M/mancozeb application.

3.4.3 Experiment 3: Fungicide, fertiliser and insecticide foliar application

Seeds were planted and *Trichoderma* drench was applied to trays containing potting mix on 19 June 2020. Seedlings emerged on 20 July 2020. *Bacillus thuringiensis,* chlorothalonil, copper hydroxide, cuprous oxide, disodium octaborate tetrahydrate (1 and 3g/l), fluazinam, iprodione, phosphorous acid, prochloraz and pyrimethanil were applied 110 days after emergence when the seedlings were approximately 150 to 250mm tall. Seedlings were harvested 11 days later, on 18 November 2020.

3.4.4 Experiment 4: Seed coat treatment

Unsterilised seeds (100g lots) were coated separately with nine treatments on 9 August 2020:

1. ipconazole/metalaxyl-M (Rancona Dimension at 0.32ml)
2. captan (Fruitfed Captan 80WP at 0.1g)
3. thiram (Thiram 40F at 0.16g)
4. metalaxyl-M (Apron at 0.075ml)
5. methiocarb (Mesurol 200SC at 1.5ml)
6. White paint (Dulux Weathershield) adherent (0.2ml)
7. PVA glue (polyvinyl acetate; Bic White Glue) adherent (0.2ml)
8. Control (*Trichoderma* and no agrichemical)
9. Control (no *Trichoderma* or agrichemical)

PR6 mixture *Trichoderma* spores (0.0417g, being 2.5 x 10^9 spores equal to 1x10^6 spores/seed) and sterile distilled water (0.2ml) were added to nine sterile plastic 200ml vials and mixed with a vortex mixer (Labnet Vortex Mixer VX-200). Seed was added to each vial and mixed with the spore suspension using the vortex mixer, to coat evenly.

Stock solutions of PVA glue and paint were made by hand-mixing 2ml of each product with 3ml sterile distilled water in a 15ml microcentrifuge tube. The agrichemicals were weighed and added to individual 2ml microcentrifuge tubes. Sterile distilled water (0.3ml) was added to each microcentrifuge tube, apart from the methiocarb tube, and mixed using a vortex mixer. The total volume of the agrichemicals and 0.5ml of the adherent solutions were pipetted out of the tubes and put into the vials containing the seed and spores, while continuing to mix to coat the seed evenly. Treatment 8 received spores only and no agrichemical, whilst Treatment 9 received no spores or agrichemicals. Seeds were dried in sterile petri dishes, subsampled, and sown in trays containing potting mix on 9 August 2020. Seedlings emerged on 24 August 2020 and were harvested on 5 September and 24 October 2020.

3.4.5 Experiment 5: Herbicide ground preparation

Atrazine, glyphosate, hexazinone and terbuthylazine (Assett, 6.7ml/l) were applied to trays containing soil on 16 July 2020, 78 days before seeding and *Trichoderma* drench application (2 October 2020), whilst simazine was applied to bare soil on 24 September 2020, 8 days before seeding. Seedlings emerged on 15 October 2020 and were harvested on 22 October 2020.

3.4.6 Experiment 6: Herbicide soil drench 4 days after sowing

Oxyfluorfen and propazine were applied to trays of bare soil on 20 July 2020, four days after seeding and *Trichoderma* drench application on 16 July 2020. Seedlings emerged on 6 August 2020 and were harvested on 17 August and 24 October 2020.

3.4.7 Experiment 7: Herbicide aerial application (potting mix)

Seeds were planted and *Trichoderma* drench applied to trays containing potting mix on 26 June 2020. Seedlings emerged on 20 July 2020. Clopyralid (6.7ml/l), haloxyfop-P (AGPRO Haloxyfop-P), hexazinone and terbuthylazine (1.67 and 16.7ml/l), propazine, simazine and terbuthylazine (Assett, 6.7ml/l) were applied on 7 November 2020, 110 days after emergence when the seedlings were approximately 150 to 250mm tall. Seedlings were harvested 11 days later, on 18 November 2020.

3.4.8 Experiment 8: Herbicide aerial application (soil)

Seeds were planted and *Trichoderma* drench applied to trays containing soil on 26 June 2020. Seedlings emerged on 26 July 2020. Haloxyfop-P (AGPRO Haloxyfop-P), simazine, terbuthylazine (AGPRO Terbuthylazine, 6.7ml/l), terbuthylazine (Assett, 6.7ml/l) and triclopyr were applied on 7 November 2020, 104 days after emergence when the seedlings were approximately 100 to 200mm tall. Seedlings were harvested 11 days later, on 18 November 2020.

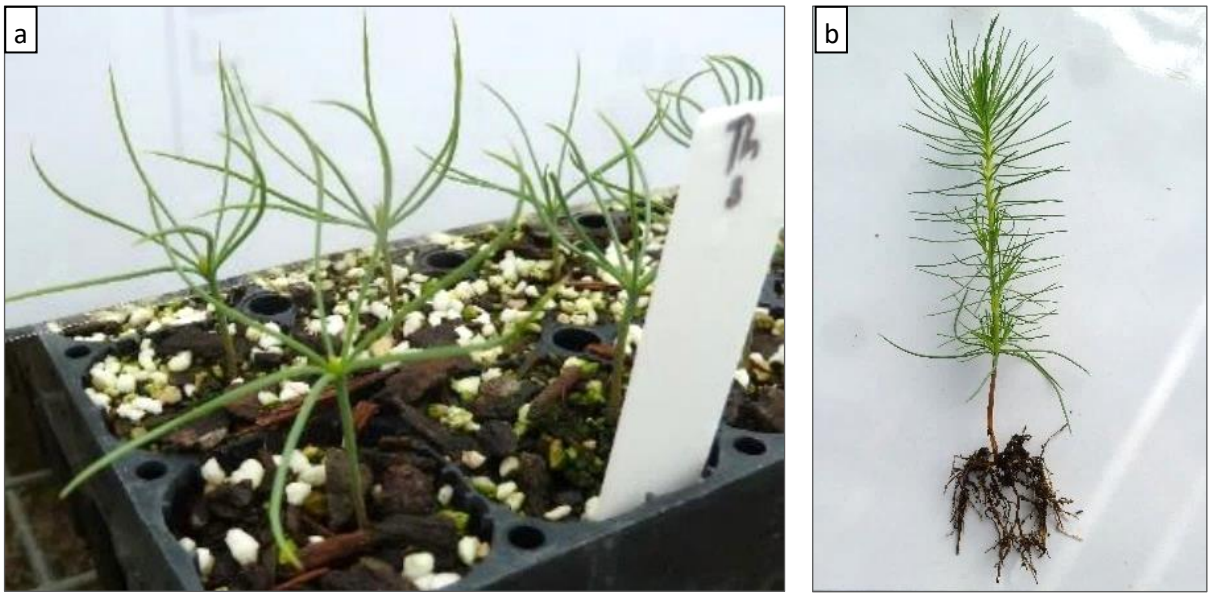

Figure 1: Seedlings a) ten days after emergence from potting mix and b) 145 days after emergence from soil media.

In each experiment, twelve seedlings were harvested 7 to 12 days (Figure 1a) and sometimes 60 to 145 days (Figure 1b) after emergence (see sections 3.4.1 to 3.4.8 for dates), to measure *Trichoderma* colonisation and persistence respectively. Seedlings were sampled from rows that were not next to another treatment. Roots were washed and between 36 to 48 10mm pieces were sampled avoiding the very immature sections of roots. Root pieces were surface sterilised with Virkon (1%w/v) for 10 minutes and placed on petri dishes containing *Trichoderma* isolation malt yeast extract agar with rose bengal (MRB; Appendix A). Dishes were incubated on a laboratory bench top with ambient light and temperature conditions for 14 days. The total number of *Trichoderma* colonies was counted, and percentage root colonisation calculated.

Trays for individual experiments were arranged in a RCBD in a greenhouse, then transferred to a shade house for Experiments 3, 7 and 8, at Lincoln University. Trays were hand-watered to maintain moistness.

In the greenhouse experiments, the uninoculated control seedlings (Control – *Trichoderma* treatment) had low levels (mean of 7.8% for potting mix and 6.4% for soil media) of *Trichoderma* from environmental sources (*eg*. from the growing media and/or airborne spores in the nursery), therefore these sources only had a minimal impact on the results.

Data for each agrichemical group or greenhouse experiment were analysed for significance by analysis of variance (ANOVA) and a least significant difference (LSD) test (GenStat, v20). Differences in sensitivity between the mean of PR6 and PR3a isolates were determined by amalgamating data for all agrichemicals and analysing for significance using ANOVA and LSD tests.

Table 2. Agrichemicals (registration status in New Zealand forestry nurseries or plantations, rate of active or principle ingredient) tested for effect on *Trichoderma* isolates.

Active or Principle Ingredient registered for use in NZ forestry [a]	Active or Principle Ingredient	Product	Chemical Group	Mode of action, recommended timing of spray and diseases, weeds or pests controlled [b]	Quantity of Active or Principle Ingredient in Product (g/kg, g/l, ml/l, %)	Agrichemical Field Rate (g, ml or l per hectare or kg seed or 100 litres or 100m^2)	Water rate per ha	Rate Used in Experiments (g or ml per l of agar or water) [c]
Fungicides:								
No (N)	captan	Fruitfed Captan 80WG	cyclic imide	Seed coat treatment - control of damping off disease in peas. Protectant - soil drench before sowing of nursery seeds for control of damping off diseases.	800 g/kg	1g/kg seed 125 g in 100L water for 100m^2	-	1.3 g
No	chlorothalonil	Cavalry 720SC	chloronitrile	Protectant. Broad-spectrum, including control of *Botrytis*. At first appearance of disease.	720g/l	2.0l/ha	1000l	2.0ml
Yes N, P	copper hydroxide	Kocide Opti	inorganic copper	Broad-spectrum protectant including control of *Dothistroma* needle blight. Monthly from October or November.	300g/l	1.25 - 3.1kg/ha	600-1100l	2.0g
Yes N, P	cuprous oxide	Nordox 75WG		Protectant. At presence of *Dothistroma* needle blight.	750g/l	2.66kg/ha	400-1000l	2.7g
No	fluazinam	Gem	phenylpyridinamine	Broad-spectrum protectant, including control of *Botrytis*. Monthly from December.	500g/l	>1l/ha	>500l	1.0ml
No	iprodione	Rapid 500	dicarboximide	Broad-spectrum protectant, including control of *Botrytis*. Monthly from December.	500g/l	750ml-1l/ha	1000l	1.0ml
No	ipconazole/ metalaxyl-M	Rancona Dimension	triazole phenylamide	Seed coat treatment, Systemic - control or suppression of seed and soil borne diseases in cereals, maize and sweetcorn.	25g/l 20g/l	3.2ml/kg seed	-	3.2ml
No	metalaxyl-M	Apron	phenylamide	Seed coat treatment. Systemic - control of *Pythium* root disease in lucerne, brassicas and peas.	350g/kg	0.75ml/kg seed	-	1.0ml
No (N)	metalaxyl-M	Ridomil Gold SL	phenylamide	Systemic – control of *Phytophthora* root disease. Soil drench before seedling emergence.	480g/l	1-1.3l/ha	300l	1.5ml
Yes (N), P	metalaxyl-M/ mancozeb	Ridomil Gold MZ WG	phenylamide and dithiocarbamate	Systemic and protectant – control of *Phytophthora* root disease. Soil drench before seedling emergence. Apply 10mm irrigation to wash agrichemical onto the soil.	40g/kg 640g/kg	12.5-50kg/ha	200-300l	0.05 (L) [d] 56.0g (L & GH)
No (N)	phosphorous acid	Foschek	inorganic	Systemic and protectant– control of *Phytophthora* and *Pythium* root disease. Apply before disease first appears then 2-4 weekly.	400g/l	5l/ha	1000l	5.0ml
Yes N	prochloraz	Sportak EW	imidazole	Broad spectrum protectant and systemic (eradicant) – control of Terminal crook disease. 7-14-day intervals.	450g/l	1.5l/ha	500l	3.0ml
No (N)	pyrimethanil	Scala	anilino-pyrimidine	Protectant and some systemic (curative). Maximum of 2 sprays per season. *Botrytis* control.	400g/l	2l/ha	1000l	2.0ml
No (N)	thiram	Thiram 40F	disulphide	Seed coat treatment. Broad-spectrum protectant. Soil drench before sowing for damping off diseases.	400g/l	1.6g/kg seed 800ml/100m^2	-	3.0ml

Fumigants:								
No	dazomet	Basamid Granular	triadiazine	Broad-spectrum soil fumigant - fungal diseases, nematodes, insects and weeds. Plant 4 weeks after treatment.	970g/kg	400-450kg/ha	-	0.17 g
No	metam	Fumasol	thiocarbamate	Broad-spectrum soil fumigant - fungal diseases, nematodes, insects and weeds. Plant 4 weeks after treatment.	510g/l	350-500l/ha	400-700l	0.5ml
Herbicides:								
Yes P	atrazine	Atrazine 900 WG	triazine	Pre- and post-emergence control of broadleaf and some grass weeds. Apply pre- or post-planting.	900g/l	4.5-7kg/ha	100-300l	15 ml
Yes N, P	clopyralid	Void	Substituted pyridine	Post-weed emergence control of selective control of broadleaf and brush weeds. Apply post-planting.	300g/l	1-2.5l/ha	300l	0.7ml (L) 6.7ml (L & GH)
Yes P	glyphosate	Deal 510 RF	N-(phosphonomethyl) glycine	Non-selective pre-planting control of broadleaf and grasses weeds.	510g/l	2-6l/ha	100-200l	7.0ml
Yes N, P	haloxyfop-P	Hurricane AGPRO Haloxyfop 100	carboxylic acid derivative	Post-emergence control of grasses in seedling trees. Tree release spray for broadleaf and grass weeds.	100g/l	0.5-7.5l/ha	100-400l	5.0ml
Yes (N), P	Hexazinone/ terbuthylazine	AGPRO Valzine 500	triazine and heterocyclic triazine	Ground preparation or over newly planted trees. Post-emergence control of broadleaf, brush and grass weeds.	75g/l 425g/l	0.5-5l/ha 10-20l/ha	150-300l	1.7ml (L & GH) 16.7ml (L & GH)
Yes (N), P	hexazinone	Viper 90DF	heterocyclic triazine	Ground preparation or tree release spray for control of broadleaf, brush and grass weeds.	900g/l	2.1-8.3kg/ha	220-450l	4.7ml
Yes N	oxyfluorfen	Goal Advanced	nitrophenyl ether	Pre- and post-emergence control of broadleaf weeds.	480g/l	1-2l/ha	300-400l	2.5ml
Yes P	picloram	AGPRO Picloram 200	substituted pyridine	Aerial and spot-treatment of perennial and brush weeds. Long residual life in soil.	200g/l	1-2l/ha	>400l	2.5ml
Yes N	propazine	Gesamil 500FW	triazine	2-3 days after sowing for post-emergence control of annual broadleaf and grass weeds.	500g/l	2l/ha	500l	4.0ml
Yes N, P	simazine	AGPRO Simazine 500	triazine	Ground preparation. Post-emergence control of annual and perennial broadleaf and grass weeds.	510g/l	1.25-6l/ha	300l	7.5ml
Yes (N), P	terbuthylazine	Assett AGPRO Terbuthylazine 500	triazine	Pre-planting or tree release spray prior to spring growth. Broadleaf and grass weeds.	500g/l	2l/ha 12-20l/ha	200-300l	6.7ml (L & GH) 6.7ml (L & GH) and 25ml (L)
Yes P	triclopyr	AGPRO Triclop 600	substituted pyridine	Broad-spectrum systemic, aerial release spray. Broadleaf and brush weeds.	600g/l	1-2l/ha	200-400l	2.5ml
Insecticides:								
Yes N	*Bacillus thuringiensis sp. kurstaki*	Bactur WDG	bacteria	Bacterial toxin produced after ingestion. Apply at presence of insects (caterpillars, fall webworm).	>32000 International Units	500g – 1kg/ha	2000l	1.0g
No	chlorpyrifos	Lorsban 50EC	organophosphate	Contact and vapour action, stomach poison and acts on nervous system. Apply at presence of insects (caterpillars, thrips, beetles, leafrollers, aphids).	500g/l	500ml/ha	1000l	0.5ml
Yes N	cypermethrin	Ripcord	synthetic pyrethroid	Contact action and stomach poison. Apply at presence of insects (caterpillars, beetles).	200g/l	50ml/100l	-	0.5ml

Yes N	deltamethrin	Ballistic	synthetic pyrethroid	Contact action, stomach poison and acts on nervous system. Apply at presence of insects (caterpillars, thrips, beetles, tomato fruitworm).	27.5g/l	36ml/100l	-	0.36ml
No	lambda-cyhalothrin	Karate Zeon	synthetic pyrethroid	Contact action, stomach poison and acts on nervous system. Apply at presence of insects (caterpillars, thrips, beetles, tomato fruitworm, cutworms).	250g/l	40ml/ha	1000l	0.04ml
No	tau-fluvalinate	Mavrik Aquaflo	synthetic pyrethroid	Contact action and acts on nervous system. Apply at presence of insects (caterpillars, thrips, leafrollers, aphids, tomato fruitworm).	240g/l	400ml/ha	1000l	0.4ml
Adjuvants, Fertilisers and Biostimulants:								
	disodium octaborate tetrahydrate	AGPRO Sprayable Boron	micro-nutrient fertiliser.		21%	200-500g/100L (≤1kg/ha)	-	1.0g (GH) 3.0g (L and GH)
	macro- and micro-nutrients, see Appendix B	Nitrophoska Extra	macro-nutrient fertiliser.		-	150-200kg	-	10g
	magnesium, nitrogen	grochem Trace-It Magnesium	macro-nutrient fertiliser.		8% N, 9% Mg	2-4l	200-500l	6.0ml
	methiocarb	Mesurol 200SC	Seed coat treatment – bird repellent. Carbamate.		200 g/l	15 ml/kg seed	-	3.6ml
	mineral oil	AGPRO Crop Oil	adjuvant that improves penetration, adhesion and wetting of agrichemicals.		>950ml/l	0.5-4l/ha	-	3.0ml
	multiple: see Appendix B	AgriSea Foliar	liquid seaweed concentrate fertiliser.		-	5l	500l	10.0ml
	multiple; see Appendix B	Ag Concepts AgZyme	soil microbe growth catalyst.		-	1l	400l	2.5ml
	multiple; see Appendix B	Ag Concepts SuperHume	liquefied organic carbon (humic acids) and seaweed extract from *Ascophyllum nodosom*. Improves soil structure and a food source for soil microbes.		6%, 0.6%	5l	-	12.5ml
	nitrogen/ phosphate/ sulphur	Ravensdown Cropmaster DAP	macro-nutrient fertiliser.		17.6% N, 20% P, 1% S	100-300kg	-	1.0g
	paraffinic oil/ polyol fatty acid esters	Orion Agriscience Synoil	adjuvant that improves penetration, adhesion and wetting and reduces volatilisation of agrichemicals.		100%	0.5% of spray mix	-	5.0ml
	polyether modified polysiloxane	Penatra	organosilicone – adjuvant that improves penetration adhesion and wetting of certain herbicides on brush and woody weeds.		1020g/l	250ml	100l	2.5ml
	polyether modified polysiloxane	AGPRO Green Organosilicone Batch 10187#5 [e]	organosilicone - adjuvant that improves penetration, adhesion and wetting of certain herbicides on brush and woody weeds.		1010g/l	100-250ml	100l	2.5ml
	polyether modified polysiloxane	AGPRO Organosilicone	organosilicone - adjuvant that improves penetration, adhesion and wetting of certain herbicides on brush and woody weeds.		1010g/l	100-250ml	100l	2.5ml

[a] According to NZ Novachem Agrichemical Manual 2020/2021; N = registered for nursery, (N) = unregistered for forestry nurseries but used by some nurseries, P = registered for plantation use

[b] **Protectant or Contract**: active ingredients that act in the infection stage by providing a protective barrier (*ie:* no mobility of active ingredient) that prevents fungal spores from germinating or penetrating the plant tissue. **Systemic** = active ingredients that can penetrate and be mobile in the plant tissue. They can be defined as a) **curative**: can stop or reduce mycelial growth in the early colonisation stage (before symptoms are present) or b) **eradicant**: act on the later stages of colonisation (when symptoms are present) and can suppress spore production; may have some activity on new growth depending on the mobility of the product, or a combination of both (https://www.horticulture.com.au/globalassets/hort-innovation/resource-assets/ny15002_fungicides_factsheet.pdf).

[c] L = laboratory, GH = greenhouse [d] Ridomil Gold MZ WG recommended rate is 12.5-50 kg/ha; assuming an even distribution of agrichemical to a depth of 100mm, this would be 50mg/l (*ie*. 50 kg/ha = 50 kg/10,000 m2 = 5g/m2 ; since 1m2 area x 100mm depth = 100l volume then 5 g/100l= 50 mg/l) [e] product under development at AGPRO New Zealand Ltd.

Table 3: Details of greenhouse *in-vivo* experiments.

Agrichemical type	Experiment		Growing media	Treatment	Agrichemical application date	Seed planting date; *Trichoderma* spore application date and method	Seedling root sampling date
	Name	Number					
Fungicide, Fumigant, Fertiliser and Insecticide	Fumigant	1	soil	dazomet, metam, C+T, C-T [a]	3 July 2020	9 August 2020 (seed coat)	7 September 2020 24 October 2020
	Fungicide soil drench before planting	2	potting mix	captan, thiram, C+T, C-T	17 July 2020	24 July 2020 (drench)	24 August 2020 27 October 2020
	Fungicide soil drench before emergence			metalaxyl-M/mancozeb	4 August 2020	16 July 2020 (drench)	17 August 2020 27 October 2020
	Fungicide, fertiliser and insecticide foliar application 3.5 months after emergence	3	potting mix	*Bacillus thuringiensis,* chlorothalonil, copper hydroxide, cuprous oxide, disodium octaborate tetrahydrate (1 and 3g/l), fluazinam, iprodione, phosphorous acid, prochloraz, pyrimethanil, C+T, C-T	7 November 2020	19 June 2020 (drench)	18 November 2020
	Seed coat treatment	4	potting mix	captan, iprodione/metalaxyl-M, metalaxyl-M, methiocarb, paint, polyvinyl acetate, thiram, C+T, C-T	9 August 2020	9 August 2020 (seed coat)	5 September 2020 24 October 2020
Herbicide	Ground preparation	5	soil	atrazine, glyphosate, hexazinone, terbuthylazine (Assett, 6.7ml/l), C+T, C-T	16 July 2020	2 October 2020 (drench)	22 October 2020
				simazine	24 September 2020		
	Soil drench 4 days after sowing	6	soil	oxyfluorfen, propazine, C+T, C-T	20 July 2020	16 July 2020 (drench)	17 August 2020 24 October 2020
	Aerial application 3.5 months after emergence	7	potting mix	clopyralid (6.7ml/l), haloxyfop-P (AGPRO Haloxyfop-P), hexazinone/terbuthylazine (1.67 and 16.7ml/l), propazine, simazine, terbuthylazine (Assett, 6.7ml/l), C+T, C-T	7 November 2020	26 June 2020 (drench)	18 November 2020
		8	soil	haloxyfop-P (AGPRO Haloxyfop-P), simazine, terbuthylazine (AGPRO Terbuthylazine, 6.7ml/l), terbuthylazine (Assett, 6.7ml/l), triclopyr, C+T, C-T	7 November 2020	26 June 2020 (drench)	18 November 2020

[a] C+T = control with *Trichoderma* but no agrichemical treatment; C-T = control with no *Trichoderma* or agrichemical treatment.

4.0 RESULTS AND DISCUSSION

Spore germination was 100% in all *Trichoderma* isolates at 24 and 48 hours (Table 4 to 7 and Appendix C, D, F, and H). Mycelial radial growth at 68 hours at 25°C was 15.2, 14.8, 15.2, 14.8, 14.4, 14.6, 14.4 and 14.0 mm/day for isolates FCC55, FCC318, FCC327, FCC340, FCC13, FCC14, FCC15 and FCC180 respectively.

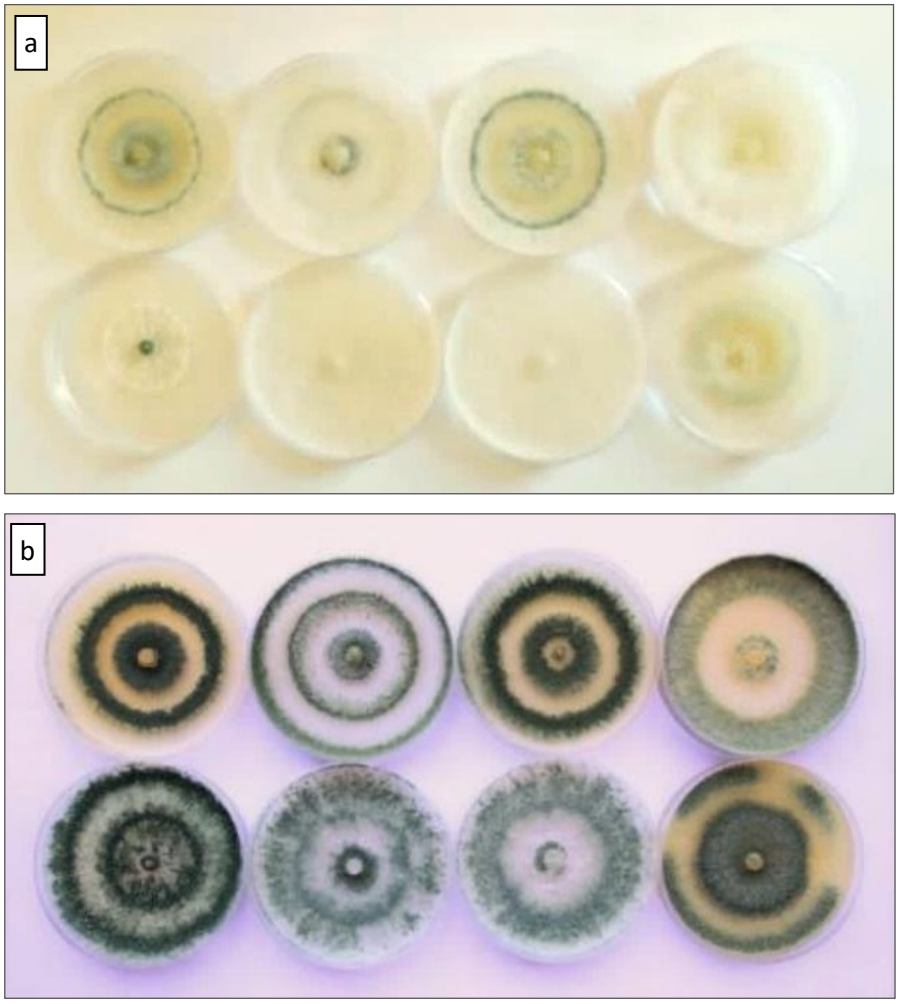

Figure 2: Mycelial growth of *Trichoderma* isolates FCC55, FCC318, FCC327, FCC340 (top rows) and FCC13, FCC14, FCC15 and FCC180 (bottom rows) on MYE with no agrichemical addition, (a) 68 hours and (b) 130 hours after inoculation with mycelium agar plugs.

4.1 Insecticides

Mean spore germination (%) was significantly ($P<0.05$) lower in two insecticide treatments cypermethrin and *Bacillus thuringiensis* 24 hours after exposure (Table 4), compared to the controls. However, the reductions were small (≤4%) compared to the mean spore germination of the controls (100%), therefore these insecticides were not considered to have a negative effect on spore viability. By 48 hours, germinations for all insecticide treatments were ≥ 99% (Table 4).

Spore germination at 24 hours was significantly lower in isolates FCC340 (96.1%), FCC327 (97.0%) and FCC318 (97.7%) compared to the other isolates (Table 4). Spore germination for each isolate was also dependent on the individual insecticide tested (*ie*: the interaction between isolate and agrichemical was significantly different at P<0.05; Appendix C). However, the reductions at 24 hours were small (the largest being 9.7% for isolate FCC340 in the presence of *Bacillus thuringiensis* (Appendix C) and by 48 hours all germinations were ≥ 98%. Overall, no insecticides were considered to have detrimental effects on spore germination of the isolates tested.

Lambda-cyhalothrin and tau-fluvalinate significantly (P<0.05) increased mycelial growth by up to 2% compared to the mean of the controls (Table 4). However, this was not considered to be a beneficial effect when selecting insecticides for use with *Trichoderma* biocontrol agents. Mycelial growth on cypermethrin and *Bacillus thuringiensis*-amended agar petri dishes was significantly (P<0.05) reduced by 33% and 48% respectively (Table 4, Figure 3). A similar antagonistic result was also found when Kim *et. al.* (2008) placed *T. harzianum* mycelium onto agar dishes amended with *Bacillus thuringiensis.*

Mycelial growth of each isolate was also dependent on the individual insecticide tested (*ie:* the interaction between isolate and insecticide was significantly different at P<0.05; Appendix C). For example, the mycelial growth of isolate FCC14 was inhibited the most by cypermethrin (41.6%) but the least by *Bacillus thuringiensis* (27.9%, Appendix C). In contrast, the mycelial growth of isolate FCC180 was inhibited the least by cypermethrin (26.2%) but had one of the highest inhibitions by *Bacillus thuringiensis* (50.5%).

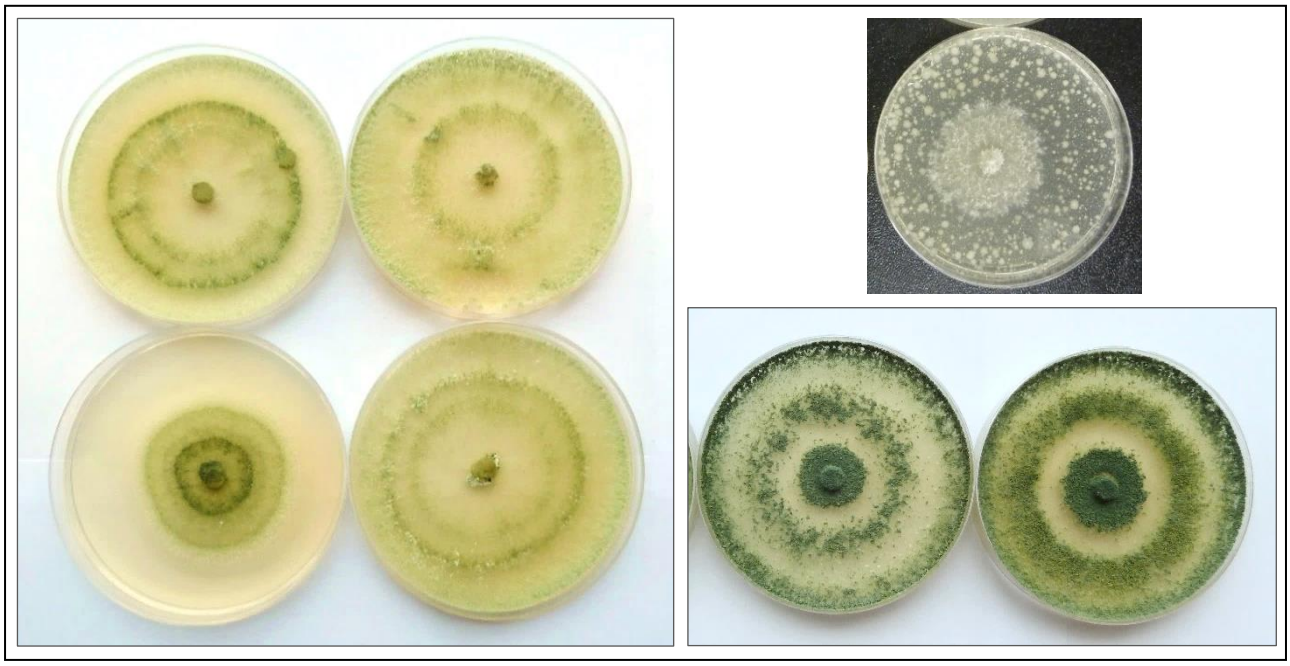

Figure 3: Mycelial growth of *Trichoderma* isolates FCC327 on MYE (top left to top right) with no agrichemical addition, Karate Zeon, Bactur and (bottom left to right) Ripcord , Mavrik Aquaflo, Lorsban and Ballistic, 68 hours after inoculation with mycelium agar plugs.

Insecticides had limited or no effect on isolate sporulation after 68- and 130-hours exposure (Appendix J).

No insecticides, apart from *Bacillus thuringiensis*, had sufficient negative effects on *Trichoderma* isolate development to be considered for further testing in plant studies. The tolerance of the eight isolates to the insecticides may be due to the ability of *Trichoderma* to degrade insecticides (Senthilkumar *et. al*., 2011), including chlorpyrifos (Harish *et. al*., 2012, Jayaraman *et. al*., 2012), when mixed in solutions.

Bacillus thuringiensis was included in the foliar fungicide spray greenhouse experiment (Experiment 3; see section 4.3). The presence of established *Trichoderma* (of PR6 mixture) in seedling roots was not significantly affected 11 days after foliar application of *Bacillus thuringiensis* (Bactur WDG), compared to the untreated control seedlings (Figure 11). Further testing could involve multiple applications of foliar *Bacillus thuringiensis* during the growing season to confirm it does not affect root *Trichoderma* persistence.

Table 4: Effect of insecticides on *Trichoderma* isolate spore germination (%) and inhibition (%) of mycelial growth.

Main Effect Means		**Spore Germination (%)** [a]		**Inhibition (%) of mycelial growth** [b]
		24 hours	**48 hours**	
Treatment:				
Active Ingredient	**Product**			
Bacillus thuringiensis	Bactur WDG	96.0 b	99.3 a	44.5 a
chlorpyrifos	Lorsban 50EC	(100.0)	(100.0)	0.0 c
cypermethrin	Ripcord	99.3 a	99.8 a	33.4 b
deltamethrin	Ballistic	(100.0)	(100.0)	0.7 c
lambda-cyhalothrin	Karate Zeon	99.9 a	(100.0)	-1.2 d
tau-fluvalinate	Mavrik Aquaflo	(100.0)	(100.0)	-2.1 d
control *(Trichoderma* isolates with no treatment)		(100.0)	(100.0)	-
LSD (5%)		0.7	0.5	1.0
LSE (5%)		0.5	0.4	-
Isolate:				
FCC55		98.8 [99.4] b	100.0 [100.0] a	15.4 a
FCC318		97.7 [98.8] c	99.5 [99.8] ab	8.6 e
FCC327		97.0 [98.5] cd	99.0 [99.7] b	15.3 a
FCC340		96.1 [98.1] d	99.3 [99.8] ab	12.5 c
FCC13		99.6 [99.8] b	99.3 [99.8] ab	14.1 b
FCC14		99.3 [99.7] ab	99.8 [99.9] ab	12.1 c
FCC15		99.0 [99.4] ab	99.3 [99.8] ab	10.6 d
FCC180		99.9 [99.9] a	100.0 [100.0] a	11.9 c
LSD (5%)		1.1 (-)	1.0 (-)	1.2

[a] Data with zero variability (*ie.* consistently 0 or 100) (in round-brackets) were omitted from ANOVA analysis. Statistical analysis of data with variability was by ANOVA using the unprotected least significant difference (LSD) procedure at the 5% level. Two means with no letters in common differed significantly at $P<0.05$. Statistical comparison of unbracketed and round-bracketed data was by least significant effect (LSE) at the 5% level. Data in square-brackets represented means calculated using all data.

[b] To allow for different growth rates of each isolate, inhibition (%) was calculated by ((X-Y)/X) x 100, where X and Y are the radial growth rate (mm/day) of isolate in the control and agrichemical-amended petri dishes, respectively.

4.2 Adjuvants, fertilisers and biostimulants

Mean spore germination (%) was not significantly affected by the adjuvants, fertiliser and biostimulants tested, apart from disodium octaborate tetrahydrate, that caused a 98% reduction after 48 hours of agrichemical exposure (Table 5). Mean spore germination was not significantly different in the isolates when tested with these products (Table 5). However, a significant ($P<0.05$) interaction between treatments and isolates was found (Appendix D) but was not considered important because most treatments did not affect spore germination.

Disodium octaborate tetrahydrate inhibited mycelial growth the most (by 63.9%) out of the tested products, followed by the three surfactants, AGPRO Green Organosilicone, AGPRO Organosilicone and Penatra (43.7, 34.3 and 31.4% respectively, Table 5). Mesurol 200SC, a bird repellent commonly used in seed coating, reduced mycelial growth by less than 10% (Table 5). Reglinski *et. al.* (2009) also found mycelial growth of *T. atroviride* was not affected by methiocarb. In contrast, two fertilisers (Nitrophoska Extra and Trace-It Magnesium) and two oils (AGPRO Crop Oil and Synoil) increased mycelial growth by up to 3% (Table 5), compared to the controls, possibly due to the provision of extra nutrients.

Significant differences ($P<0.05$) in mean mycelial growth were found in the isolates but the range was relatively small (from 13.7 to 17.4%, Table 5). Mycelial growth of each isolate was also dependent on the individual adjuvants, fertiliser and biostimulants tested (*ie:* the interaction between isolate and treatment was significantly different at $P<0.05$; Appendix E). For example, mycelial growth of isolate FCC14 was inhibited the most by Cropmaster DAP (45.7%) but the least by AgriSea Foliar (-2.3. In contrast, mycelial growth of isolate FCC327 was inhibited the least by Cropmaster DAP (14.9%) but the most by AgriSea Foliar (13.5%).

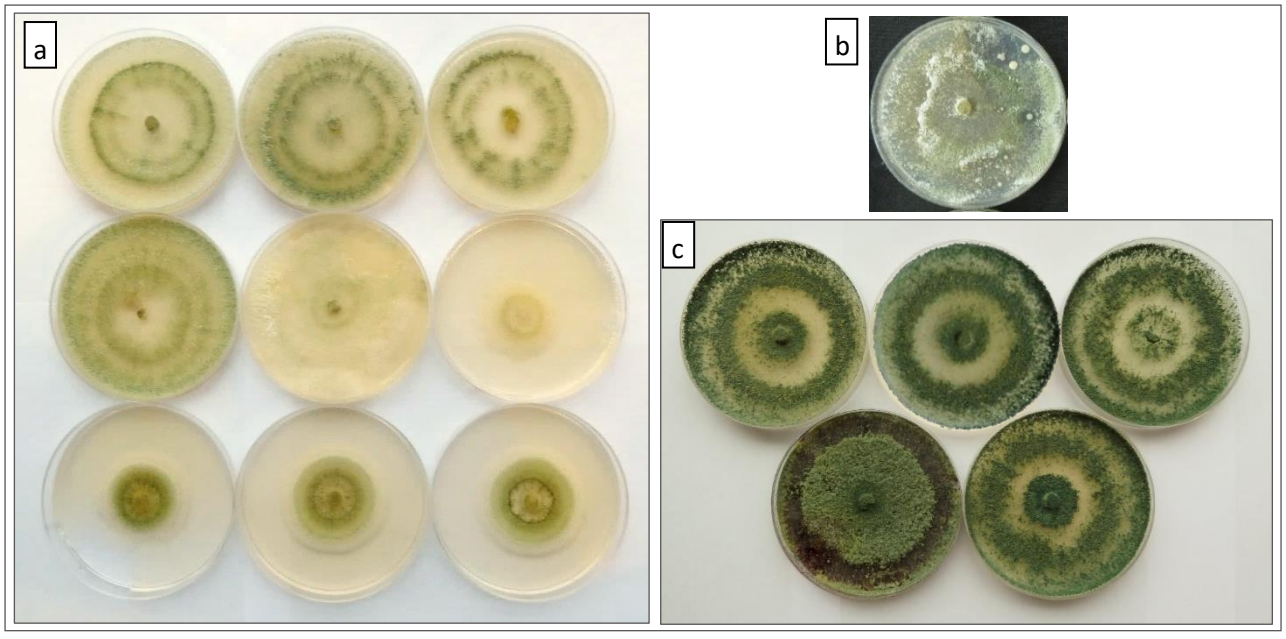

Figure 4: Mycelial growth of *Trichoderma* isolate FCC327 on MYE with (a) no agrichemical, Nitrophoska Extra and Trace-It Magnesium (top row), AGPRO Crop Oil, Cropmaster DAP and AGPRO Sprayable Boron (middle row) and AGPRO Green Organosilicone, AGPRO Organosilicone and Penatra (bottom row) addition, 68 hours after inoculation with mycelium agar plugs; (b) AgriSea Foliar and (c) no agrichemical, Trace-It Magnesium and Synoil (top row) and SuperHume and AgZyme (bottom row) addition, 130 hours after inoculation with mycelium agar plugs.

However, these differences were not important when selecting isolates for use as biocontrol organisms because most of the adjuvants, fertiliser and biostimulants tested were not harmful.

Adjuvants, fertilisers and biostimulants had limited or no effect on isolate sporulation after 68- and 130-hours exposure (Appendix J).

Overall, no adjuvants, fertilisers and biostimulants, apart from disodium octaborate tetrahydrate, had sufficiently large negative effects on *Trichoderma* isolate development to be considered for further testing in plant studies. AGPRO Sprayable Boron (at 1 and 3g/l) was included in the foliar fungicide spray greenhouse experiment (Experiment 3; see section 4.3). The presence of established *Trichoderma* (PR6 mixture) in seedling roots was not significantly affected 11 days after foliar application of disodium octaborate tetrahydrate, compared to the inoculated control seedlings (Figure 11). Further testing could involve multiple applications of foliar boron during the growing season to confirm it does not affect root *Trichoderma* persistence. It is recommended that spray tank mixing of *Trichoderma* and boron (disodium octaborate tetrahydrate) should be avoided due to the fungicidal effects of boron on mycelial initiation and development (Ang *et. al.,* 2011).

Table 5: Effect of adjuvants, fertilisers and biostimulants on *Trichoderma* isolate spore germination (%) and inhibition (%) of mycelial radial growth.

Main Effect Means		Spore Germination (%) [a]		Inhibition (%) of mycelial growth [b]
		24 hours	48 hours	
Treatment:				
Active Ingredients and product rate	**Product**			
disodium octaborate tetrahydrate (3g/l)	AGPRO Sprayable Boron	1.2 b	2.0 {±0.2}	63.9 a
humic acid/kelp extract/organic acids	SuperHume	(100.0)	(100.0)	0.5 h
macro- and micro-nutrients; see Appendix B	Nitrophoska Extra	(100.0)	(100.0)	-2.5 k
magnesium/nitrogen	Trace-It Magnesium	99.9 a	(100.0)	-3.0 j
methiocarb	Mesurol 200SC	99.9 a	(100.0)	7.8 f
mineral oil	AGPRO Crop Oil	(100.0)	(100.0)	-2.9 j
multiple; see Appendix B	AgriSea Foliar	(100.0)	(100.0)	6.7 g
multiple; see Appendix B	AgZyme	(100.0)	(100.0)	0.1 i
nitrogen/phosphate/sulphur	Cropmaster DAP	(100.0)	(100.0)	22.2 e
paraffinic oil	Synoil	(100.0)	(100.0)	-0.5 j
polyether modified polysiloxane	AGPRO Green Organosilicone	(100.0)	(100.0)	43.7 b
polyether modified polysiloxane	AGPRO Organosilicone	99.9 a	(100.0)	34.3 c
polyether modified polysiloxane	Penatra	(100.0)	(100.0)	31.4 d
control *(Trichoderma* isolates with no treatment)		(100.0)	(100.0)	-
LSD (5%)		0.3	-	0.6
LSE (5%)		0.2	-	-
Isolate:				
FCC55		75.2 [92.4] a	2.0 {±0.0} [92.5]	15.4 c
FCC318		75.3 [92.4] a	1.7 {±0.3} [92.4]	13.7 d
FCC327		75.0 [92.3] a	2.0 {±0.6} [92.5]	16.3 b
FCC340		75.4 [92.4] a	1.3 {±0.3} [92.4]	13.7 d
FCC13		75.3 [92.4] a	3.0 {±0.0} [92.5]	16.4 b
FCC14		75.0 [92.3] a	1.7 {±0.3} [92.4]	17.4 a
FCC15		75.4 [92.4] a	1.3 {±0.3} [92.4]	16.1 b
FCC180		75.0 [92.3] a	3.3 {±0.7} [92.6]	15.1 c
LSD (5%)		0.4 (-)	-	0.5

[a] Data with zero variability (*ie.* consistently 0 or 100) (in round-brackets) were omitted from ANOVA analysis. Statistical analysis of data with variability was by ANOVA using the unprotected least significant difference (LSD) procedure at the 5% level. Two means with no letters in common differed significantly at $P<0.05$. Statistical comparison of unbracketed and round-bracketed data was by least significant effect (LSE) at the 5% level. Data in square-brackets represent means calculated using all data, whilst data in curly-brackets represent the standard error of means.

[b] To allow for different growth rates of each isolate, inhibition (%) was calculated by ((X-Y)/X) x 100, where X and Y are the radial growth rate (mm/day) of isolate in the control and agrichemical-amended petri dishes, respectively.

4.3 Fumigants and Fungicides

The two fumigants, dazomet and metam, were 100% fungicidal on *Trichoderma* spore germination, mycelial growth (Table 6 and Appendix F and G) and sporulation (Appendix J). When inoculated seeds were planted into metam-treated soil (Figure 6), the presence of *Trichoderma* in seedling roots was significantly (P<0.05) reduced (by 72%) 29 days after sowing and 66 days after metam application, compared to the inoculated control seedlings (Figure 5). However, by approximately 3.5 months after sowing, root colonisation had recovered to levels (76.3%) approaching those found in the unamended soil; 86.1%, Figure 5). The initial reduction in root colonisation may have been caused by sensitivity of applied *Trichoderma* spores to insufficient degradation of metam in the first month after application and/or additional gas release when the trays were placed in the warm greenhouse for seedling sowing. In contrast, seedlings grown in dazomet-treated soil had root colonisation levels similar to the unamended soil, 29 days after sowing (Figure 5). By approximately 3.5 month after sowing, persistence of root *Trichoderma* was still high (53%), although significantly (P<0.05) less than the inoculated soil (86.1%). Overall, persistence of root *Trichoderma* was not substantially affected by the application of fumigants, but this experiment demonstrated the care required when using these soil sterilant products with biocontrol agents.

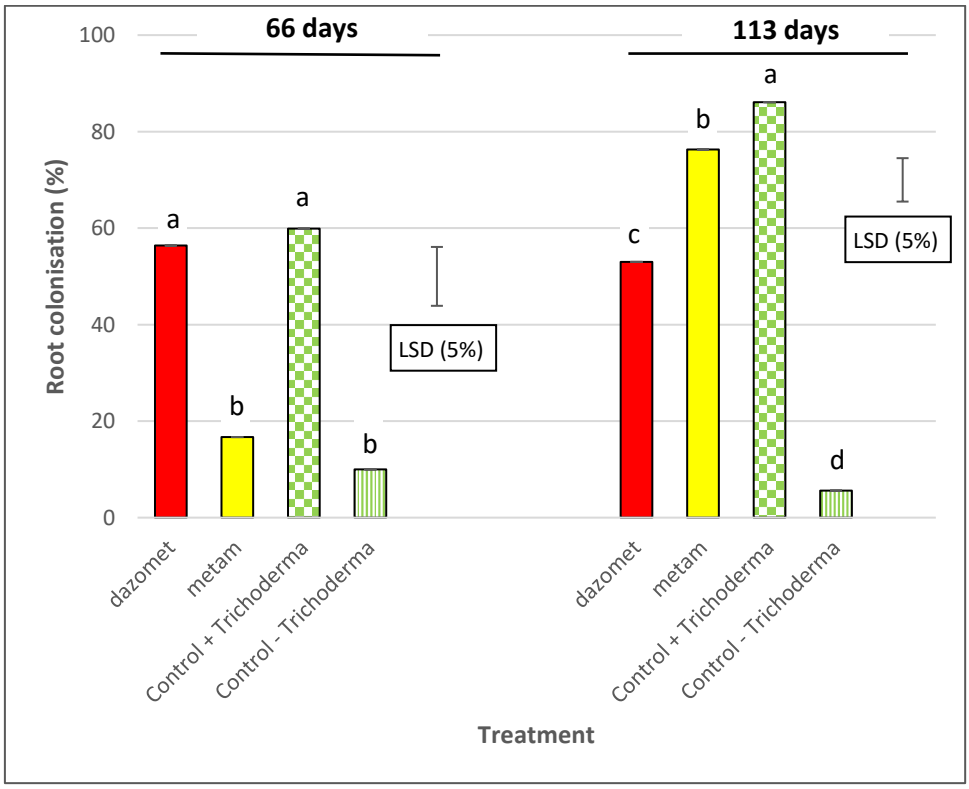

Figure 5: Effect of soil fumigants applied 37 days before sowing of *Trichoderma* inoculated and uninoculated *P. radiata* seeds, on *Trichoderma* root colonisation (%) sampled 66 and 113 days after fumigant application (Experiment 1).

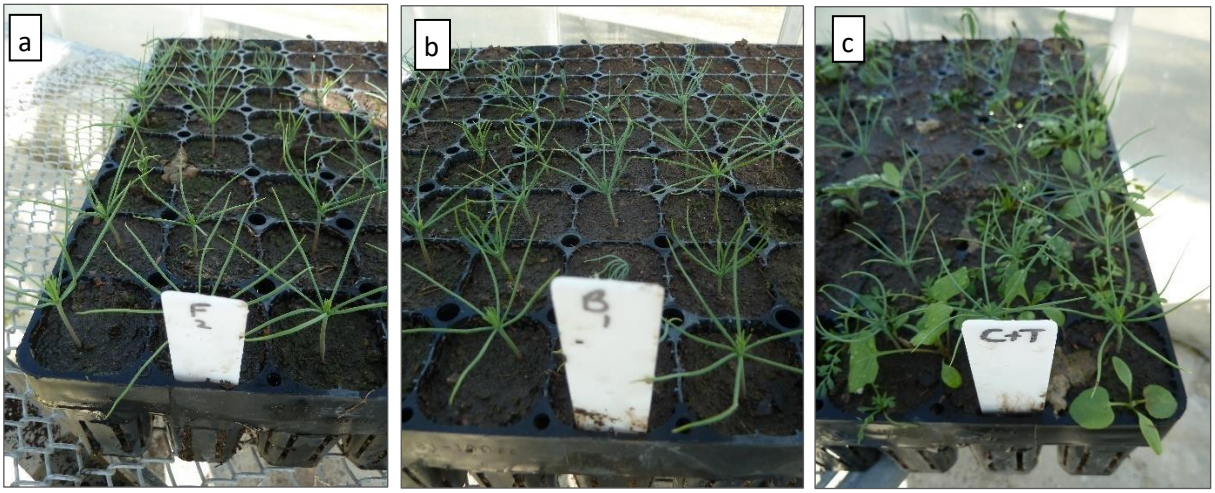

Figure 6: *Trichoderma* inoculated *P. radiata* seedlings 13 days after emergence in fumigant a) Fumasol and b) Basamid Granular treated and c) untreated soil.

Six (captan, chlorothanonil, copper hydroxide, fluazinam, prochloraz and thiram) of the eleven fungicides with protectant activity, delayed spore germination after 24 hours of agrichemical exposure (Table 6). Interestingly, after 48-hours of exposure, spore germination was near maximum levels (≥ 95%) for captan, chlorothanonil, copper hydroxide and fluazinam, suggesting these agrichemicals were fungistatic rather than fungicidal. Only two protectants, prochloraz and thiram, were fungicidal at 48-hours of exposure.

In contrast, five of the protectant-activity fungicides, cuprous oxide, iprodione, metalaxyl-M/mancozeb, phosphorous acid and pyrimethanil had limited effect on spore germination (≤25% inhibition) but inhibited mycelial growth by 25 to 100% compared to the controls (Table 6, Figure 7). Six other protectants, captan, chlorothanonil, copper hydroxide, fluazinam, thiram and prochloraz also had significant ($P<0.05$) inhibitory effects on mycelial growth (between 34.7 to 100%; Table 6 and Figure 7). The additional systemic action of some fungicides (metalaxyl-M/mancozeb, phosphorous acid, prochloraz and pyrimethanil) have may have contributed to the reduction in mycelial growth.

Apron slightly promoted (+0.8%) mycelial growth compared to Ridomil Gold SL, the other agrichemical containing metalaxyl-M, possibly because of the slightly lower rate selected to represent seed coat application, and/or formulation differences.

Spore germination of the PR6 isolates were generally less sensitive (mean of 47.1%) to the fungicides tested compared to the PR3a isolates (mean of 43.2%; Table 6). Similarly, mycelial growth was generally less sensitive in the PR6 isolates (mean of 66.5% inhibition) compared to the PR3a isolates (73.5%). Spore germinations and mycelial growth for each isolate were also dependent on the individual fungicide or fumigant treatment tested (*ie*: the interaction between isolate and treatment was significantly different at $P<0.05$; Appendix F and G). For example, mycelial growth of isolate FCC340 was inhibited the most by chlorothalonil (90.5%) but the least by phosphorous acid (9.0%). In contrast, mycelial growth of isolate FCC180 was inhibited the least by chlorothalonil (35.9%) but the most by phosphorous acid (75.7%). Selection of isolates for use as biocontrol organisms may depend on the fungicides likely to be used.

Table 6: Effect of fumigants and fungicides on *Trichoderma* isolate spore germination (%) and inhibition (%) of mycelial growth.

Main Effect Means		Spore Germination (%) [a]		Inhibition (%) of mycelial growth [b]
		24 hours	48 hours	
Treatment: [c]				
Active Ingredients	**Product**			
captan	Fruitfed Captan 80WG	2.5 d	98.0 b	89.5 b
chlorothalonil	Cavalry 720SC	0.5 e	99.9 a	70.2 e
copper hydroxide	Kocide Opti	4.6 c	99.8 a	94.7 a
cuprous oxide	Nordox 75WG	75.2 b	95.8 c	(100.0)
dazomet	Basamid Granular	(0.0)	(0.0)	(100.0)
fluazinam	Gem	0.5 e	94.5 d	37.5 g
iprodione	Rapid 500	93.0 a	98.1 b	25.3 i
metalaxyl-M	Apron	(100.0)	(100.0)	-0.8 j
metalaxyl-M	Ridomil Gold SL	(100.0)	(100.0)	43.0 f
metalaxyl-M/mancozeb	Ridomil Gold MZ WG (0.05g/l)	(100.0)	(100.0)	(0.0)
metalaxyl-M/mancozeb	Ridomil Gold MZ WG (56g/l)	(100.0)	(100.0)	87.9 c
metam	Fumasol	(0.0)	(0.0)	(100.0)
phosphorous acid	Foschek	(100.0)	(100.0)	34.7 h
prochloraz	Sportak EW	(0.0)	(0.0)	(100.0)
pyrimethanil	Scala	(100.0)	(100.0)	78.3 d
thiram	Thiram 40F	0.7 e	1.5 e	89.7 b
control *(Trichoderma* isolates with no treatment)		(100.0)	(100.0)	(0.0)
LSD (5%)		1.1	0.9	1.0
LSE (5%)		0.8	0.6	0.7
Isolate:				
FCC55		29.1 [46.9] ab	84.6 [72.8] a	51.3 [64.3] e
FCC318		29.6 [47.1] ab	85.1 [73.1] a	56.4 [68.0] d
FCC327		29.9 [47.3] a	84.8 [72.9] a	49.6 [63.0] f
FCC340		29.2 [47.0] ab	84.8 [72.9] a	60.0 [70.6] c
FCC13		28.5 [46.6] b	84.5 [72.8] a	71.7 [79.2] a
FCC14		17.1 [41.3] d	82.6 [71.9] b	62.5 [72.5] b
FCC15		17.5 [41.5] d	82.6 [71.9] b	62.4 [72.4] b
FCC180		21.5 [43.4] c	82.7 [71.9] b	59.0 [69.9] c
LSD (5%)		1.2 (-)	0.9 (-)	0.9 (-)

a Data with zero variability (*ie.* consistently 0 or 100) (in round-brackets) were omitted from ANOVA analysis. Statistical analysis of data with variability was by ANOVA using the unprotected least significant difference (LSD) procedure at the 5% level. Two means with no letters in common differed significantly at $P<0.05$. Statistical comparison of unbracketed and round-bracketed data was by least significant effect (LSE) at the 5% level. Data in square-brackets represent means calculated using all data.

b To allow for different growth rates of each isolate, inhibition (%) was calculated by ((X-Y)/X) x 100, where X and Y are the radial growth rate (mm/day) of isolate in the control and agrichemical-amended petri dishes, respectively.

c Rancona Dimension arrived after the spore germination and mycelia growth assays but was used in the greenhouse experiment.

Figure 7: Mycelial growth of *Trichoderma* isolate FCC327 on MYE (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 (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.

Sporulation of the isolates was not affected by eight of the thirteen fungicides tested but was by copper hydroxide, cuprous oxide, fluazinam, prochloraz and pyrimethanil (Appendix J).

The impact of fungicides with negative effects on laboratory isolate development, on colonisation and persistence of seedling root *Trichoderma*, was determined in three greenhouse experiments (refer to Table 3). Fungicides were applied either by seed coat, before sowing, before seedling emergence or as a foliar spray.

Seed coat application of fungicides captan, ipconazole/metalaxyl-M, metalaxyl-M and thiram resulted in significantly ($P<0.05$) higher root *Trichoderma* colonisation (mean of 79.2%), compared to the inoculated control treatment (61.9%), 27 days after application of agrichemicals and the *Trichoderma* PR6 mixture (Figure 8 and 9a). The enhanced root colonisation in the presence of captan and thiram (that caused low spore germination and mycelial growth in the laboratory assays), may have been due to:

- some *Trichoderma* spores being unexposed to the agrichemicals whilst harbouring in the rough coat of the seed,
- the ability of *Trichoderma* to grow quickly into the potting mix and later infect the developing root and/or
- the suppression of competitive fungal species resident in the potting mix.

The enhancement of root *Trichoderma* with thiram applied as a seed coat was also measured in a *P. radiata* containerised tray trial with three *Trichoderma* mixtures, PR6, PR3a and a mixture of BPRC's isolates FCC320, FCC327 and LU633 (N. Heron, Timberlands Te Ngae Nursery, *pers. comm.*). The tolerance of root *Trichoderma* to thiram was also found in field grown brassica plants that had seed coated thiram applied at a high rate of 9g Thiram 40F/kg seed (D. Kandula, Bio-Protection Research Centre, Lincoln University *pers. comm.*).

Trichoderma was also tolerant to methiocarb (70.6%) and paint (65.1%) but PVA had significantly lower root colonisation levels (41.3%) compared to the inoculated control (Figure 8). The lower tolerance to PVA is contrary to previous laboratory experiments that indicated no intolerance and may be due to formulation differences of PVA brands. Persistence of root *Trichoderma* in all treatments declined (28 to 42%) 76 days after seed coating, but all treatments were similar to the inoculated control (Figure 8).

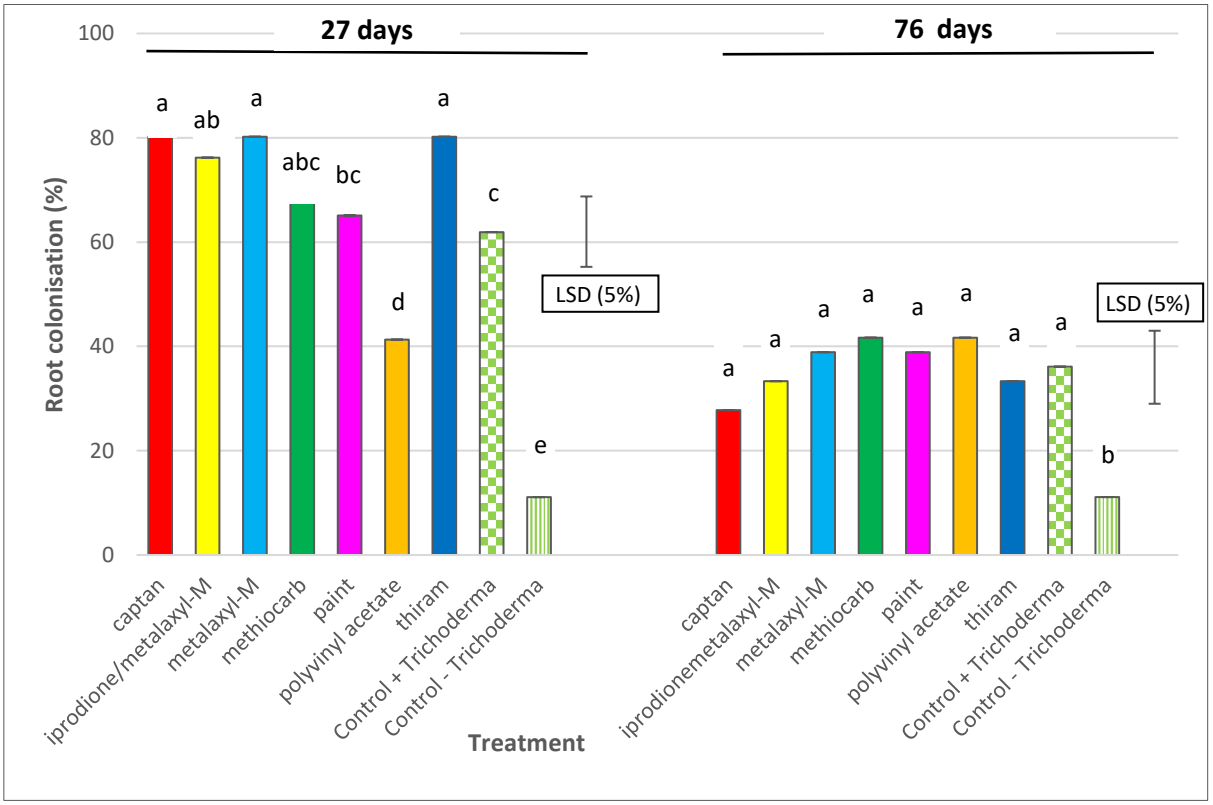

Figure 8: Effect of fungicides, adherents and a bird repellent applied as a seed coat, on *P. radiata Trichoderma* root colonisation (%) 27 and 76 days after seed coating and sowing.

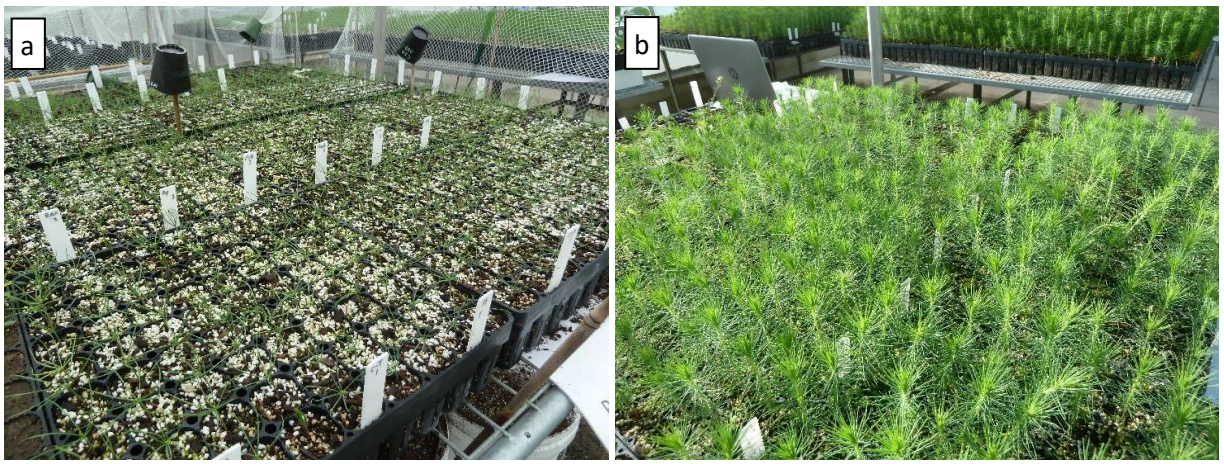

Figure 9: The seed coat experiment a) 27 and b) 76 days after sowing in the greenhouse (Experiment 4).

The enhancement of root *Trichoderma* was also measured in captan and thiram treatments applied as soil drenches before seeding and in metalaxyl-M/mancozeb applied 3 days before seedling emergence, compared to the inoculated control (Figure 10). By approximately 3 months after fungicide application, root colonisation levels had reduced in all treatments, but fungicide treatments were not significantly different ($P<0.05$) from the inoculated control (Figure 10).

Fungicides that caused large reductions in laboratory *Trichoderma* development were sprayed onto PR6 mixture inoculated seedlings 141 days after seeding. The presence of established root *Trichoderma* was not affected by any of the fungicide treatments (Figure 11), compared to the inoculated control. Persistence of root *Trichoderma* in the presence of foliar fungicides was not determined due to insufficient time left to complete this project in 2020.

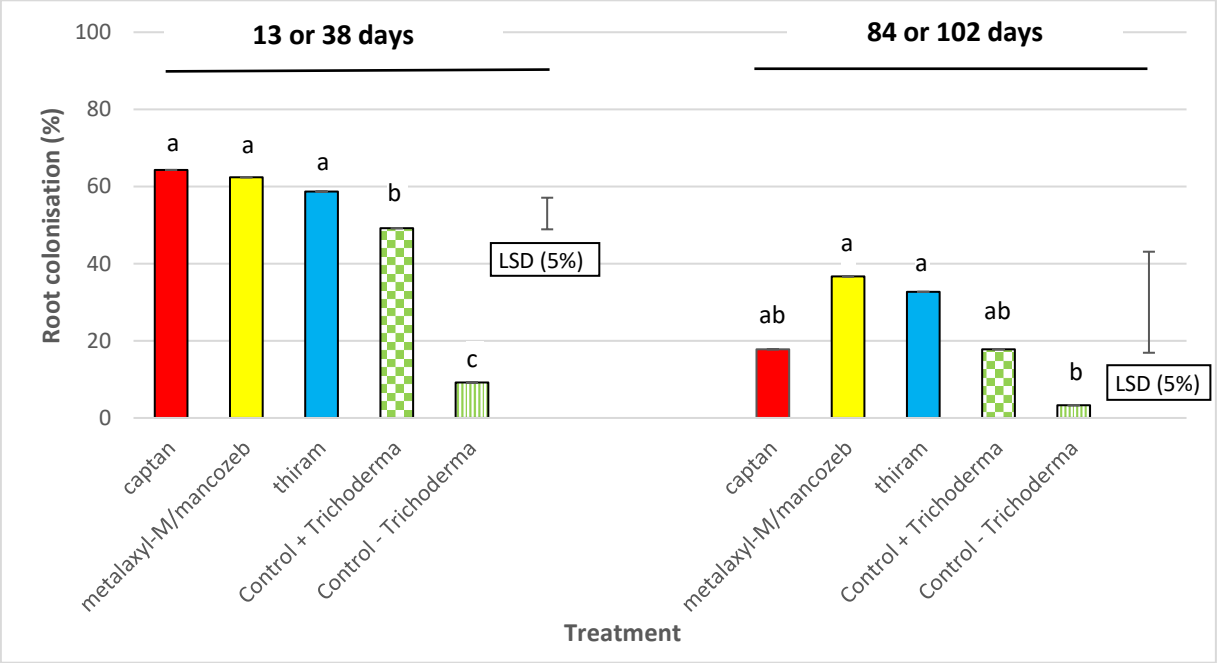

Figure 10: Effect of soil drench fungicides applied 7 days before sowing (captan and thiram) and 7 days before emergence (metalaxyl-M/mancozeb) of *Trichoderma* inoculated and uninoculated *P. radiata* seeds, on *Trichoderma* root colonisation (%) sampled 38 and 102 days (captan and thiram) or 13 and 84 days (metalaxyl-M/mancozeb) after fungicide application (Experiment 2).

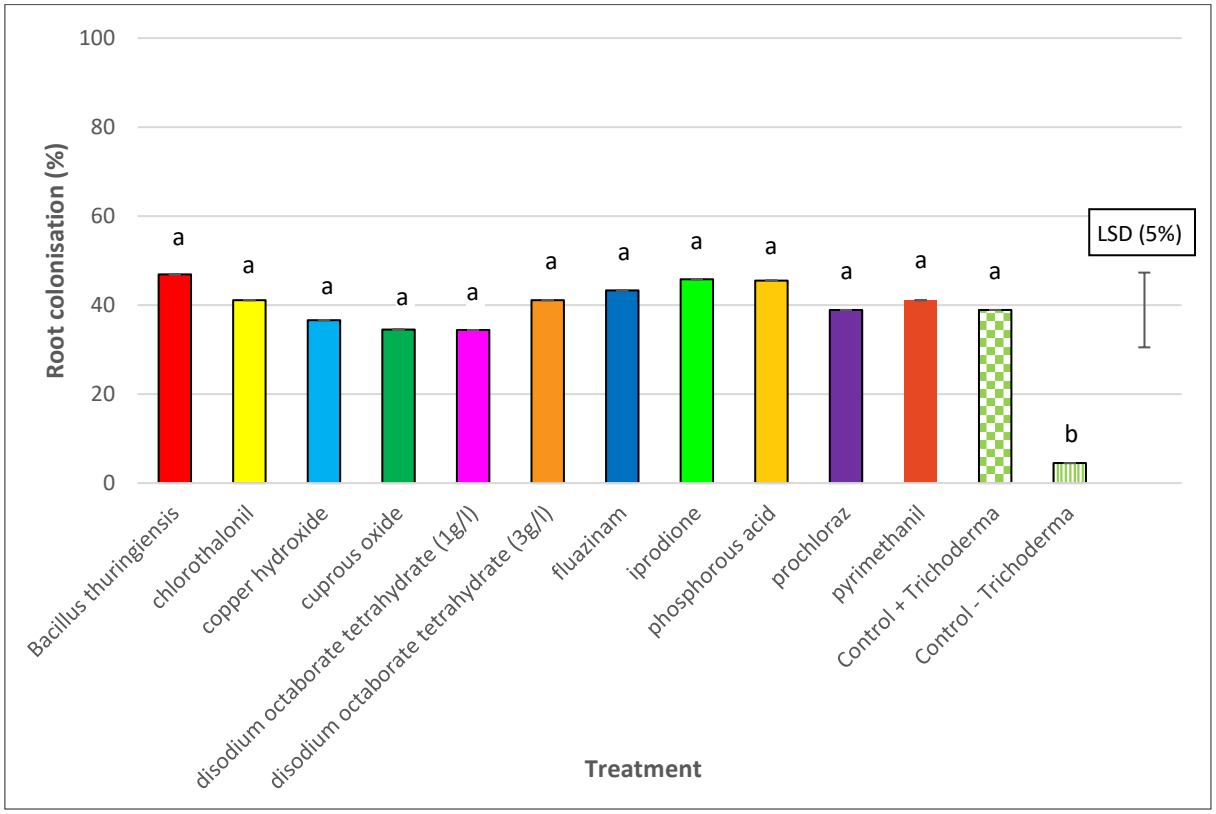

Figure 11: Effect of foliar fungicides, disodium octaborate tetrahydrate and *Bacillus thuringiensis*, applied 141 days after sowing of *Trichoderma* inoculated and uninoculated *P. radiata* seeds, on *Trichoderma* root colonisation (%) sampled 11 days after fungicide application (Experiment 3).

Overall, these results suggested that many of the fungicides tested could not be mixed with the *Trichoderma* isolates in spray tanks because of potential loss of spore viability. This confirms the recommendation to avoid mixing fungal biocontrol and most fungicide products, for example, Bioworks (2020) guideline. However, when *Trichoderma* was established in the seedling roots (by seed coat or soil drench inoculation) it was tolerant (and sometimes enhanced) to the soil- or foliar-applied fungicides in this study. In addition, the seven seed coat treatments investigated are recommended for coating of radiata pine seeds in combination with the PR6 *Trichoderma* spores. Seed coating with *Trichoderma* spores is also recommended as the most practical, efficient, low cost and socially acceptable method for application of this biocontrol agent in the nursery and plantation.

4.4 Herbicides

Spore germination was highly inhibited (>97%) by three (glyphosate, propazine and terbuthylazine (Assett)) of the fourteen herbicides tested (Table 7). Inhibition of spore germination was low (<4%) in the remainder herbicides. Mycelial growth, compared to spore germination, was affected more by the herbicides (Figure 13). Glyphosate, propazine and terbuthylazine (Assett), as well as hexazinone/terbuthylazine (16.7ml/l) and atrazine, had significantly ($P<0.05$) reduced growth by ≥ 73% (Table 7). Seven other herbicides (haloxyfop-P (AGPRO Haloxyfop 100 and Hurricane), terbuthylazine (AGPRO Terbuthylazine 500), clopyralid (Void 6.7ml/l), triclopyr, hexazinone and simazine) significantly ($P<0.05$) reduced growth but by a smaller amount (between 48.7 to 18.9%). Product formulation may have contributed to the large differences in spore germination and mycelial growth (Figure 12) between the two terbuthylazine products, Assett and AGPRO Terbuthylazine 500 at the rate

of 6.7ml/l. Higher rates of hexazinone/terbuthylazine and terbuthylazine also significantly (<P<0.05) reduced mycelial growth, compared to the lower rates (Table 7).

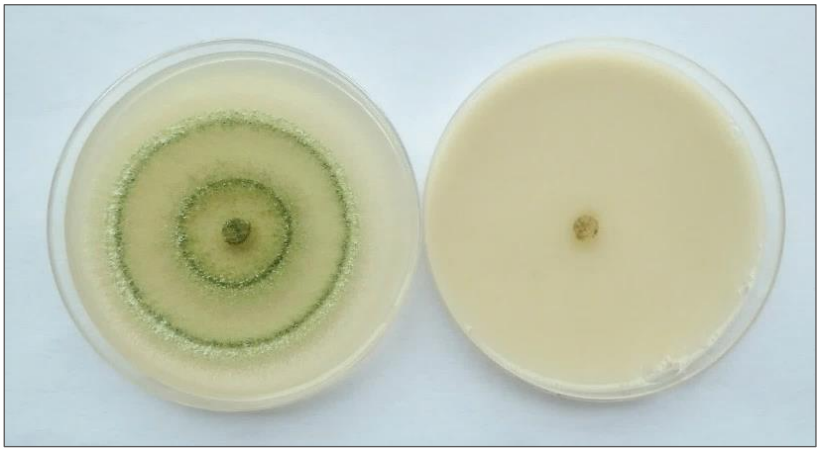

Figure 12: Mycelial growth of *Trichoderma* FCC327 isolate on MYE amended with AGPRO Terbuthylazine 500 and Assett at 6.7ml per litre.

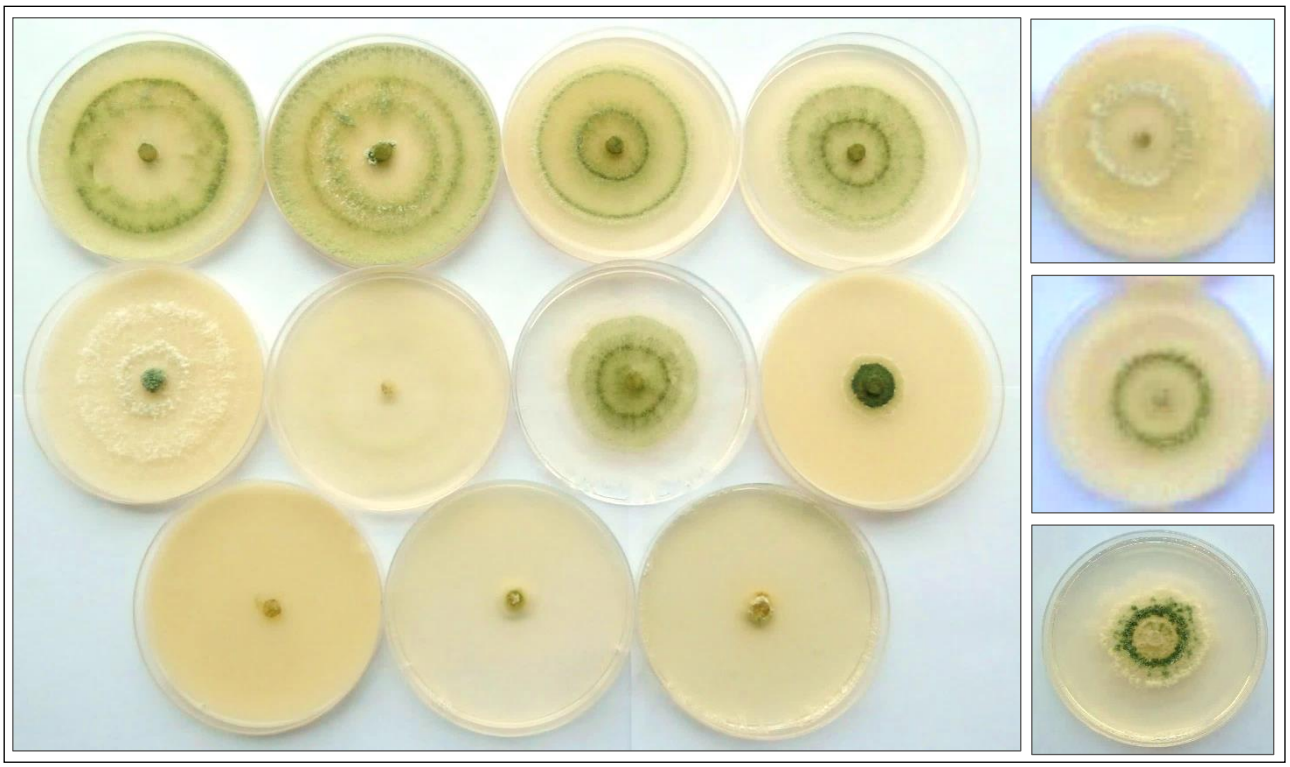

Figure 13: Mycelial growth of *Trichoderma* isolate FCC327 on MYE with no agrichemical, picloram, terbuthylazine (AGPRO Terbuthylazine 500 (6.7ml/l)), triclopyr and oxyfluorfen (top row), simazine, haloxyfop-P, clopyralid (Void (6.7ml/l), atrazine and hexazinone/terbuthylazine (AGPRO Valzine 500 1.7ml/l)) (middle row), and terbuthylazine (Assett, 6.7ml/l), glyphosate, propazine and hexazinone (bottom row), 68 hours after inoculation with mycelium agar plugs.

Although there were significant (P<0.05) differences in spore germination and mycelial growth for each isolate, the differences were generally small (maximum difference of 5.6% in mycelial growth) and not considered important when selecting isolates for use with these herbicides (Table 7). The interactions between isolate and agrichemical for spore germination and mycelial growth were also significantly different at P<0.05 (Appendix H and I). However,

these interactions were not considered important when selecting isolate/herbicide combinations, because few of the herbicides had large detrimental effects.

The impact of herbicides with negative effects on laboratory isolate development, on colonisation and persistence of seedling root *Trichoderma*, was determined in four greenhouse experiments (refer to Table3). Additional herbicides, with less negative effects were also included to reaffirm the laboratory results in a plant system.

Root *Trichoderma* colonisation in eleven day old seedlings, was significantly ($P<0.05$) enhanced or not affected by application of propazine (65.5%) and oxyfluorfen (42.5%) four days after seeding, compared to the inoculated control (46.7%; Figure 14 and 15). At 79 days after application, root colonisation was similarly enhanced (oxyfluorfen, 68.5%) or unaffected (propazine, 44.4%) by the herbicide treatments, compared to the inoculated control (50.0%; Figure 14).

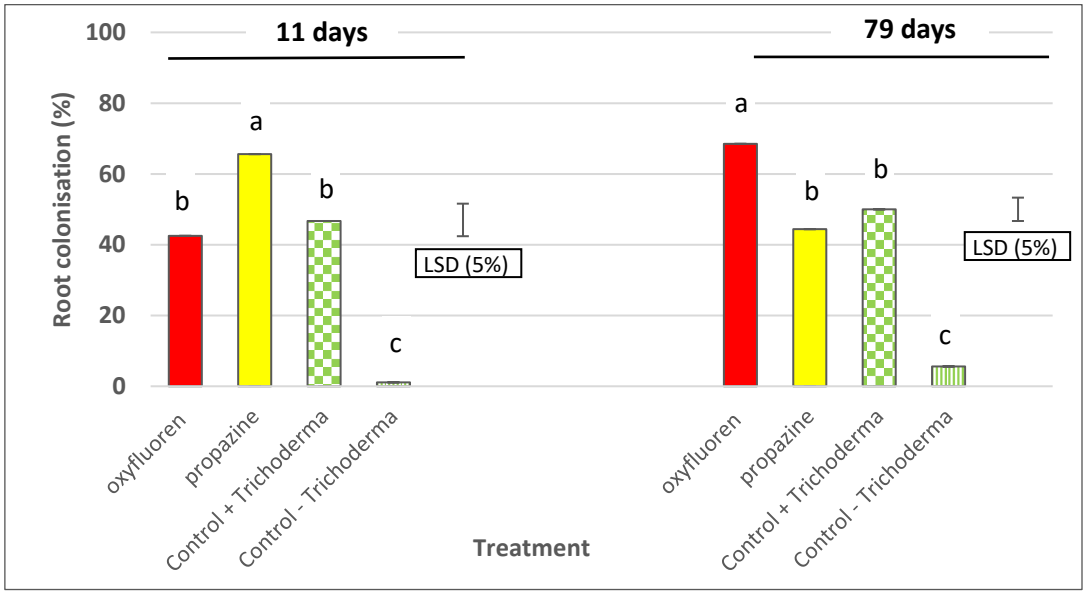

Figure 14: Effect of herbicides, applied to soil four days after sowing of *Trichoderma* inoculated and uninoculated *P. radiata* seeds, on *Trichoderma* root colonisation (%) sampled 11 and 79 days after emergence (Experiment 6).

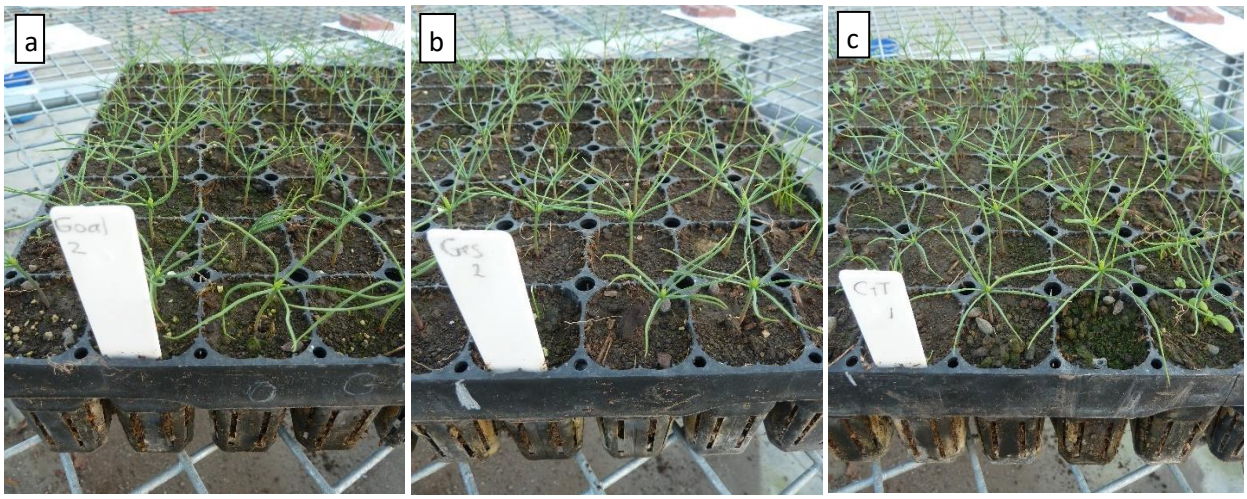

Figure 15: *Trichoderma* inoculated *P. radiata* seedlings 11 days after emergence in soil treated with herbicide (a) oxyfluorfen and b) propazine) four days after sowing. Image c) is *Trichoderma* inoculated seedlings with no herbicide treatment (Experiment 6).

Root *Trichoderma* colonisation in one week old seedlings, was significantly (P<0.05) enhanced (simazine, 86.6%), or similar (terbuthylazine, 73.0%, atrazine, 70.6%, hexazinone, 65.9%) to the inoculated control (70.6%), when grown in soil herbicide-treated 2.5 months prior to seeding (Figures 16 and 17). Although the herbicide glyphosate significantly (P<0.05) reduced root colonisation (57.1%) compared to the inoculated control, it was still at relatively high levels.

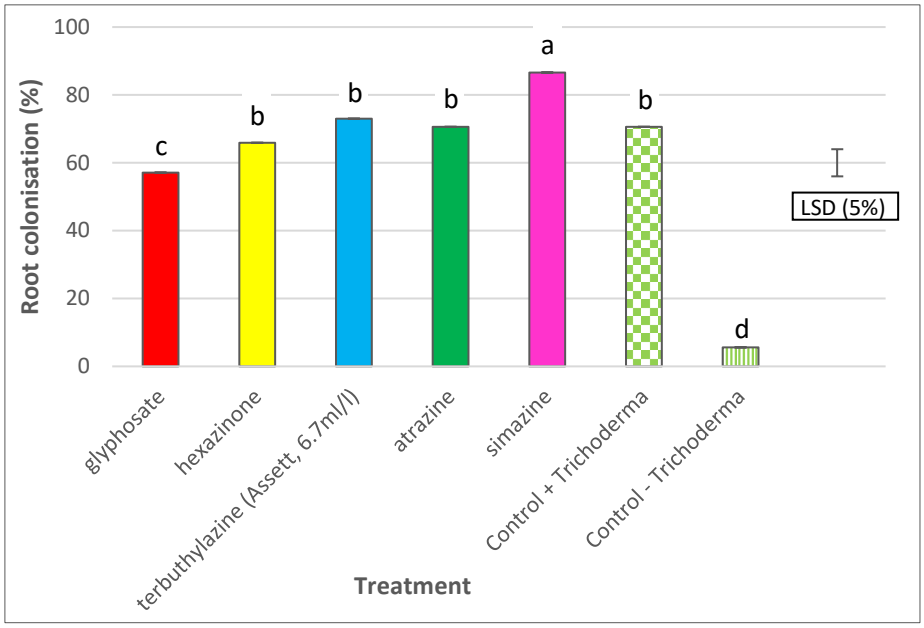

Figure 16: Effect of ground preparation herbicides, applied 8 (simazine) and 78 (glyphosate, hexazinone, terbuthylazine(Assett) and atrazine) days before sowing of *Trichoderma* inoculated and uninoculated *P. radiata* seeds, on *Trichoderma* root colonisation (%) sampled one week after emergence (Experiment 5).

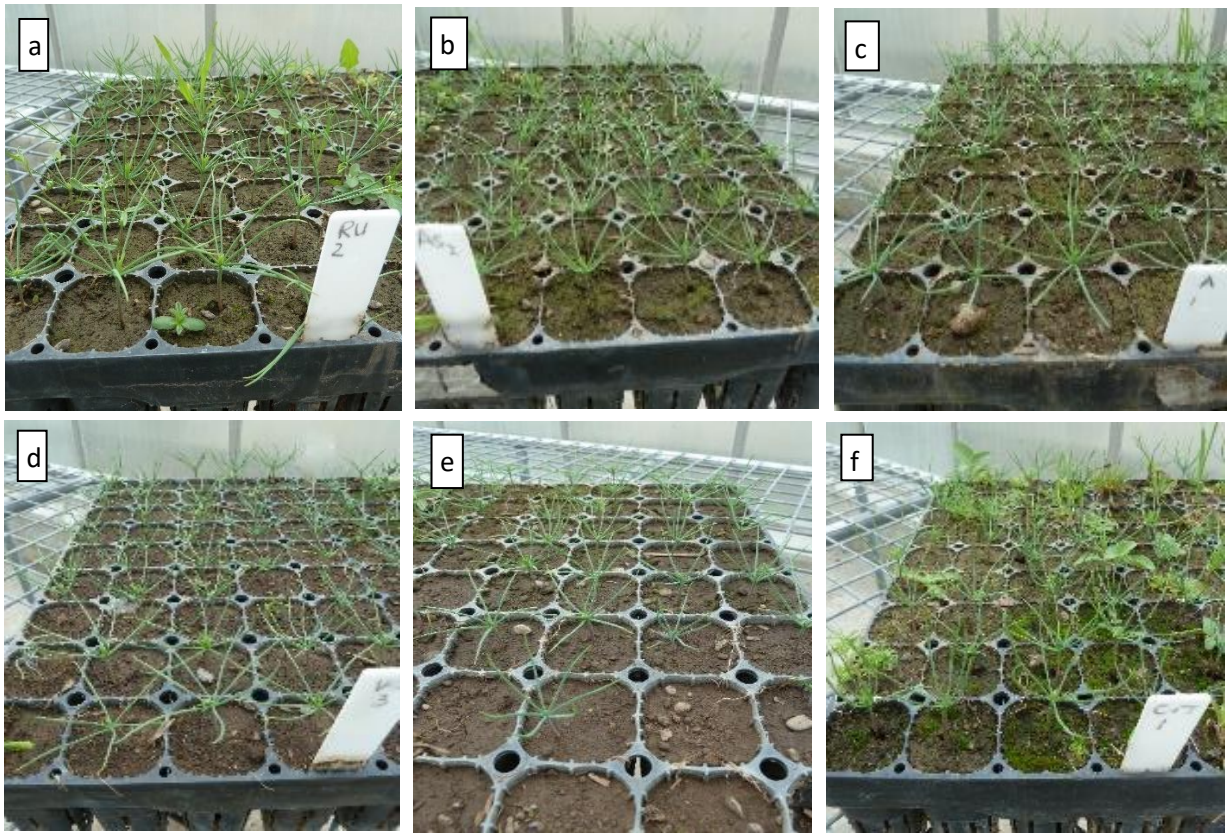

Figure 17: *Trichoderma* inoculated *P. radiata* seedlings one week after emergence from soil treated with herbicides a) glyphosate, b) terbuthylazine, c) atrazine and d) hexazinone 78 days before sowing and e) simazine 8 days before sowing. Image f) is *Trichoderma* inoculated seedlings with no herbicide treatment (Experiment 5).

Table 7: Effect of herbicides on *Trichoderma* isolate spore germination (%) and inhibition (%) of mycelial growth.

Main Effect Means		Spore Germination (%) [a]		Inhibition (%) of mycelial growth [b]
		24 hours	48 hours	
Treatment:				
Active Ingredients and product rate	**Product**			
atrazine	Atrazine 900WG	99.6 a	(100.0)	73.4 c
clopyralid (0.7ml/l)	Void	(100.0)	(100.0)	0.0 l
clopyralid (6.7ml/l)	Void	99.6 a	99.8 ab	32.2 f
glyphosate	Deal 510 RF	(0.0)	0.1 e	96.4 a
haloxyfop-P	AGPRO Haloxyfop 100	98.6 b	99.5 bc	48.7 d
haloxyfop-P	Hurricane	99.3 ab	99.8 ab	46.4 e
hexazinone	Viper 90DF	99.8 a	99.9 a	24.7 h
hexazinone/terbuthylazine (1.7ml/l)	AGPRO Valzine 500	(100.0)	(100.0)	2.9 k
hexazinone/terbuthylazine (16.7ml/l)	AGPRO Valzine 500	97.5 c	99.3 c	87.7 b
oxyfluorfen	Goal Advanced	(100.0)	(100.0)	0.0 l
picloram	AGPRO Picloram 200	(100.0)	(100.0)	-2.3 m
propazine	Gesamil 500FW	0.7 e	2.8 d	(100.0)
simazine	AGPRO Simazine 500	(100.0)	(100.0)	18.6 j
terbuthylazine (6.7ml/l)	Assett	(0.0)	(0.0)	(100.0)
terbuthylazine (6.7ml/l)	AGPRO Terbuthylazine 500	(100.0)	(100.0)	23.2 i
terbuthylazine (26.7ml/l)	AGPRO Terbuthylazine 500	96.5 d	99.2 c	48.6 d
triclopyr	AGPRO Triclop 600	(100.0)	(100.0)	28.1 g
control *(Trichoderma* isolates with no treatment)		(100.0)	(100.0)	-
LSD (5%)		0.6	0.4	0.6
LSE (5%)		0.4	0.3	0.4
Isolate:				
FCC55		87.4 [82.3] a	75.3 [82.5] ab	33.9 [41.7] f
FCC318		86.8 [82.0 ab	74.9 [82.3] bc	35.5 [43.1] c
FCC327		87.0 [82.1] ab	75.4 [82.6] a	34.4 [42.1] e
FCC340		86.8 [82.0] ab	75.3 [82.5] ab	33.3 [41.1] g
FCC13		87.2 [82.2] ab	74.9 [82.3] bc	38.9 [46.1] a
FCC14		84.0 [80.7] d	74.7 [82.2] c	36.6 [44.0] b
FCC15		85.9 [81.6] c	74.9 [82.3] bc	34.9 [42.6] d
FCC180		86.6 [81.9] b	75.0 [82.4] abc	34.4 [42.1] ef
LSD (5%)		0.6 (-)	0.4 (-)	0.4 (-)

[a] Data with zero variability (*ie.* consistently 0 or 100) (in round-brackets) were omitted from ANOVA analysis. Statistical analysis of data with variability was by ANOVA using the unprotected least significant difference (LSD) procedure at the 5% level. Two means with no letters in common differed significantly at $P<0.05$. Statistical comparison of unbracketed and round-bracketed data was by least significant effect (LSE) at the 5% level. Data in square-brackets represent means calculated using all data.

[b] To allow for different growth rates of each isolate, inhibition (%) was calculated by ((X-Y)/X) x 100, where X and Y are the radial growth rate (mm/day) of isolate in the control and agrichemical-amended petri dishes, respectively.

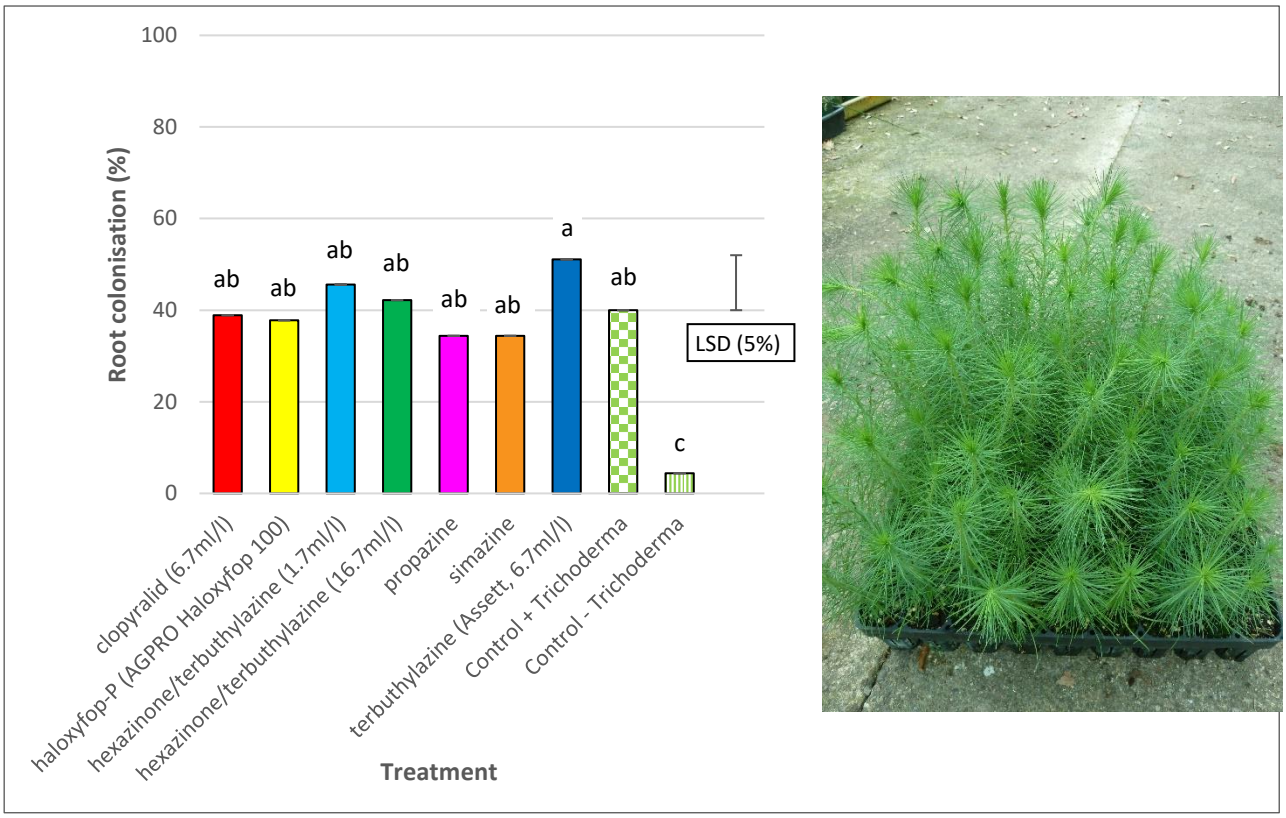

Figure 18: Effect of aerial herbicides, applied 134 days after sowing of *Trichoderma* inoculated and uninoculated *P. radiata* seeds in potting mix, on *Trichoderma* root colonisation (%) sampled 11 days after herbicide application. The image is seedlings immediately prior to spraying (Experiment 7).

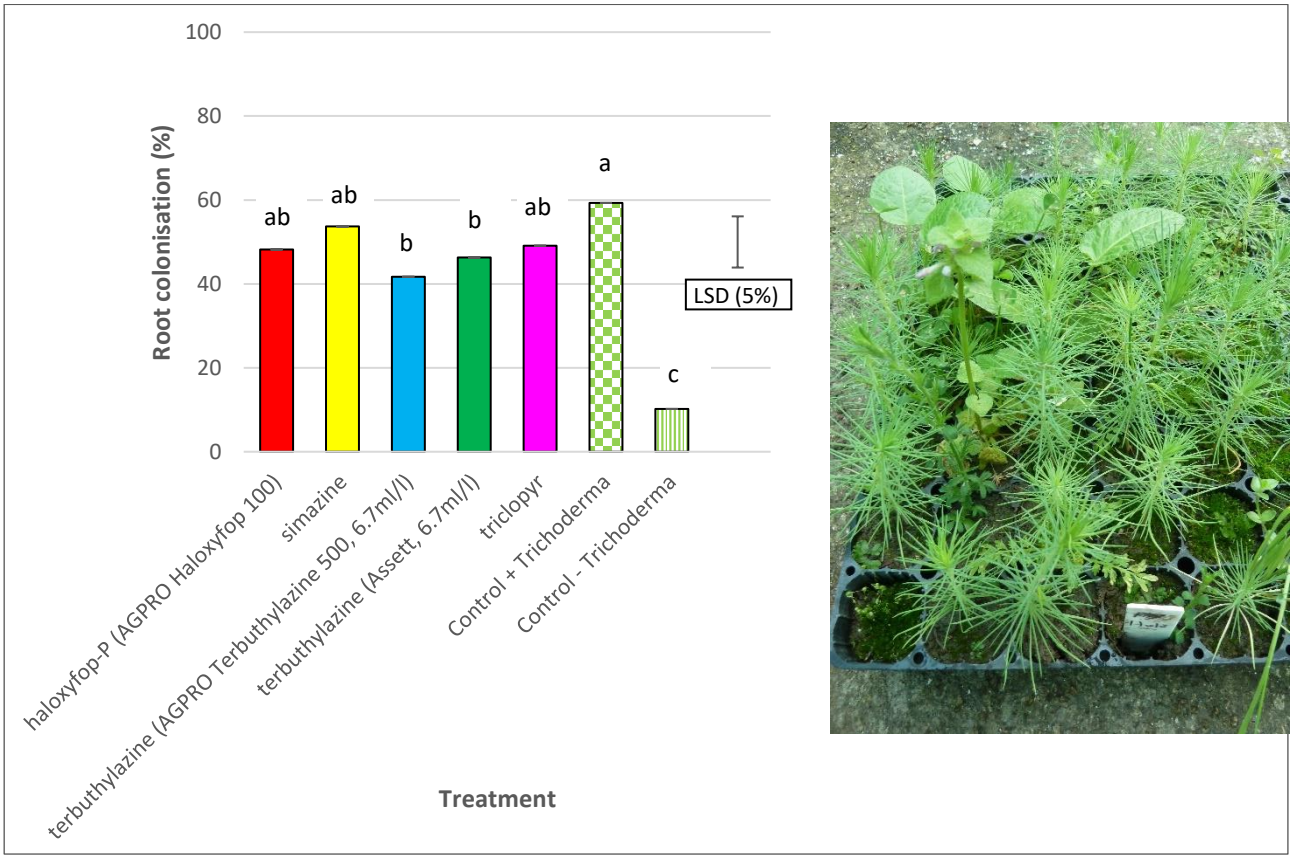

Figure 19: Effect of aerial herbicides, applied 134 days after sowing of *Trichoderma* inoculated and uninoculated *P. radiata* seeds in soil, on *Trichoderma* root colonisation (%) sampled 11 days after herbicide application. The image is seedlings immediately prior to spraying (Experiment 8).

Root colonisation of inoculated 3.5-month-old seedlings planted in potting mix and sprayed with six herbicides (terbuthylazine, hexazinone/terbuthylazine, clopyralid, haloxyfop-P, propazine and simazine) was not significantly ($P<0.05$) different from the inoculated control (Figure 18). When herbicides were sprayed onto seedlings planted in soil, three herbicides (haloxyfop-P, 48.2%, triclopyr, 49.1% and simazine, 53.7%) had similar root colonisation to the control (59.3%; Figure 19). The other two terbuthylazine herbicides, Assett and AGPRO Terbuthylazine 500, had significantly ($P<0.05$) reduced root colonisations (46.3% and 41.7% respectively) but they were not substantially lower than the inoculated control.

Overall, these results suggested that many of the herbicides tested could be mixed with the *Trichoderma* isolates in spray tanks because spore viability was not affected. The exceptions were glyphosate, propazine and terbuthylazine (as Assett). This contrasts with the recommendation to avoid mixing fungal biocontrol and herbicides products, for example, Bioworks (2020) guidelines. When *Trichoderma* was established in the seedling roots it was tolerant to and sometimes enhanced by the soil- or aerially applied herbicides in this study.

In conclusion, the sensitivity of *Trichoderma* PR6 established in the radiata pine roots, to the single application of agrichemicals at recommended rates, was minimal or nil. This result contrasted to results for some of the agrichemicals found to be harmful in the laboratory studies, where the agrichemicals were in direct contact with *Trichoderma*. 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 (*eg.* Sportak EW, Ridomil Gold SL), including those that inhibit fungal mycelial growth (*eg.* Scala, Foschek), there still may been insufficient contact to cause harm to the fungus. In addition, some growing media-applied agrichemicals may have degraded over time, reducing the potential negative impact on *Trichoderma* survival and development. Tolerance of PR6 and PR3a mixtures was found in a commercial containerised nursery with high levels of agrichemicals, with *Trichoderma* colonisation of radiata pine seedling roots at 49%, 32% and 4% in PR6, PR3a and untreated control treatments respectively, measured eight months after inoculation.

Future work could include:

- testing more, or multiple applications of fungicides or herbicides, particularly in commercial nursery seed 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. An understanding of this role in biological systems could lead to safer products with lower toxicities (C. Rowse, AGPRO New Zealand Ltd *pers. comm*.).
- the interaction between bacterial and *Trichoderma* biocontrol organisms, particularly with recent emphasis to increase biocontrol inputs and lower chemical usage in nurseries.
- understanding the complex interactions between pesticides (combination, timing and rates) and *Trichoderma* or other biocontrol organisms (eg. bacteria), either separately or in combination, may lead to lower chemical usage in nurseries (Ons *et. al.,* 2020).
- development of *Trichoderma* formulations which contain encapsulated or microencapsulated spores and/or mycelium to provide better protection against agrichemicals in spray tanks and increase the viability of *Trichoderma*.

4.5 Isolate Selection

PR6 mixture isolates had significantly (P<0.05) increased mean spore germination and mycelial growth, compared to the PR3a mixture, when analysed using all agrichemical data (Table 8). However, the difference between mixtures was not large in a biological sense. Both mixtures are therefore recommended for use as biocontrol agents in nurseries that use agrichemicals.

Sensitivity of *Trichoderma* species to agrichemicals, could be ordered from less to more, as: *T. harzianum* and *T. atrobrunneum* > *T. atroviride, T. crassum* and *T. asperellum,* with the main effectors being the applied fungicides (Table 6) and herbicides (Table 7).

Table 8: Effect of all agrichemicals tested on *Trichoderma* isolate spore germination (%) and inhibition (%) of mycelial growth.

Isolate	Species	24hr germination [a]	48hr germination	Inhibition (%) of mycelial growth
PR6 mixture:				
FCC55	*T. harzianum*	68.2 [79.6]	77.6 [87.7]	30.4 [36.0]
FCC318	*T. atrobrunneum*	68.0 [79.5]	77.6 [87.7]	30.7 [36.4]
FCC327	*T. harzianum*	68.0 [79.5]	77.5 [87.7]	30.3 [36.0]
FCC340	*T. harzianum*	67.7 [79.4]	77.5 [87.7]	31.3 [36.9]
PR3a mixture:				
FCC13	*T. asperellum*	68.0 [79.5]	77.4 [87.6]	37.1 [42.3]
FCC14	*T. atroviride*	63.1 [77.3]	76.5 [87.3]	34.1 [39.5]
FCC15	*T. atroviride*	64.0 [77.7]	76.5 [87.3]	33.0 [38.4]
FCC180	*T. crassum*	65.6 [78.4]	76.8 [87.4]	31.8 [37.4]
PR6 mixture mean		**68.0 [79.5] a**	**77.6 [87.7] a**	**30.7 [36.3] b**
PR3a mixture mean		**65.2 [78.2] b**	**76.8 [87.4] b**	**34.0 [39.4] a**
LSD (5%)		2.64 [1.18]	0.52 [0.17]	2.83 [2.64]
Significance of difference		*	*	*

[a] Data with zero variability (*eg.* 0 or 100% values) were omitted from ANOVA analysis. Statistical analysis of data with variability was by ANOVA using the unprotected least significant difference (LSD) procedure at the 5% level. Two means with no letters in common differed significantly at P<0.05. Data in square-brackets represent means calculated using all data.

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John Oliver, Ravensdown Limited
Jason Trethewey, Syngenta Crop Protection Limited and
Nigel Heron, Timberlands Te Ngae Nursery.

7.0 APPENDICES

APPENDIX A: Malt Yeast Extract Agar with Rose Bengal (MRB) Recipe:

Malt extract	10g
Yeast extract	1g
Rose Bengal (50mg/ml)	3ml
Terrachlor 75WP	0.2g
Agar	20
Chloramphenicol stock solution (100mg/ml)	1ml

Make up to 1000ml with distilled water.

APPENDIX B: Chemical analysis of Ag Concepts AgZyme and SuperHume, Nitrophoska Extra and AgriSea Foliar.

Ag Concepts AgZyme:

0.02%	Boron
0.0005%	Cobalt
0.10%	Iron
0.0005%	Manganese
0.05%	Zinc
<1.00%	Thiamine
<1.00%	Riboflavin
<1.00%	Carrageenan
<1.00%	Kelp Extract
<1.00%	Bromelain
<1.00%	Papain
<1.00%	Sucrose

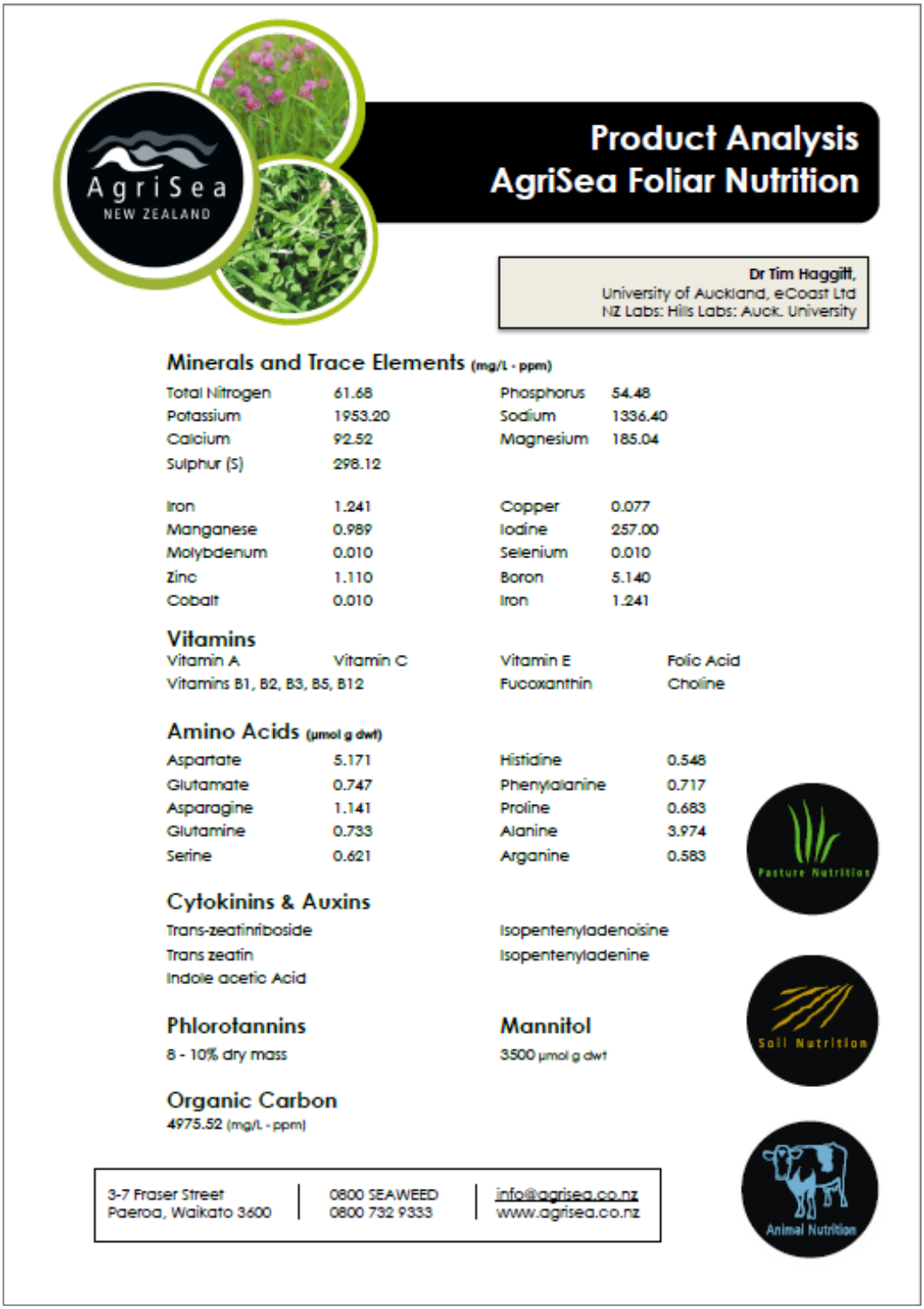
The chelating agent is EDTA (ethyleneduaminetetraaceic acd) and HEDTA (hyroxyethylenediaminetetraacetic acid).

Ag Concepts SuperHume:

6%	Humic acids, derived from Leonardite
0.6%	Kelp Extract and organic acids
93.4%	Inert Ingredients

Nitrophoska Extra:
12% Nitrogen (N) - 4.8% Nitrate N - 7.2% Ammonium N
5.2% Phosphorus (P)
14.1% Potassium (K) as Potassium Sulphate
8.0% Sulphur (S)
1.2% Magnesium (Mg) as Kieserite
3.8% Calcium (Ca)
0.4% Iron (Fe)
0.02% Boron (B)
0.01% Zinc (Zn)

AgriSea Foliar:



AgriSea NEW ZEALAND

Product Analysis
AgriSea Foliar Nutrition

Dr Tim Haggitt,
University of Auckland, eCoast Ltd
NZ Labs: Hills Labs: Auck. University

Minerals and Trace Elements (mg/L - ppm)

Total Nitrogen	61.68	Phosphorus	54.48
Potassium	1953.20	Sodium	1336.40
Calcium	92.52	Magnesium	185.04
Sulphur (S)	298.12		
Iron	1.241	Copper	0.077
Manganese	0.989	Iodine	257.00
Molybdenum	0.010	Selenium	0.010
Zinc	1.110	Boron	5.140
Cobalt	0.010	Iron	1.241

Vitamins

Vitamin A	Vitamin C	Vitamin E	Folic Acid
Vitamins B1, B2, B3, B5, B12		Fucoxanthin	Choline

Amino Acids (µmol g dwt)

Aspartate	5.171	Histidine	0.548
Glutamate	0.747	Phenylalanine	0.717
Asparagine	1.141	Proline	0.683
Glutamine	0.733	Alanine	3.974
Serine	0.621	Arganine	0.583

Cytokinins & Auxins

Trans-zeatinriboside — Isopentenyladenoisine
Trans zeatin — Isopentenyladenine
Indole acetic Acid

Phlorotannins

8 - 10% dry mass

Mannitol

3500 µmol g dwt

Organic Carbon

4975.52 (mg/L - ppm)

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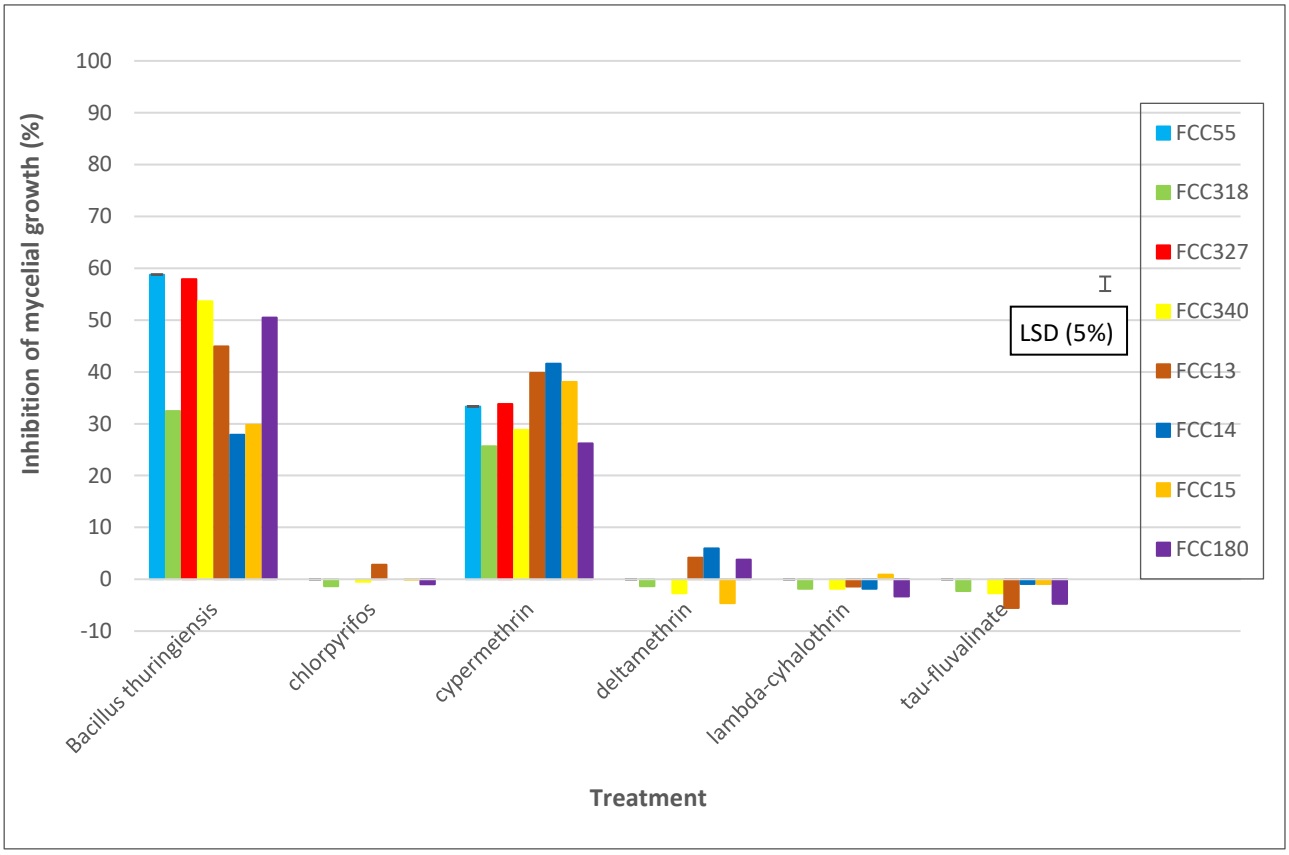
APPENDIX C: Effect of insecticides on spore germination (%), A) 24 hours and 48 hours after spore and agrichemical mixing, and B) inhibition of mycelial growth of eight *Trichoderma* isolates. Bar in graph is LSD at 5% level.

A

Treatment		Time	*Trichoderma* Isolates							
Active Ingredient	**Product**		**FCC55**	**FCC318**	**FCC327**	**FCC340**	**FCC13**	**FCC14**	**FCC15**	**FCC180**
Bacillus thuringiensis	Bactur WDG	24hr	97.7	93.0	91.0	90.3	99.7	99.0	97.7	100.0
		48hr	100.0	99.0	98.0	99.3	99.7	99.3	99.3	100.0
chlorpyrifos	Lorsban 50EC	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
cypermethrin	Ripcord	24hr	100.0	100.0	100.0	100.0	99.3	96.3	98.7	100.0
		48hr	100.0	100.0	100.0	99.3	99.3	99.3	100.0	100.0
deltamethrin	Ballistic	24hr	100.0	100.0	100.0	100.0	100.0	100.0	97.7	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
lambda-cyhalothrin	Karate Zeon	24hr	100.0	100.0	99.7	99.7	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
tau-fluvalinate	Mavrik Aquaflo	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
control (*Trichoderma* isolates with no agrichemicals)		24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Treatment x isolate interaction LSD (5%) at 24 and 48 hours = 1.9 and 1.3 respectively Significance of Isolate x Treatment = P<0.001										

B

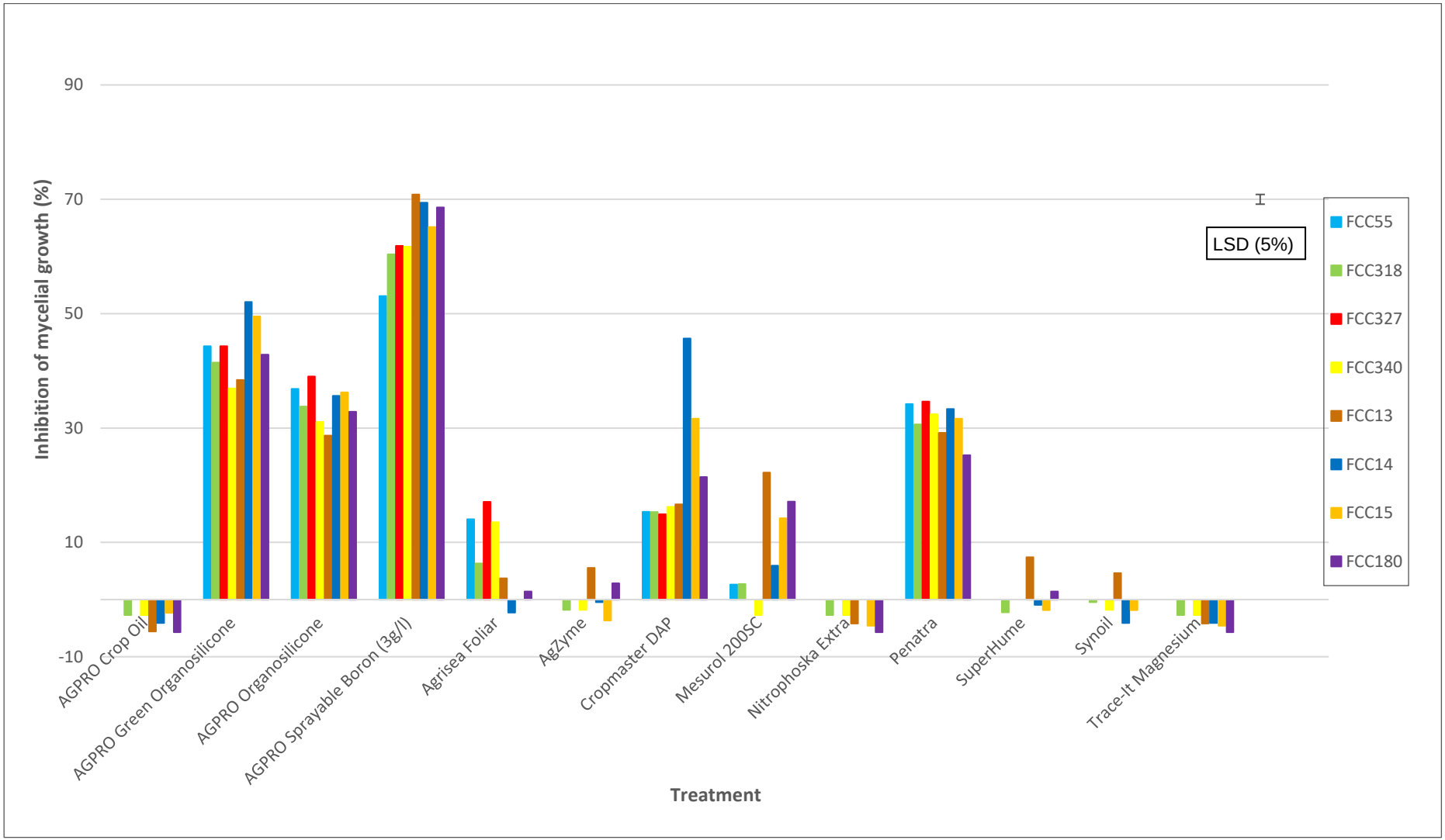


APPENDIX D: Effect of adjuvants, fertilisers and biostimulants on spore germination (%) of eight Trichoderma isolates 24 hours and 48 hours after agrichemical and spore mixing.

Treatment		Time	*Trichoderma* Isolates							
			FCC55	FCC318	FCC327	FCC340	FCC13	FCC14	FCC15	FCC180
Active Ingredient	**Product**									
disodium octaborate tetrahydrate	AGPRO Sprayable Boron (3g/l)	24hr 48hr	0.7 2.0 (±0.0) [a]	1.0 1.7 (±0.3)	0.7 2.0 (±0.6)	2.3 1.3 (±0.3)	1.0 3.0 (±0.0)	0.7 1.7 (±0.3)	2.3 1.3 (±0.3)	0.7 3.3 (±0.7)
humic acid/kelp extract/organic acids	SuperHume	24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
macro- and micro-nutrients; see Appendix B	Nitrophoska Extra	24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	99.7 100.0	100.0 100.0	100.0 100.0	100.0 100.0
magnesium/nitrogen	Trace-It Magnesium	24hr 48hr	100.0 100.0	100.0 100.0	99.3 100.0	99.3 100.0	100.0 100.0	100.0 100.0	99.3 100.0	100.0 100.0
methiocarb	Mesurol 200SC	24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	99.7 100.0
mineral oil	AGPRO Crop Oil	24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
multiple; Appendix B	AgriSea Foliar	24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
multiple; Appendix B	AgZyme	24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
nitrogen/phosphate /sulphur	Cropmaster DAP	24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
paraffinic oil	Synoil	24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
polyether modified polysiloxane	AGPRO Green Organosilicone	24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
polyether modified polysiloxane	AGPRO Organosilicone	24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	99.7 100.0	100.0 100.0	99.7 100.0
polyether modified polysiloxane	Penatra	24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
control *(Trichoderma* isolates with no agrichemicals)		24hr 48hr	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
Treatment x isolate interaction LSD (5%) at 24 hours = 0.9 Significance of Isolate x Treatment = P<0.001										

[a] Standard error of mean in brackets

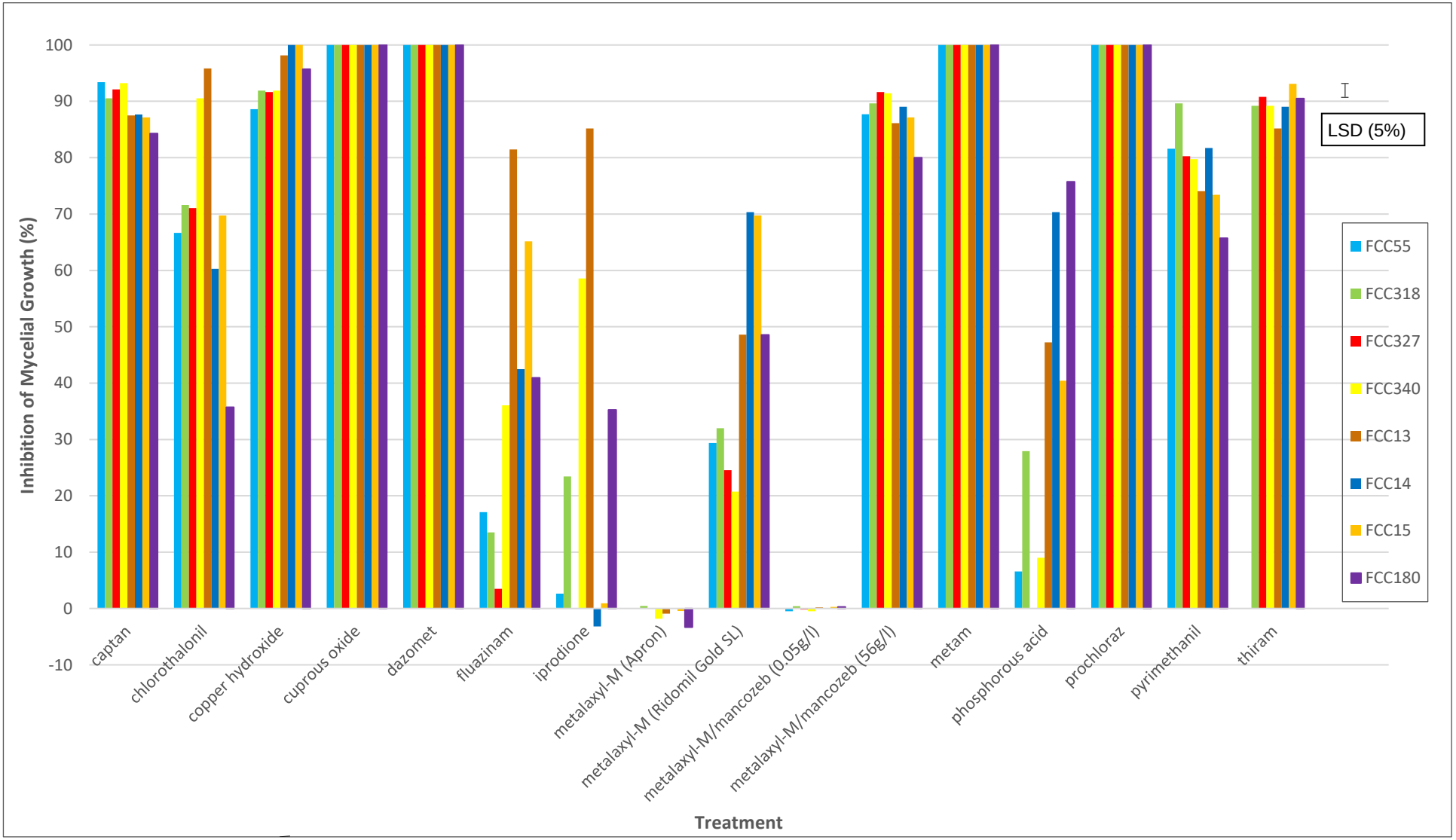
APPENDIX E: Effect of adjuvants, fertilisers and biostimulants on inhibition (%) of mycelial growth of eight *Trichoderma* isolates compared to un-amended *Trichoderma* controls. Bar is LSD at 5% level



APPENDIX F: Effect of fungicides on spore germination (%) of eight Trichoderma isolates 24 and 48 hours after agrichemical and spore mixing.

Treatment		Time	*Trichoderma* Isolates							
			FCC55	FCC318	FCC327	FCC340	FCC13	FCC14	FCC15	FCC180
Active Ingredient	**Product**									
captan	Fruitfed Captan 80WG	24hr	2.3	2.0	4.3	5.0	2.3	2.0	1.3	1.0
		48hr	100.0	98.7	99.3	97.7	100.0	91.7	97.0	100.0
chlorothalonil	Cavalry 720SC	24hr	1.0	0.7	0.7	0.3	1.0	0.0	0.0	0.3
		48hr	100.0	100.0	100.0	99.7	100.0	99.7	100.0	100.0
copper hydroxide	Kocide Opti	24hr	4.7	10.0	5.3	6.0	4.7	0.7	1.3	4.0
		48hr	100.0	100.0	99.3	100.0	100.0	99.3	100.0	100.0
cuprous oxide	Nordox 75WG	24hr	95.0	93.7	96.7	98.0	94.7	34.0	36.3	53.0
		48hr	100.0	100.0	99.7	100.0	100.0	99.3	91.0	76.7
dazomet	Basamid Granular	24hr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		48hr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
fluazinam	Gem	24hr	0.3	0.0	0.7	0.0	0.7	1.0	0.7	0.3
		48hr	91.7	96.0	93.7	94.3	90.0	93.7	96.7	100.0
iprodione	Rapid 500	24hr	100.0	99.7	100.0	95.0	96.0	80.7	80.0	91.3
		48hr	100.0	100.0	100.0	100.0	100.0	93.3	91.7	100.0
metalaxyl-M	Apron	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
metalaxyl-M	Ridomil Gold SL	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
metalaxyl-M/ mancozeb	Ridomil Gold MZ WG (0.05g/l)	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
metalaxyl-M/ mancozeb	Ridomil Gold MZ WG (56g/l)	24hr	100.0	100.0	100.0	100.0	100.0	99.7	100.0	99.7
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
metam	Fumasol	24hr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		48hr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
phosphorous acid	Foschek	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
prochloraz	Sportak EW	24hr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		48hr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
pyrimethanil	Scala	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
thiram	Thiram 40F	24hr	0.0	1.0	1.3	0.0	0.3	1.0	1.3	0.7
		48hr	0.3	1.3	1.7	2.0	1.7	1.0	1.7	2.0
control *(Trichoderma* isolates with no agrichemicals)		24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Treatment x isolate interaction LSD (5%) at 24 and 48 hours = 3.1 and 2.3 respectively Significance of Isolate x Treatment = P<0.001										

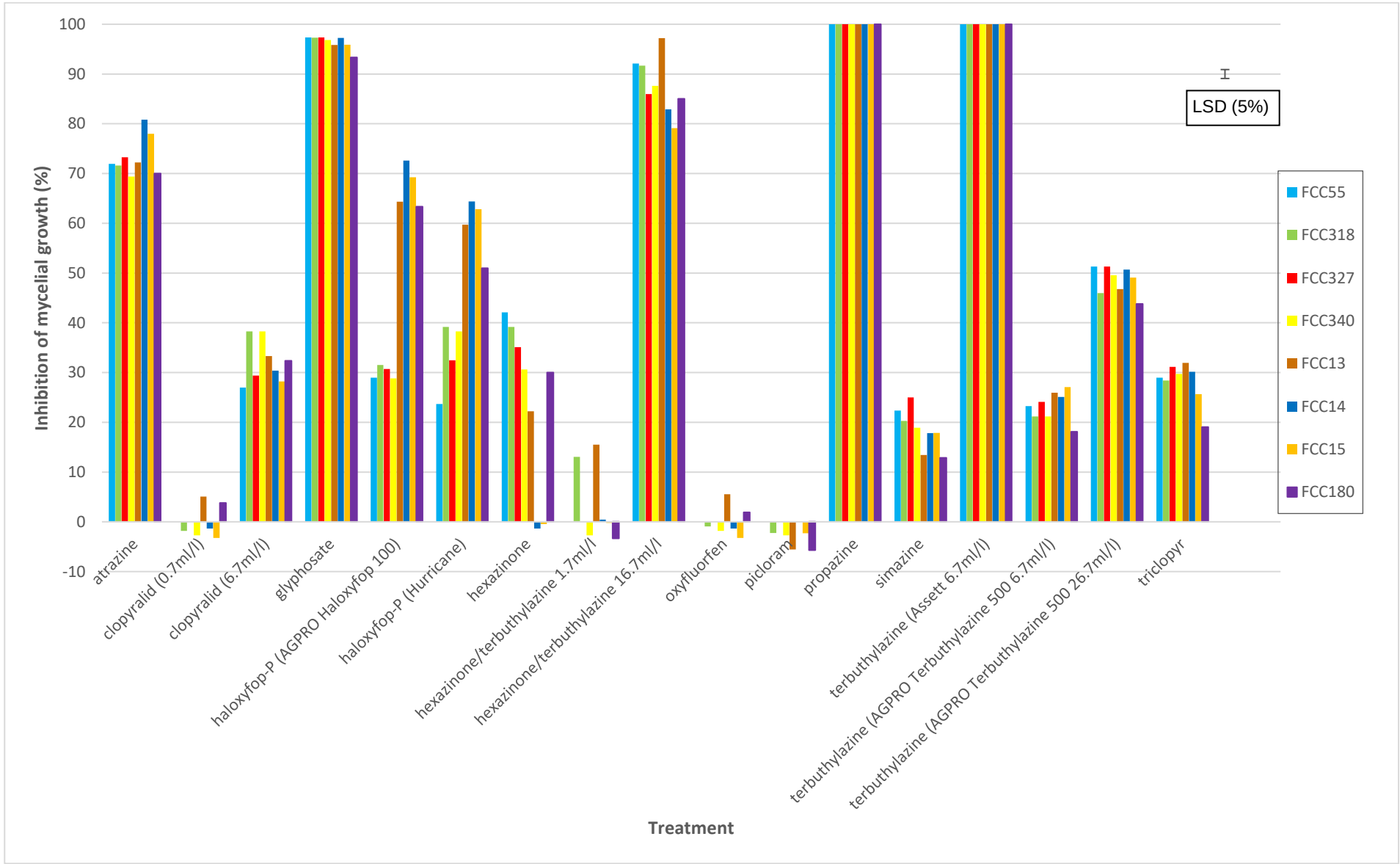
APPENDIX G: Effect of fungicides and fumigants on inhibition (%) of mycelial growth of eight *Trichoderma* isolates. Bar is LSD at 5% level.



APPENDIX H: Effect of herbicides on spore germination (%) of eight Trichoderma isolates 24 and 48 hours after agrichemical and spore mixing.

Treatment		Time	*Trichoderma* Isolates							
			FCC55	FCC318	FCC327	FCC340	FCC13	FCC14	FCC15	FCC180
Active Ingredient	**Product**									
atrazine	Atrazine 900WG	24hr	100.0	100.0	99.7	99.3	98.7	99.3	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	99.7	100.0	100.0
clopyralid (0.7ml/l)	Void	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
clopyralid (6.7ml/l)	Void	24hr	100.0	99.0	99.7	99.0	100.0	100.0	100.0	99.0
		48hr	100.0	100.0	99.7	99.3	100.0	99.3	91.0	99.7
glyphosate	Deal 510 RF	24hr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		48hr	0.0	0.0	0.3	0.0	0.3	0.3	0.0	0.0
haloxyfop-P	AGPRO Haloxyfop 100	24hr	100.0	100.0	100.0	95.0	100.0	98.7	9.0	96.3
		48hr	100.0	100.0	100.0	98.0	100.0	99.0	99.3	99.3
haloxyfop-P	Hurricane	24hr	100.0	98.7	99.7	100.0	99.7	100.0	100.0	96.0
		48hr	100.0	100.0	100.0	99.7	100.0	100.0	100.0	99.0
hexazinone	Viper 90DF	24hr	100.0	99.7	100.0	99.3	100.0	99.7	100.0	100.0
		48hr	100.0	100.0	100.0	99.7	100.0	99.7	100.0	100.0
hexazinone/ terbuthylazine (1.7ml/l)	AGPRO Valzine 500	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
hexazinone/ terbuthylazine (16.7ml/l)	AGPRO Valzine 500	24hr	97.7	96.7	99.3	100.0	98.3	91.7	96.0	100.0
		48hr	100.0	98.7	100.0	100.0	100.0	98.0	97.3	100.0
oxyfluorfen	Goal Advanced	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
picloram	AGPRO Picloram 200	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
propazine	Gesamil 500FW	24hr	1.3	0.0	0.3	1.3	1.0	0.0	0.0	1.3
		48hr	2.0	1.0	3.3	5.0	2.3	3.7	3.0	2.0
simazine	AGPRO Simazine 500	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
terbuthylazine (6.7ml/l)	Assett	24hr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		48hr	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
terbuthylazine (6.7ml/l)	AGPRO Terbuthylazine 500	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
terbuthylazine (26.7ml/l)	AGPRO Terbuthylazine 500	24hr	100.0	100.0	97.3	100.0	100.0	82.3	92.0	100.0
		48hr	100.0	100.0	99.3	100.0	100.0	97.0	97.0	100.0
triclopyr	AGPRO Triclop 600	24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
control *(Trichoderma* isolates with no agrichemicals)		24hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		48hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Treatment x isolate interaction LSD (5%) at 24 and 48 hours = 1.8 and 1.2 respectively Significance of Isolate x Treatment = P<0.001										

APPENDIX I: Effect of herbicides on inhibition (%) of mycelial growth of eight *Trichoderma* isolates. Bar is LSD at 5% level.



APPENDIX J: Effect of insecticides, adjuvants, fertilisers, biostimulants, fungicides, fumigants and herbicides on observed sporulation of eight Trichoderma isolates 68 and 130 hours after placement of mycelium plug on agrichemical amended MYE.

Treatment		Time	*Trichoderma* Isolates (FCC-) [a]							
			55	318	327	340	13	14	15	180
Active Ingredient	**Product**									
control *(Trichoderma* isolate with no agrichemicals)		68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
Insecticides:										
Bacillus thuringiensis	Bactur WDG	68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
chlorpyrifos	Lorsban 50EC	68hr	✓	✓	✓	✓	✓	✓	×	×
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
cypermethrin	Ripcord	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
deltamethrin	Ballistic	68hr	✓	×	✓	×	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
lambda-cyhalothrin	Karate Zeon	68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
tau-fluvalinate	Mavrik Aquaflo	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
Adjuvants, Fertilsers and Biostimulants*:*										
disodium octaborate tetrahydrate	AGPRO Sprayable Boron (3g/l)	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
humic acid/kelp extract/organic acids	SuperHume	68hr	×	×	✓	×	✓	×	×	×
		130hr	✓	✓	✓	×	✓	×	✓	✓
macro- and micro-nutrients	Nitrophoska Extra	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
magnesium/nitrogen	Trace-It Magnesium	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
methiocarb	Mesurol 200SC	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
mineral oil	AGPRO Crop Oil	68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
nitrogen/phosphate /sulphur	Cropmaster DAP	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
paraffinic oil	Synoil	68hr	✓	✓	✓	✓	✓	×	×	×
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
polyether modified polysiloxane	AGPRO Green Organosilicone	68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
polyether modified polysiloxane	AGPRO Organosilicone	68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
polyether modified polysiloxane	Penatra	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
see Appendix 1	AgriSea Foliar	68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
see Appendix 1	AgZyme	68hr	✓	✓	✓	✓	✓	×	×	×
		130hr	✓	✓	✓	✓	✓	×	✓	✓
Fungicides and Fumigants:										
captan	Fruitfed Captan 80WG	68hr	✓	✓	✓	✓	✓	×	×	×
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
chlorothalonil	Cavalry 720SC	68hr	✓	✓	✓	✓	×	×	✓	×
		130hr	✓	✓	✓	✓	×	✓	✓	✓
copper hydroxide	Kocide Opti	68hr	×	✓	×	×	×	×	×	×
		130hr	×	✓	×	×	×	×	×	×
cuprous oxide	Nordox 75WG	68hr	×	×	×	×	×	×	×	×
		130hr	×	×	×	×	×	×	×	×
dazomet	Basamid Granular	68hr	×	×	×	×	×	×	×	×
		130hr	×	×	×	×	×	×	×	×

fluazinam	Gem	68hr	✓	✓	×	×	×	×	×	×
		130hr	✓	✓	×	×	×	×	×	×
iprodione	Rapid 500	68hr	✓	✓	✓	×	×	×	×	×
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
metalaxyl-M	Apron	68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
metalaxyl-M	Ridomil Gold SL	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
metalaxyl-M/mancozeb (0.05g/l)	Ridomil Gold MZ WG	68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
metalaxyl-M/mancozeb (56g/l)	Ridomil Gold MZ WG	68hr	✓	✓	✓	✓	✓	×	×	×
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
metam	Fumasol	68hr	×	×	×	×	×	×	×	×
		130hr	×	×	×	×	×	×	×	×
phosphorous acid	Foschek	68hr	✓	✓	✓	✓	×	×	×	×
		130hr	✓	✓	✓	✓	×	✓	✓	✓
prochloraz	Sportak EW	68hr	×	×	×	×	×	×	×	×
		130hr	×	×	×	×	×	×	×	×
pyrimethanil	Scala	68hr	×	×	×	×	×	×	×	×
		130hr	×	×	×	×	×	×	×	×
thiram	Thiram 40F	68hr	×	×	×	×	×	✓	✓	×
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
Herbicides:										
atrazine	Atrazine 900WG	68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
clopyralid (0.7ml/l)	Void	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
clopyralid (6.7ml/l)	Void	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
glyphosate	Deal 510 RF	68hr	×	×	×	×	×	×	×	×
		130hr	×	×	×	×	×	×	×	×
haloxyfop-P	AGPRO Haloxyfop 100	68hr	×	×	×	×	✓	×	×	×
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
haloxyfop-P	Hurricane	68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
hexazinone	Viper 90DF	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
hexazinone/terbuthylazine (1.7ml/l)	AGPRO Valzine 500	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
hexazinone/terbuthylazine (16.7ml/l)	AGPRO Valzine 500	68hr	×	×	×	×	×	✓	✓	×
		130hr	×	×	×	×	×	✓	✓	×
oxyfluorfen	Goal Advanced	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
picloram	AGPRO Picloram 200	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
propazine	Gesamil 500FW	68hr	×	×	×	×	×	×	×	×
		130hr	×	×	×	×	×	×	×	×
simazine	AGPRO Simazine 500	68hr	✓	✓	✓	✓	✓	✓	✓	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓
terbuthylazine (6.7ml/l)	Assett	68hr	×	×	×	×	×	×	×	×
		130hr	×	×	×	×	×	×	×	×
terbuthylazine (6.7ml/l)	AGPRO Terbuthylazine 500	68hr 130hr	✓ ✓	✓ ✓	✓ ✓	✓ ✓	✓ ✓	✓ ✓	✓ ✓	✓ ✓
terbuthylazine (26.7ml/l)	AGPRO Terbuthylazine 500	68hr 130hr	✓ ✓	✓ ✓	✓ ✓	✓ ✓	✓ ✓	✓ ✓	✓ ✓	✓ ✓
triclopyr	AGPRO Triclop 600	68hr	✓	✓	✓	✓	✓	×	×	✓
		130hr	✓	✓	✓	✓	✓	✓	✓	✓

[a] presence (✓) or absence (×) of sporulation on the three replicate agar petri dishes