Plant Pathology (2009) **58**, 723–730 Doi: 10.1111/j.1365-3059.2009.02021.x

Management of phytophthora root rot in radiata pine seedlings

T. Reglinski[a]*, T. M. Spiers[a], M. A. Dick[b], J. T. Taylor[a] and J. Gardner[b]

[a]*The New Zealand Institute for Plant and Food Research Limited, Ruakura Research Centre, Private Bag 3123, Hamilton; and* [b]*NZ Forest Research Institute (Scion), Private Bag 3020, Rotorua, New Zealand*

Chemical and biological agents were evaluated for their ability to suppress root rot, caused by *Phytophthora cactorum*, in field-grown radiata pine seedlings in New Zealand. Trials were conducted over two seasons in an area of a forest nursery with a natural infestation of *P. cactorum*, and a history of root rot. In each season, symptoms of root rot developed during April, one month after root pruning, when seedlings were approximately six months old. In trial one, root rot incidence by mid July 2007 was 9·1% in untreated plots and 8·4% in plots that had been treated with metalaxyl-M/mancozeb (14 kg ha^{-1}) at seedling emergence. Disease incidence was lowest (2·1%) in plots that received seven monthly applications of phosphorous acid (6·5 L ha^{-1}). Other treatments, including seed coating with thiram or *Trichoderma* spp., and foliar applications of methyl jasmonate, did not control disease. In trial two, effects of treatment timing relative to root pruning were investigated. By late June 2008, three months after root pruning, root rot incidence was 22·2% in the untreated plots. Phosphorous acid was the most effective treatment and almost completely suppressed disease (0·1% incidence) when applied fortnightly from February until May (seven applications). Metalaxyl-M/mancozeb (15 kg ha^{-1}) was not effective (21·4% incidence) when applied five months before root pruning. However, disease incidence was reduced when the chemical was applied one week after root pruning (14·9% incidence) and greater control was achieved (8·2% incidence) when the application rate was increased to 50 kg ha^{-1}.

Keywords: metalaxyl-M/mancozeb, phosphorous acid, *Phytophthora cactorum*, *Pinus radiata*

Introduction

Forest nurseries in New Zealand produce over 50 million radiata pine (*Pinus radiata*) seedlings annually and operate with a considerable level of chemical intervention in order to manage the diseases caused by fungi and oomycetes that affect these seedlings. Root disease caused by *Phytophthora* spp. can be problematic for seedling production in bare-root nurseries (Dumroese & James, 2005). *Phytophthora cinnamomi* is frequently associated with root rot and has been isolated from soils in forest nurseries and indigenous forests in New Zealand (Johnston *et al*., 2003), Australia (Cahill *et al*., 2008), the US (Balci *et al*., 2007) and Europe (Vettraino *et al*., 2005). Phytophthora root rot occurs most readily in poorly drained soils where free water can disseminate motile zoospores (Hardham, 2005). There is evidence that root hypoxia can increase plant susceptibility to infection by *P. cinnamomi* (Messenger *et al*., 2000). Although not a major pathogen of forest plantations in New Zealand, *P. cinnamomi* has been reported to cause root and collar rot of a number of tree species in waterlogged soils (Johnston *et al*., 2003). Similarly, *P. cactorum* has also been reported to cause root rot of trees in New Zealand (Newhook, 1959) but is more commonly associated with disease in pine seedlings (Gadgil, 2005). In the early 1970s, *P. cactorum* was associated with high incidence of root rot in *P. radiata* seedlings at the Milton Forest Service Nursery, Otago, New Zealand, that eventually led to the closure of the nursery.

Chemical control remains an important component in the management of *Phytophthora* in plant nurseries. Phenylamides such as metalaxyl and metalaxyl-M (also called mefenoxam) have been used extensively for control of phytophthora root rots of a wide range of plants, including conifers (Humphrey, 1980; Erwin & Ribeiro, 1996). Salts of phosphorous acid, also referred to as phosphonate or phosphite, have been shown to provide effective control of *Phytophthora*-related diseases in several plant species (Hardy *et al*., 2001). These compounds exhibit direct activity against the pathogen and can activate host resistance against infection (Hardy *et al*., 2001; Daniel *et al*., 2005). Application of potassium phosphonate caused a reduction in *P. cinnamomi* root rot in glasshouse-grown radiata pine (Ali *et al*., 2000). More recently, phosphorous

*E-mail: treglinski@hortresearch.co.nz

Published online 8 March 2009

acid-based products, Foli-R-Fos® 400, and Fosject® 400, have demonstrated efficacy against *P. cinnamomi* in mature *Banksia* and *Eucalyptus marginata* trees (Shearer & Fairman, 2007a,b).

Careful management of chemical control options is necessary to minimise the risk of resistance development among pathogen populations and to reduce the impact of chemicals upon the environment. Integrated management strategies have been developed to use chemicals in a sustainable manner by combining them with biological and cultural methods. Biological options, including microbial antagonists such as *Trichoderma* spp., and activators of host disease resistance such as methyl jasmonate (MeJA), have demonstrated potential to contribute to disease management in forest nurseries (Reglinski & Dick, 2005). There is evidence that the amendment of the growing medium with organic compounds and/or minerals may also contribute to the suppression of root pathogens. For example, humate (a mixture of mineral salts of humic and fulvic acids formed from the microbial degradation of plant and animal tissues) has been proposed to encourage the proliferation of beneficial microbes, including trichoderma spp., (Pascual *et al.*, 2002; Vawdrey *et al.*, 2002) whilst calcium, either as gypsum (calcium sulphate) or lime (calcium carbonate) has been used to suppress phytophthora root rot in susceptible crops (Messenger *et al.*, 2000; Campanella *et al.*, 2002).

In this study, disease management schedules involving combinations of metalaxyl-M/mancozeb, phosphorous acid, *Trichoderma* spp., humate plus lime and methyl jasmonate were evaluated for their ability to control root rot caused by *P. cactorum* in radiata pine seedlings.

Materials and methods

Trial design

Two trials were carried out at the Kaingaroa Timberlands Nursery, Te Ngae, Rotorua, in the central North Island, New Zealand: trial one over the period September 2006 to July 2007, and trial two over the period October 2007 to June 2008. The soil type was Rotomahana shallow sandy loam. Seedbeds were prepared in an area that was prone to water run-off from blocks of higher elevation during heavy rain and that had suffered over 30% losses to phytophthora root rot during the 2005/06 season. Radiata pine seed supplied by PF Olsen Ltd., New Zealand, was a control pollinated mix of 15 parents with a growth and form (GF) 24 rating (seed lot 05/203) in trial one and was an open pollinated mix with a GF19 rating (seed lot 05/611) in trial two. Before sowing, seed was stratified by soaking for 48 h and then storing for 14 days at 4°C in moist conditions.

Trial one

The trial area utilized nine beds spaced 0·75 m apart, each 1 m wide and 95 m long, with each bed containing eight rows of seedlings at a density of 120 per square metre. A randomized split plot experimental design was used. Three experimental treatments were applied to seed before sowing. The seed treatments were randomly applied to whole beds, with three replications of each treatment, and were: thiram (Thiram 40F, NuFarm Ltd., applied at 40 mL kg^{-1} seed), *Trichoderma* spp. (Arbor-Guard™, P F Olsen Ltd., applied at 2·5 g kg^{-1} seed) and an untreated control. In addition to the experimental treatments, all seed was treated with methiocarb to mitigate against losses to birds. The effect of methiocarb on *Trichoderma* was assessed by measuring the growth of the fungus on potato dextrose agar that was amended with methiocarb. The seed was sown on 28 September 2006.

Each bed was divided into 21 plots, each measuring 4·5 m long, with seven soil and/or foliar treatments randomly applied in triplicate in each bed. The soil treatments: metalaxyl-M/mancozeb (Ridomil® Gold MZ WG, Syngenta Crop Protection Ltd., 14 kg ha^{-1}) or humate (NZ Humates Ltd., 550 kg ha^{-1}) plus lime (NZ Humates Ltd., 275 kg ha^{-1}) were applied 20 days after sowing; whilst the foliar treatments: phosphorous acid (Foli-R-Fos® 400, Key Industries Ltd., 6·5 L ha^{-1}) or methyl jasmonate (Sigma Aldrich Ltd., 0·4 L ha^{-1}) were each applied seven times at monthly intervals commencing 54 days after sowing. The specific soil/foliar treatment combinations were: 1) untreated, 2) metalaxyl-M/mancozeb, 3) metalaxyl-M/mancozeb/phosphorous acid, 4) metalaxyl-M/mancozeb/methyl jasmonate, 5) humate-lime/phosphorous acid, 6) humate-lime/methyl jasmonate, 7) phosphorous acid.

Trial two

Ten beds, each 1 m wide and 53 m long, were used. Seedling density was the same as in trial one. The design was a randomized complete block, with eight treatments randomly applied in duplicate to single plots 3·3 m long in each bed, to give a total of 20 replicates. All seed was treated with *Trichoderma* spp. and methiocarb before being sown on 16 October 2007. The soil treatments were: metalaxyl-M/mancozeb (15 kg ha^{-1}) or humate (1000 kg ha^{-1}) plus lime (500 kg ha^{-1}) applied 16 days after sowing, or metalaxyl-M/mancozeb (15 and 50 kg ha^{-1}) applied one week after root pruning, 162 days after sowing. The foliar treatments were phosphorous acid applied either six times at monthly intervals commencing 58 days from sowing, four times at monthly intervals commencing 134 days from sowing or seven times at two-weekly intervals commencing 120 days from sowing. The specific soil/foliar combinations were: 1) untreated, 2) metalaxyl-M/mancozeb (15 kg ha^{-1} at emergence), 3) metalaxyl-M/mancozeb (15 kg ha^{-1} at root pruning), 4) metalaxyl-M/mancozeb (50 kg ha^{-1} at root pruning), 5) phosphorous acid (six monthly applications from 58 days after sowing), 6) humate-lime (at emergence)/phosphorous acid (six monthly applications from 58 days after sowing), 7) phosphorous acid (four monthly applications from 134 days after sowing), 8) phosphorous acid (seven fortnightly applications from 120 days after sowing).

In both trials the seedlings were managed according to normal nursery practice and this included the application of fungicides (prochloraz, fluazinam, captan, copper hydroxide) to control terminal crook caused by *Colletotrichum acutatum* f. sp. *pineum* and botrytis caused by *Botrytis cinerea*. Foliar sprays were applied using a backpack pressure sprayer. Humate and lime were spread by hand. Treatments were applied to the full length of each plot, but to avoid cross-contamination, assessments usually excluded a buffer zone of 0·5 m at each end of the plot. To supplement rainfall, irrigation was applied using overhead sprinklers in conformance with commercial practice.

The seedling roots were pruned at a depth of 8–10 cm using a reciprocating cutter bar in March of each year, to encourage lateral root development. Lateral roots were pruned using cutting discs approximately one month after root pruning each year. In trial one the seedlings were topped at a height of 40 cm in May. No topping was done in trial two. A timeline of crop management and experimental operations is listed in Table 1.

Trial assessments

The trials were inspected monthly to monitor seedling health and the number of dead and wilting plants recorded. In trial one, on 10 May 2007 (224 days from sowing), the height of 100 seedlings per plot was measured. On 17 May, *Phytophthora* incidence was estimated by counting the number of seedlings in the whole plot (out of approximately 500 seedlings per plot) that showed above ground symptoms: needles exhibiting a dry chlorotic appearance with wilting of the growing tip. On 18 July, the seedlings within the centre 3·5 m of each plot were assessed by physically removing seedlings exhibiting foliar symptoms of root rot (symptoms ranged from fading and drooping of needles to brown, dead needles) and inspecting their roots. Only seedlings with signs of poor root health (reduced root volume, brown root tissue) were counted as having disease caused by *Phytophthora* spp. A sub-sample of ten seedlings was examined more closely in the laboratory. Roots were washed to remove soil and examined under a stereo microscope and the lower stem of each was peeled to determine the extent of discoloured, dead tissue. Disease incidence for each plot was calculated from the number of diseased seedlings and an estimate of total seedling number (380 seedlings) in the assessed area of each plot.

In trial two, visual assessments of the number of seedlings exhibiting symptoms described above within the centre 2·3 m of each plot were carried out on 10 April (177 days after sowing), and repeated on 7 May, 5 June and 30 June 2008. Estimates of the number of seedlings in each plot were made, which ranged from 110 to 220, and used to calculate disease incidence.

To confirm the presence of *Phytophthora* in the trial sites, samples of brown root and stem tissue and soil were taken from affected plots in each trial. Samples were collected only at the end of the growing season for trial 1 and at monthly intervals from November 2007 until June 2008 for trial 2. Soil samples were flooded with sterile distilled water and then baited by floating leaves of *Rhododendron catawbiense* on the surface. After 14 days the leaves were washed, surface sterilized for 2 min in 10% hydrogen peroxide and then sections were cut from the leaves and placed onto Petri dishes containing either carrot agar (Erwin & Ribeiro, 1996) or a modified *Phytophthora*-selective medium SMA (synthetic mucor agar) (Brasier *et al.*, 2005). Sections of diseased root and stem material were either surface sterilized as described for the rhododendron leaves before plating, or if there was a clear interface of live and dead tissue, this was dissected and placed directly onto the *Phytophthora*-selective medium and also onto malt extract agar. Petri dishes were incubated at 18°C in the dark for seven days. Developing colonies of pythiaceous organisms were sub-cultured on carrot agar and isolates were identified through morphological examination and verified by sequencing of the ITS region of the genome (an aligned sequence ITS6/ITS4).

Disease incidence data for each date were expressed as percentages and subjected to angular transformation prior to analysis. Seedling height and incidence data were

Table 1 Timeline of *Pinus radiata* crop management and experiment operations for evaluation of *Phytophthora cactorum* control

	Trial one		Trial two	
Operation	Date	Days from sowing	Date	Days from sowing
Seed sown	28 Sep 2006		16 Oct 2007	
Emergence	12 Oct 2006	14	5 Nov 2007	20
Soil treatment	18 Oct 2006	20	1 Nov 2007	16
First foliar treatment	21 Nov 2006	54	13 Dec 2007	58
Root pruning	16 Mar 2007	169	19 Mar 2008	155
Soil treatment	–		26 Mar 2008	162
Lateral pruning	16 Apr 2007	200	19 Apr 2008	186
Height measurement	10 May 2007	224	–	
Topping	15 May 2007	229	–	
Root rot assessments	17 May 2007	231	10 Apr 2008	177
	18 Jul 2007	293	7 May 2008	204
			5 Jun 2008	233
			30 Jun 2008	258

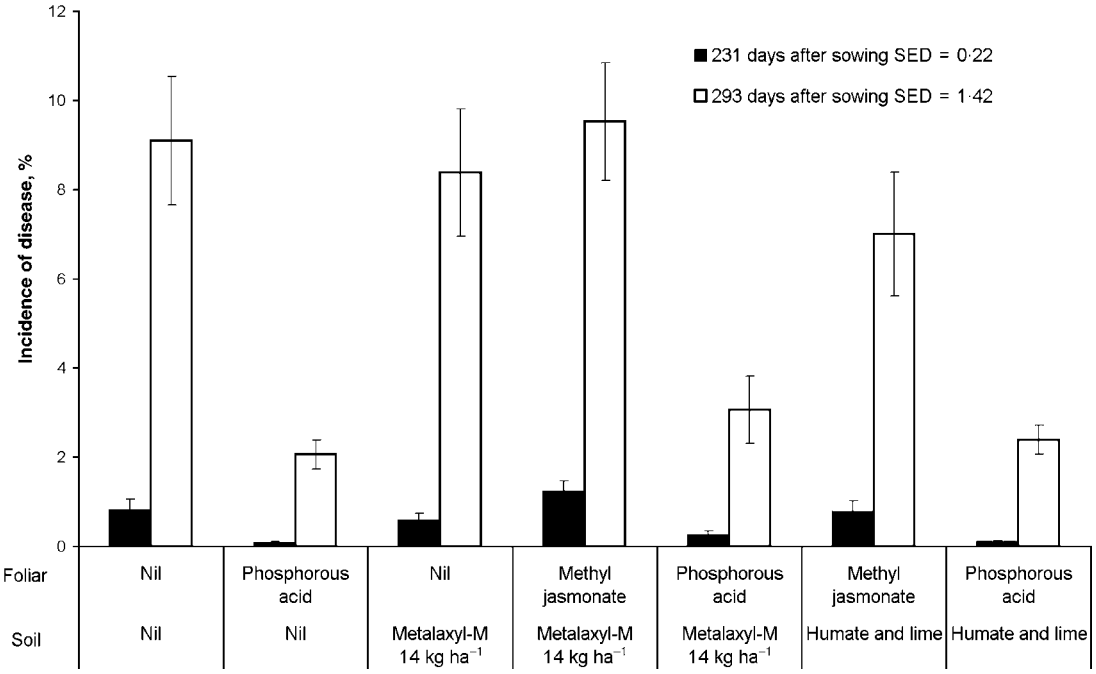

Figure 1 Incidence (cumulative) of phytophthora root rot in pine seedlings assessed on 17 May (231 days after sowing) and 18 July 2007 (293 days after sowing). Soil treatments were applied at seedling emergence on 18 October 2006 (20 days after sowing). Foliar treatments were applied monthly from 21 November 2006 (54 days after sowing) until 22 May 2007 (236 days after sowing). SED = standard error of the difference between means; bars indicate standard error of the mean.

analysed separately as a split-plot ANOVA in GenStat v9. Analysis of the raw and transformed data gave the same conclusions, so the raw means only have been presented for simplicity. Planned non-orthogonal treatment contrasts for metalaxyl-M/mancozeb vs humate and lime, methyl jasmonate vs phosphorous acid, metalaxyl-M/mancozeb vs control and phosphorous acid vs control were included in the analysis. Since the overall treatment effect was significant ($P < 0{\cdot}001$) and the treatment contrasts were planned, Bonferroni's correction was not applied.

Results

Trial one

Foliar symptoms typical of phytophthora root rot were first observed in April 2007, one month after root pruning when seedlings were approximately seven months old. However, because incidence was very low at that time, no formal assessment was carried out until 17 May 2007 (Fig. 1). A further more detailed disease assessment (including root symptoms) was carried out on 18 July. *Phytophthora cactorum* was isolated from 80% of seedlings exhibiting disease symptoms on this date, confirming this as the causal agent. No other fungi or oomycetes were consistently isolated from diseased tissue.

None of the seed treatments affected seedling height ($P = 0{\cdot}439$) or root rot incidence ($P = 0{\cdot}734$) and there were no significant interactions between seed treatments and sub-plot treatments ($P = 0{\cdot}734$). Plate assays indicated that methiocarb had no effect on the growth of the *Trichoderma* used to treat the seed (data not shown). Seedling height was significantly ($P < 0{\cdot}001$) reduced (by approximately 14%) in each of the methyl jasmonate treatments (401 mm ± 2·4), compared with the height in the other treatments (465 mm ± 2·3). None of the other treatments affected seedling height, compared with the untreated controls ($P > 0{\cdot}1$).

Disease incidence in untreated seedlings increased from 0·8% to 9·1% between 17 May and 18 July 2007. The standard nursery management treatment (metalaxyl-M/mancozeb applied at seedling emergence) did not reduce disease incidence compared with that in the untreated control. Phosphorous acid, whether used alone or in combination with soil-applied treatments, was the only treatment that significantly ($P < 0{\cdot}001$) reduced disease incidence (Fig. 1). On 18 July 2007, the lowest disease incidence (2·1%) was recorded in plots treated with phosphorous acid alone, a reduction of *ca.* 75% compared with that in the metalaxyl-M/mancozeb treatment. However, this was not significantly different from that in plots treated with phosphorous acid in combination with metalaxyl-M/mancozeb (3·1%) or humate-lime (2·4%). Humate-lime reduced the incidence of root rot slightly when contrasted with metalaxyl-M/mancozeb ($P = 0{\cdot}009$ and 0·062 at 17 May and 18 July, respectively). Methyl jasmonate, used in combination with metalaxyl-M/mancozeb or humate-lime, did not affect root rot incidence

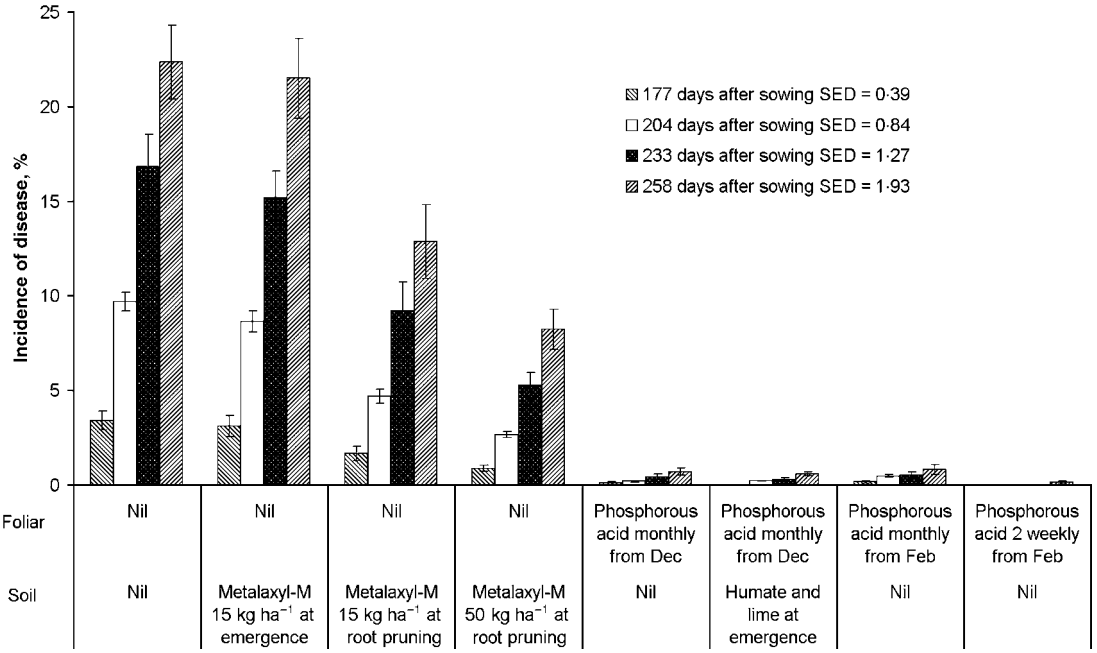

Figure 2 Incidence (cumulative) of phytophthora root rot in pine seedlings assessed on 10 April (177 days after sowing), 7 May (204 days after sowing), 5 June (233 days after sowing) and 30 June 2008 (258 days after sowing). Soil treatments were applied at seedling emergence on 1 November 2007 (16 days after sowing) or at root pruning on 26 March 2008 (162 days after sowing). Foliar treatments were applied monthly from 13 December 2007 (58 days after sowing) or 13 February (121 days after sowing) until 7 May 2008 (204 days after sowing) or fortnightly from 13 February until 7 May 2008. SED = standard error of the difference between means; bars indicate standard error of the mean.

compared with the untreated control, and performed significantly less well ($P < 0{\cdot}001$) than the equivalent phosphorous acid combinations.

Trial two

Phytophthora-symptoms were first recorded on 10 April 2008, 177 days after sowing and three weeks after root pruning. Further assessments were recorded on 7 May, 5 June and 30 June (Fig. 2). *Phytophthora cactorum* was isolated from soil and root tissue samples taken from plots exhibiting disease symptoms on 7 April, 7 May, 5 June and 26 June. *Phytophthora*-incidence in untreated plots was 3·4% on 10 April and this increased to 22·2% by 30 June. Disease incidence in plots treated with metalaxyl-M/mancozeb (15 kg ha^{-1}) at seedling emergence was not significantly different ($P > 0{\cdot}05$) from that in the untreated controls on any of the assessment dates. However, metalaxyl-M/mancozeb significantly reduced disease ($P < 0{\cdot}05$) when applied one week after root pruning, and on 30 June root rot incidence was 14·9% in plots treated with 15 kg ha^{-1}. Disease control was further enhanced (8·2% incidence) when metalaxyl-M/mancozeb was applied at 50 kg ha^{-1}. Phosphorous acid was the most effective treatment, reducing disease incidence to 0·8% or below when applied monthly from December (six applications) or February (four applications), and almost completely suppressed disease (0·1% incidence) when applied fortnightly from February (seven applications). Although disease incidences in the phosphorous acid treatments did not differ significantly from each other, all phosphorous acid treatments were significantly ($P < 0{\cdot}05$) more effective than the metalaxyl-M/mancozeb treatments.

Discussion

Chemical and biological disease control methods were compared for their ability to suppress phytophthora root rot in radiata seedlings in a New Zealand forest nursery. Field experiments were conducted over two seasons in an area that was naturally infested with *P. cactorum* and had a history of root rot. In both seasons, root rot symptoms only became evident after root pruning when seedlings were six to seven months old. In New Zealand, bare root nursery seedlings are mechanically conditioned to produce a compact fibrous root system and to slow down height growth. This is done by passing a sharp blade through the seed bed to a depth of 10 cm to sever vertical roots. A rotating blade travelling between the rows is also used to trim root laterals. Thereafter, a blunt blade is passed through the beds at 2–3 week intervals in order to prevent further vertical root formation before lifting. Sowing is timed so that seedlings are large enough (20 cm tall) for conditioning to begin in late summer or early autumn (Menzies *et al.*, 2005). There is evidence that mechanical injury to roots may facilitate disease development, since it has been shown that fresh wounds rendered pepper roots more susceptible to infection by *P. capsici* (Adorada *et al.*,

2000). Furthermore, zoospores of *P. ramorum* were attracted to wounds on rooted rhododendron plantlets where they encysted and germinated before infecting the root tissue (Parke, 2007).

Metalaxyl and metalaxyl-M (syn. mefanoxam) have been shown to be highly effective against phytophthora root rot caused by *P. cinnamomi* and *P. cactorum* (Erwin & Ribeiro, 1996). In this study, soil treatment with metalaxyl-M/mancozeb was not effective when applied at seedling emergence, but suppressed phytophthora root rot when applied six months later, one week after root pruning. The poor performance of the early season treatment may be attributable to the degradation of the active ingredient in the soil during the interval between application and root pruning. Half-life of metalaxyl is strongly influenced by soil type and has been reported to range from 82 days in sandy soil with a pH of 5·8 (Davison & McKay, 1999) to less than one day in clay soil with a pH of 3·7 (Monkiedje *et al.*, 2007). Furthermore, degradation of metalaxyl can be accelerated in soils with a history of metalaxyl usage (Davison & McKay, 1999). The half-life of metalaxyl-M was not determined in this study, but given the potential for rapid degradation in the field, it is likely that efficacy could be further improved by scheduling fungicide application immediately before root pruning.

Although MeJA did not affect disease incidence, seedlings in the MeJA-treated plots were significantly smaller ($P < 0{\cdot}001$) than their counterparts throughout the trial area. A recent study by Gould *et al.* (2008) demonstrated that treatment of radiata pine seedlings with one single application of MeJA resulted in an elevation of resistance to *Diplodia pinea* and a concomitant reduction in seedling growth rate. There, the authors proposed that the reduction in growth rate was attributable to the reallocation of resources towards active defence mechanisms. It is possible that monthly applications of MeJA have similarly affected resource partitioning in the radiata seedlings in this study, without measurably enhancing resistance to root rot.

Trichoderma are common soil fungi and are antagonistic to many plant pathogens, so making them good candidates for the suppression of root diseases (Vinale *et al.*, 2008). Amendment of growth media with *T. harzianum* resulted in suppression of damping-off caused by *P. cinnamomi* in *Pinus echinata* seedlings (Kelley, 1976) and also reduced seedling mortality caused by *Fusarium oxysporum* in container-grown Douglas fir (Mousseaux *et al.*, 1998). In this study, *Trichoderma* seed treatment did not suppress the development of phytophthora root rot, possibly because wet conditions in the trial area that would favour pathogen development would be unfavourable to *Trichoderma* spp. Moisture is a key factor in the establishment, spread and longevity of phytophthora, and Kelley (1976) reported that *T. harzianum* was unable to suppress damping off in *P. echinata* seedlings when soil moisture content was approaching saturation.

Soils that are low in organic matter and that drain poorly can provide a favourable environment for *Phytophthora* (Hardham, 2005). Incorporation of calcareous soil amendments such as gypsum (Messenger *et al.*, 2000) and lime (Campanella *et al.*, 2002), or humic fractions (Pascual *et al.*, 2002) have been shown to reduce pathogen populations in soil and to suppress root disease. Furthermore, humic acid has also been reported to encourage the proliferation of beneficial microbes, including *Trichoderma* (Pascual *et al.*, 2002; Vawdrey *et al.*, 2002). The integrated use of humate and lime with *Trichoderma* seed treatment in this study did not suppress root rot. Recent studies have shown that *T. viride* and *T. harzianum* exhibited a differential growth response to various humic fractions (Loffredo *et al.*, 2008) suggesting that there may be some merit in the development of specific humic acid-*Trichoderma* combinations for field evaluation.

The most effective treatment in this study was phosphorous acid. This chemical was applied in accordance with the product label for use in nursery stock, which recommends application every 2–4 weeks during active growth. In trial one, seven monthly applications of this chemical suppressed root rot by up to circa 78% compared with the untreated control. Ali *et al.* (2000) reported that control of phytophthora root rot in bare-rooted radiata seedlings using phosphonate was enhanced when used in combination with the plant activator Bion® (Syngenta). No such benefit was observed in this study when phosphorous acid was combined with metalaxyl-M/mancozeb or with humate-lime. There was, however, evidence of treatment interactions, with contrast analysis in trial one indicating that combinations of phosphorous acid with humate-lime were more effective ($P < 0{\cdot}10$) than with metalaxyl-M/mancozeb.

In trial two, phosphorous acid suppressed root rot by 97% and 99% when applied monthly from December or fortnightly from February, respectively. There was no significant difference in disease control between these two regimes, suggesting that early season applications are unnecessary. Studies in Australia have shown that a single foliar application of phosphite (syn. phosphorous acid) was sufficient to protect native species from stem inoculation with *P. cinnamomi* for between five months and two years (Tynan *et al.*, 2001; Shearer & Fairman, 2007b). Phosphite is translocated systemically in plants to actively growing tissues and acts directly against the pathogen and potentiates host defences to attempted infection (Jackson *et al.*, 2000). However, there is considerable variation in the longevity of phosphite effectiveness between plant species (Shearer *et al.*, 2007) and this can be affected by environmental and host factors including temperature, water status and plant physiology (Tynan *et al.*, 2001). Shearer & Fairman (2007b) suggested that adaptations of some native species to drought and infertile soils may result in slow growth and so maximise retention of the chemical within the plant. Conversely, phosphorous acid concentration may be expected to decline rapidly in actively growing plants in fertile environments, such as the forest nursery, and further research is required to establish whether application frequency can be reduced without loss of efficacy.

The results of this study indicate that phosphorous acid can be used to protect pine seedlings against phytophthora

root rot in infested soils. However, pathogen survival was not determined in treated plots and so the effect of this treatment on population levels in the soil is not known. *Phytophthora* species vary in their sensitivity to phosphorous acid (Wilkinson *et al*., 2001b) and *P. cinnamomi* has been recovered from treated glasshouse plants (Wilkinson *et al*., 2001a) and from soil collected from foliar sprayed plants in infested field sites (Shearer & Fairman, 2007b). More recently, Dobrowolski *et al.* (2008) reported that prolonged use of phosphorous acid encouraged the selection of *P. cinnamomi* isolates with decreased sensitivity to the chemical. It is also important to note that this study was conducted in a commercial nursery in which other fungicides (prochloraz, fluazinam, captan, copper hydroxide) were being applied to manage terminal crook and botrytis. It is possible that one or more of these chemicals may have had an additive or synergistic interaction with phosphorous acid or conversely a negative interaction with any of the other treatments. Indeed, it would be inadvisable to rely solely on phosphorous acid for the control of this pathogen and integrated strategies combining chemical control with good crop hygiene and other biological and cultural control options should be pursued.

Acknowledgements

We thank the New Zealand Foundation for Research, Science and Technology (Bio-protection of New Zealand's productive ecosystems, LINX0304; Biosecurity, protection and risk management of New Zealand's forests, CO4X0302) for funding; Kaingaroa Timberlands; Warwick Brown for providing seed and chemicals, use of the nursery block and crop management; Robert Hill, BioDiscovery Ltd., for coating the seed with Arbor-Guard; Beccy Ganley, Ensis, for assistance with field work and *Phytophthora* spp. isolation; Catherine Cameron, AgResearch, Ruakura for statistical analyses and advice; and Stephen Hoyte for valuable editorial comments.

References

Adorada DL, Biles CL, Liddell CM, Fernandez-Pavia S, Waugh KO, Waugh ME, 2000. Disease development and enhanced susceptibility of wounded pepper roots to *Phytophthora capsici*. *Plant Pathology* **49**, 719–26.

Ali Z, Smith I, Guest DI, 2000. Combinations of potassium phosphonate and Bion (acibenzolar-S-methyl) reduce root infection and dieback of *Pinus radiata*, *Banksia integrifolia* and *Isopogon cuneatus* caused by *Phytophthora cinnamomi*. *Australasian Plant Pathology* **29**, 59–63.

Balci Y, Balci S, Eggers J *et al*., 2007. *Phytophthora* spp. associated with forest soils in Eastern and North-Central U.S. oak ecosystems. *Plant Disease* **91**, 705–10.

Brasier CM, Beales PA, Kirk SA, Denman S, Rose J, 2005. *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. *Mycological Research* **109**, 853–9.

Cahill DM, Rookes JE, Wilson BA, Gibson L, McDougall KL, 2008. *Phytophthora cinnamomi* and Australia's biodiversity: impacts, predictions and progress towards control. *Australian Journal of Botany* **56**, 279–310.

Campanella V, Ippolito A, Nigro F, 2002. Activity of calcium salts in controlling *Phytophthora* root rot of citrus. *Crop Protection* **21**, 751–6.

Daniel R, Wilson BA, Cahill DM, 2005. Potassium phosphonate alters the defence response of *Xanthorrhoea australis* following infection by *Phytophthora cinnamomi*. *Australasian Plant Pathology* **34**, 541–8.

Davison EM, McKay AG, 1999. Reduced persistence of metalaxyl in soil associated with its failure to control cavity spot of carrots. *Plant Pathology* **48**, 830–5.

Dobrowolski MP, Shearer BL, Colquhoun IJ, O'Brien PA, Hardy GEStJ, 2008. Selection for decreased sensitivity to phosphite in *Phytophthora cinnamomi* with prolonged use of fungicide. *Plant Pathology* **57**, 928–36.

Dumroese RK, James RL, 2005. Root diseases in bareroot and container nurseries of the Pacific Northwest: epidemiology, management, and effects on outplanting performance. *New Forests* **30**, 185–202.

Erwin DC, Ribeiro OK, 1996. *Phytophthora Diseases Worldwide*. St. Paul, MN, USA: APS Press.

Gadgil PD, 2005. The fungi of New Zealand Volume 4: Fungi on trees and shrubs in New Zealand. *Fungal Diversity Research Series* **16**, 1–437.

Gould N, Reglinski T, Spiers M, Taylor JT, 2008. Physiological trade-offs associated with methyl jasmonate-induced resistance in *Pinus radiata*. *Canadian Journal of Forestry* **38**, 677–84.

Hardham AR, 2005. *Phytophthora cinnamomi*. *Molecular Plant Pathology* **6**, 589–604.

Hardy GEStJ, Barrett S, Shearer BL, 2001. The future of phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. *Australasian Plant Pathology* **30**, 133–9.

Humphrey WA, 1980. Controlling phycomycetes which attack conifers. *HortScience* **15**, 397.

Jackson TJ, Burgess T, Colquhoun I, Hardy GEStJ, 2000. Action of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*. *Plant Pathology* **49**, 147–54.

Johnston PR, Horner IJ, Beever RE, 2003. *Phytophthora cinnamomi* in New Zealand's indigenous forests. In: McComb JA, Hardy GES, Tommerup IC, eds. *Phytophthora in Forests and Natural Ecosystems – 2nd International IUFRO working party meeting*. Albany, Western Australia: Murdoch University Print, 41–7.

Kelley WD, 1976. Evaluation of *Trichoderma harzianum* impregnated clay granules as a biocontrol for *Phytophthora cinnamomi* causing damping off in pine seedlings. *Phytopathology* **66**, 1023–7.

Loffredo E, Berloco M, Senesi N, 2008. The role of humic fractions from soil and compost in controlling the growth *in vitro* of phytopathogenic and antagonistic soil-borne fungi. *Ecotoxicology and Environmental Safety* **69**, 350–7.

Menzies MI, Dibley MJ, Brown W, Faulds T, 2005. Nursery procedures for radiata pine. In: Colley M, ed. *Forestry Handbook*. Christchurch, NZ: New Zealand Institute of Forestry Inc., 100–3.

Messenger BJ, Menge JA, Pond E, 2000. Effects of gypsum soil amendments on avocado growth, soil drainage, and resistance to *Phytophthora cinnamomi*. *Plant Disease* **84**, 612–6.

Monkiedje A, Zuehlke S, Maniepi SJN, Spiteller M, 2007. Elimination of racemic and enantioenriched metalaxyl based fungicides under tropical conditions in the field. *Chemosphere* **69**, 655–63.

Mousseaux MR, Dumroese RK, James RL, Wenny DL, Knudsen GR, 1998. Efficacy of *Trichoderma harzianum* as a biological control of *Fusarium oxysporum* in container-grown Douglas-fir seedlings. *New Forests* **15**, 11–21.

Newhook FJ, 1959. The association of *Phytophthora* spp. with mortality of *Pinus radiata* and other conifers I: Symptoms and epidemiology in shelterbelts. *New Zealand Journal of Agricultural Research* **2**, 808–43.

Parke JL, 2007. Root and stem infection of rhododendron from potting medium infested with *Phytophthora ramorum*. *Plant Disease* **91**, 1265–70.

Pascual JA, Garcia C, Hernandez T, Lerma S, Lynch JM, 2002. Effectiveness of municipal waste compost and its humic fraction in suppressing *Pythium ultimum*. *Microbial Ecology* **44**, 59–68.

Reglinski T, Dick M, 2005. Biocontrol of forest nursery pathogens. *New Zealand Journal of Forestry* **50**, 14–21.

Shearer BL, Crane CE, Barrett S, Cochrane A, 2007. *Phytophthora cinnamomi* invasion, a major threatening process to conservation of flora diversity in the South-west Botanical Province of Western Australia. *Australian Journal of Botany* **55**, 225–38.

Shearer BL, Fairman RG, 2007a. A stem injection of phosphite protects *Banksia* species and *Eucalyptus marginata* from *Phytophthora cinnamomi* for at least four years. *Australasian Plant Pathology* **36**, 78–86.

Shearer BL, Fairman RG, 2007b. Application of phosphite in a high-volume foliar spray delays and reduces the rate of mortality of four *Banksia* species infected with *Phytophthora cinnamomi*. *Australasian Plant Pathology* **36**, 358–68.

Tynan KM, Wilkinson CJ, Holmes JM *et al*. 2001. The long-term ability of phosphite to control *Phytophthora cinnamomi* in two native plant communities of Western Australia. *Australian Journal of Botany* **49**, 761–70.

Vawdrey LL, Martin TM, De Faveri J, 2002. The potential of organic and inorganic soil amendments, and a biocontrol agent (*Trichoderma* sp.) for the management of *Phytophthora* root rot of pawpaw in far northern Queensland. *Australasian Plant Pathology* **31**, 391–9.

Vettraino AM, Morel O, Perlerou C, Robin C, Diamandis S, Vannini A, 2005. Occurrence and distribution of *Phytophthora* species in European chestnut stands, and their association with ink disease and crown decline. *European Journal of Plant Pathology* **111**, 169–80.

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M, 2008. Trichoderma-plant-pathogen interactions. *Soil Biology & Biochemistry* **40**, 1–10.

Wilkinson CJ, Holmes JM, Dell B *et al*. 2001a. Effect of phosphite on *in planta* zoospore production of *Phytophthora cinnamomi*. *Plant Pathology* **50**, 587–93.

Wilkinson CJ, Shearer BL, Jackson TJ, Hardy GE St J, 2001b. Variation in sensitivity of Western Australian isolates of *Phytophthora cinnamomi* to phosphite *in vitro*. *Plant Pathology* **50**, 83–9.