FUTURE FORESTS RESEARCH

RADIATA MANAGEMENT TECHNICAL NOTE
Site Productivity

Number: RSPTN-016
Date: January 2011

Mycorrhizal Development and Nursery Treatments

Summary

Ectomycorrhizal (ECM) associations greatly enhance the capability of radiata pine to access soil nutrients. Fertilisers and fungicides are routinely applied in commercial radiata nurseries to enhance seedling performance, but the effect of these treatments on ECM development is largely unknown. To address this issue, a trial was established to identify any significant alterations in the diversity and extent of ECM associations caused by variations in the application rates of conventional fungicides and fertilisers. The root systems of 432 *Pinus radiata* seedlings were examined and the mycorrhizal species present on approximately 150,000 root tips were identified.

Any level of fertiliser and fungicide application significantly decreased the extent of ECM colonisation ($P < 0.05$) and significantly altered the diversity of the mycorrhizal species associated with the seedlings when compared to untreated seedlings ($P < 0.05$). The proportion of *Rhizopogon rubescens* present on the seedlings decreased with increasing levels of application of fungicide and fertiliser, and the proportion of other fungal species also varied in response to differences in the quantity of fungicide and fertiliser applied to the seedlings. Assessments of ECM colonisation and diversity on out planted seedlings will continue for at least a further two years to identify any ongoing effects of the nursery treatments. These data will be combined with growth measurements to investigate the potential relationships between ECM community structure and plant performance.

Authors: Simeon Smaill and Katrin Walbert

Background

Ectomycorrhizal (ECM) associations greatly enhance the capability of *Pinus radiata* D. Don (radiata pine) to access soil resources and are therefore critical to the successful establishment and growth of seedlings [2, 5, 8].

As some ECM associations formed in the nursery can persist for some time after out planting [6, 10] it is possible that management practices which alter the diversity and activity of ECM species in the nursery can indirectly affect the performance of seedlings for several years following out planting.

Fungicide and fertiliser use is common practice in commercial radiata pine nurseries, but the effect of these treatments on mycorrhization has not been comprehensively assessed. The effect of fertiliser application on the mycorrhization of radiata pine seedlings has been investigated only for specific ECM species (e.g. [4]), while the effects of fungicide application on the mycorrhization of radiata pine seedlings has not been examined.

To improve understanding of the effects of fungicide and fertiliser applications on ECM in New Zealand radiata pine nurseries, a trial was established at Te Ngae Nursery, Rotorua (Timberlands Ltd.), testing the effects of variations in application rates. The ECM community present at Te Ngae originates from the spores present in the soil used in the establishment of the nursery. As in most New Zealand nurseries, Te Ngae does not inoculate with specific ECM species; consequently the structure of the ECM community is wholly dependent on selection pressures generated by the nursery environment and management practices. Here we report the effects of the variations in fungicide and fertiliser application on the diversity and extent of the ECM associations formed by the seedlings.

Methods

The trial design and the fungicides and fertilisers used in the trial are described in full in Smaill and Walbert (2010). Briefly, fungicide selection was based on known problems in the nursery, and fertiliser use was determined from soil nutrient analysis. Seedlings in the trial were subjected to the treatments for approximately five months.

Fungicide and fertiliser were applied at standard rates, 25% less than standard and 25% greater than standard. This made a total of nine different treatment combinations, each replicated nine times. A further 27 plots were established as controls, receiving no treatments.

Immediately prior to lifting for out planting, four seedlings from each of the 108 plots were collected from each plot. The root section from each plant was removed and stored at -20 ^{o}C until analysis. From the root system of each seedling, three lateral root branches were randomly selected and all mycorrhizal root tips present were counted and identified.

Results

Future Forests Research Ltd, PO Box 1127, Rotorua. Ph: 07 921 1883 Email: info@ffr.co.nz Web: www.ffr.co.nz

RADIATA MANAGEMENT TECHNICAL NOTE

Site Productivity

Number: RSPTN-016
Date: January 2011

Approximately 150,000 root tips were identified as being infected with an ECM species. *Rhizopogon rubescens* was the most frequently observed ECM species (57% of all observations), followed by *Wilcoxinia mikolae*, *Hebeloma* sp., *Tuber* sp. and *Thelephora terrestris* in decreasing order of incidence. These five species accounted for 99.94% of all infected root tips.

The mean number of ECM root tips on seedlings collected from control plots was greater than seedlings treated with any combination of fertiliser and fungicide (384 ± 34 and 338 ± 24, respectively, $P < 0.05$), but the response of individual ECM species varied. The number of root tips infected with *R. rubescens* and *Tuber* sp. decreased ($P < 0.01$) with treatment while the number of tips infected with *W. mikolae* and *T. terrestris* increased ($P < 0.05$).

Table 1. Differences in mean root tips counts between control and treated plots for individual species; standard errors are given in parentheses

Species	Control	Treated	Change
R. rubescens	251 (27)	184 (21)	-27%
W. mikolae	83 (11)	105 (6)	+27%
Hebeloma sp.	42 (7)	42 (5)	0%
Tuber sp.	7.8 (2.2)	3.4 (0.8)	-56%
T. terrestris	0.9 (0.5)	3.3 (0.8)	+267%

The observed ECM species were also sensitive to differences in the rates of fertiliser and fungicide application. The incidence of *Hebeloma* sp. was greater with increasing levels of fertiliser application.

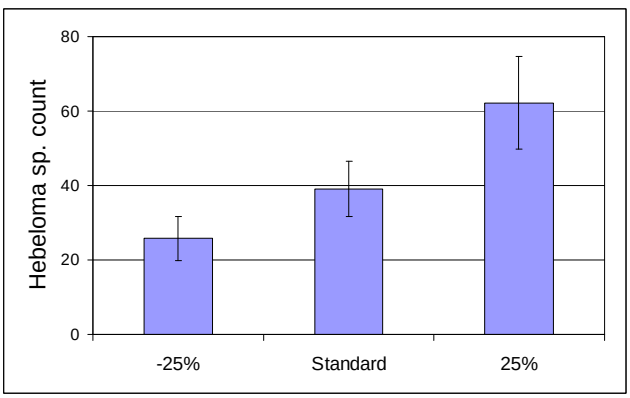

Fig 1. Mean Hebeloma sp. counts vary with fertilisation application rates (P < 0.01)

Optimum application rates were also observed in some cases, as the number of root tips infected with *Tuber* sp. was greatest in plots that received the standard rate of fungicide application.

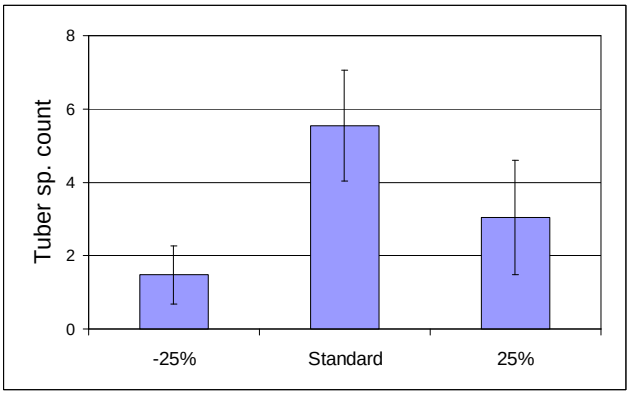

Fig 2. Mean Tuber sp. counts vary with fungicide application rates (P < 0.01)

Increased levels of fertiliser application decreased the relative proportion to total root tip colonisation by the dominant ECM species, *R. rubescens* ($P < 0.001$). This was accompanied by increases in the relative proportion of most other species, such as *W. mikolae.*

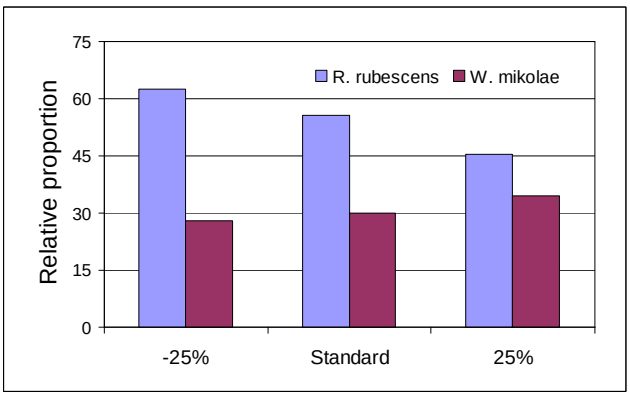

Fig 3. Variations fertiliser application rates affect the proportion of root tips infected with R. rubescens and W. mikolae.

The same trend was observed with the variations in rates of fungicide application. The numbers of root tips infected with *R. rubescens* decreased ($P < 0.05$), causing the proportion of root tips infected with other ECM species to increase.

Future Forests Research Ltd, PO Box 1127, Rotorua. Ph: 07 921 1883 Email: info@ffr.co.nz Web: www.ffr.co.nz

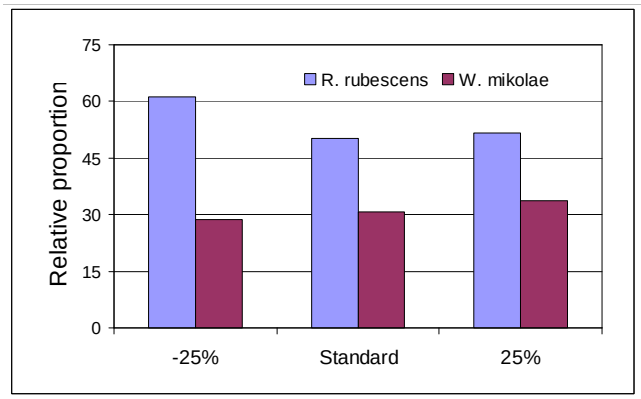

Fig 4. Variations in fungicide application rates affect the proportion of root tips infected with R. rubescens and W. mikolae.

Interpretation and Ongoing Work

Two important findings are highlighted by these results. Firstly, conventional fertiliser and fungicide treatments reduce the overall magnitude of ECM colonisation of radiata pine seedlings. Secondly, variations in the magnitude of fertiliser and fungicide treatment alter the relative diversity of the five dominant ECM species found on the seedlings. The implications of these findings are considered below.

The presence of well developed ECM associations in the nursery is fundamental to improving the survival rates and performance of out planted seedlings [8, 10]. That fertiliser and fungicide applications decreased the extent of mycorrhization is in agreement with the results of studies of other tree species in seedling nurseries (e.g. [7, 4]), and indicates that reductions in the use of fertiliser and fungicide may be advisable. However, the benefits these practices provide in terms of nutrient availability and protection from pathogens must also be considered. Furthermore, although it has been observed that increased levels of fungicide application also decrease seedling root collar diameter which may be linked to ECM development [9], this study is yet to provide concrete evidence that the number of ECM associations in the nursery affects tree performance and productivity following out planting.

The significance of the observed alterations to the relative diversity of ECM species present on the seedlings is also unknown. Different ECM species have different capabilities, and some species may prove more beneficial to the host plant species than others (e.g. [1, 6]). For example, associations between radiata pine and *Hebeloma* sp. do not appear to persist for long following out planting [11]. Whilst it is beneficial in the nursery, the presence of *Hebeloma* sp. is therefore potentially of little use in the establishment of new plantations. It possible that *T. terrestris* is also of lesser value than other ECM species as it has been demonstrated to acquire fewer resources than other ECM species [3].

Practices that favour the development of the more beneficial ECM species have the potential to optimise the productivity gains that can be made from ECM associations. However, this concept requires further investigation by comparing relative ECM diversity to meaningful measurements of radiata pine growth.

Such comparisons will be made over the next two years in a recently established stand in Kaingaroa forest planted with 2596 seedlings from the nursery trial. The effect of fertiliser and fungicide treatments and ECM development and diversity in the nursery on ongoing ECM community structure and tree growth and performance will be determined.

It is anticipated that this work will increase understanding of the relative benefits of the different ECM species present in radiata pine nurseries around New Zealand. Furthermore, this work will enable the development of nursery management plans that maximise plant growth and health by making the best use of naturally occurring ECM species while improving the cost-effectiveness of fertiliser and fungicide treatments.

References

1. Avis, PG, McLaughlin, DJ, Dentinger BC and Reich PB 2003. Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. New Phytologist 160, 239-253.

2. Chu-Chou M and Grace LJ 1987. Mycorrhizal fungi of *Pinus radiata* planted on farmland in New Zealand. New Zealand Journal of Forestry Science 17, 76-82.

3. Colpaert JV, Van Tichelen KK, Van Assche JA and Van Laere A 1999. Short-term phosphorus uptake rates in mycorrhizal and non-mycorrhizal roots of intact *Pinus sylvestris* seedlings. New Phytologist 143, 589-597.

4. Diaz G, Carrillo C and Honrubia M. Mycorrhization, growth and nutrition of *Pinus halepensis* seedlings fertilized with different

Future Forests Research Ltd, PO Box 1127, Rotorua. Ph: 07 921 1883 Email: info@ffr.co.nz Web: www.ffr.co.nz

doses and sources of nitrogen. Annals of Forestry Science 67, in press.

5. Duñabeitia MK, Hormilla S, Garcia-Plazaola JI, Txarterina K, Arteche U and Becerril JM 2004. Differential responses of three fungal species to environmental factors and their role in the mycorrhization of Pinus radiata D. Don. Mycorrhiza 14, 11-18.

6. Gagné A, Jany, J-L, Bousquet, J and Khasa, DP 2006. Ectomycorrhizal fungal communities of nursery-inoculated seedlings outplanted on clear-cut sites in northern Alberta. Canadian Journal of Forest Research 36, 1684-1694.

7. O'Neill JJM, and Mitchell DT 2000. Effects of benomyl and captan on growth and mycorrhizal colonization of Sitka-spruce (*Picea sitchensis*) and ash (*Fraxinus excelsior*) in Irish nursery soil. Forest Pathology 30, 165-174.

8. Ortega U, Duñabeitia M, Menendez S, Conzalea-Murua C and Majada J 2004. Effectiveness of mycorrhizal inoculation in the nursery on growth and water relations of *Pinus radiata* in different water regimes. Tree Physiology 24, 65-73.

9. Smaill SJ and Walbert K 2010. Seedling performance and nursery treatments. Radiata Management Technical Note 17.

10. Walbert K 2008. Ectomycorrhizal communities associated with a Pinus radiata plantation in the North Island, New Zealand. PhD Thesis. Department of Ecology, Lincoln University.

11. Walbert K, Ramsfield TD, Ridgway HJ and Jones EE 2010. Ectomycorrhizal species associated with *Pinus radiata* in New Zealand including novel associations determined by molecular analysis. Mycorrhiza 20, 209-215.

Future Forests Research Ltd, PO Box 1127, Rotorua. Ph: 07 921 1883 Email: info@ffr.co.nz Web: www.ffr.co.nz