Occurrence of arbuscular mycorrhiza and ectomycorrhiza on *Leptospermum scoparium* from the Rakaia catchment, Canterbury

Kim Weijtmans[1], Murray Davis[2]*, Peter Clinton[2], Thomas W. Kuyper[1] and Laurie Greenfield[3]
[1]Wageningen University, PO Box 8005, Wageningen, The Netherlands
[2]Ensis, PO Box 29237, Christchurch, New Zealand
[3]University of Canterbury, Christchurch, New Zealand
*Author for correspondence (Email: murray.davis@ensisjv.com)

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Abstract: *Leptospermum* is one of only three New Zealand genera that are colonised by ectomycorrhizal (EM) fungi, and *L. scoparium* is one of the very few New Zealand species that can be colonised by both arbuscular mycorrhizal (AM) and EM fungi. This study examined AM and EM colonisation on *L. scoparium* growing within AM grassland ecosystems or adjoining *Nothofagus* forest in the Rakaia catchment, Canterbury. Very low AM colonisation was found (<4%) in all samples, while EM colonisation ranged from 7 to 55% of root length colonised. These results contradict an earlier report that *L. scoparium* is mostly colonised by AM fungi. We suggest the montane environment of the study sites would favour EM rather than AM colonisation. EM colonisation was higher in mature plants than in saplings. Lowest EM colonisation (7–15%) was recorded on root samples that were from either young or mature plants occurring as separate individuals in grassland distant from other indigenous EM species, while highest colonisation (49–55%) was recorded on samples from mature closed canopy *L. scoparium* stands, irrespective of distance from other indigenous EM sources.

Keywords: *Nothofagus*; soil properties

Introduction

Most plant species form associations with mycorrhizal fungi. These associations usually confer advantages to the host plant through enhanced nutrient and water uptake and tolerance to soil pathogens (Marschner 1995). Most genera indigenous to New Zealand form associations with arbuscular mycorrhizal (AM) fungi, while only three woody plant genera (*Kunzea*, *Leptospermum* (both Myrtaceae) and *Nothofagus* (Fagaceae)) associate with ectomycorrhizal (EM) fungi (Orlovich & Cairney 2004). Cooper (1976) observed EM structures on ferns that grew in close proximity to EM plants, but Orlovich and Cairney (2004) noted that neither the structure nor their functional significance is well described.

Both *Kunzea ericoides* (A. Rich.) J. Thompson (kānuka) and *Leptospermum scoparium* J.R. et G. Forst. (mānuka, tea tree) have been reported as dual mycorrhizal, i.e. forming both AM and EM symbioses, depending on soil and other habitat conditions (Baylis 1962; Moyersoen & Fitter 1999). *Kunzea* and *Leptospermum* are dominantly Australian genera and are related to *Eucalyptus* (Myrtaceae), a number of species of which have also been reported to be dual mycorrhizal (Chen et al. 2000). Moyersoen and Fitter (1999) examined mycorrhizal colonisation of *L. scoparium* along a 100-km transect in localities dominated either by EM (*Nothofagus*) or AM (*Podocarpus*) species and found that root samples were colonised by EM fungi principally in areas dominated by *Nothofagus*, while EM colonisation was sparse in areas dominated by *Podocarpus* and absent on ultramafic soils. AM colonisation seemed independent of habitat type.

Nothofagus-dominated forests cover about two million hectares and, though they have been much reduced since human occupation, comprise almost half the remaining indigenous forest area of New Zealand (Newsome 1987). They form ectomycorrhizas and are characterised by slow dispersal, commonly only by marginal spread, into previously forested areas. Baylis (1980) suggested their slow dispersal might be due to lack of compatible EM fungi in the neighbouring communities. While *Nothofagus* spread is dominantly short distance, it has been reported to spread over greater distances into vegetation where *L. scoparium* is present (Burrows & Lord 1993). Baylis (1980) suggested *L. scoparium* might share

EM species in common with *Nothofagus*, and thus facilitate its spread. Since *L. scoparium* can form both EM and AM it may initially establish in grassland or shrubland communities dominated by AM species and subsequently host EM fungal species capable of forming associations with *Nothofagus*. It is therefore of interest to determine the incidence of EM colonisation on *L. scoparium* in environments now devoid of *Nothofagus*, but where it occurred in the past, and where it may be desired to rehabilitate *Nothofagus* forest. This article reports a study examining AM and EM colonisation on *L. scoparium* growing within dominantly grassland ecosystems or adjoining remnant *Nothofagus* forest in a montane environment in the Rakaia catchment, Canterbury.

Materials and methods

Root samples of five plants of *L. scoparium* were collected from each of seven localities within the Rakaia catchment between northern Lake Coleridge and the Torlesse Range (Table 1). The three sites at Lake Hill were in close proximity to each other and the soils were all well drained. Plants from sites 1 and 2 were saplings (as inferred from plant height and diameter; saplings being plants <0.5 m tall and <5 cm in diameter) within *Agrostis*-dominated grassland, while site 3 was a small mature closed-canopy stand of *L. scoparium* within similar grassland. The Glenthorne, Lake Selfe and Lyndon sites were poorly drained. The Glenthorne and Lake Selfe 2 sites consisted of large mature closed-canopy stands of *L. scoparium*, while the plants at the Lake Selfe 1 site were saplings among moss and sedge vegetation. The Lyndon site consisted of discrete mature plants in a grassland flush site. The sample locations at Glenthorne and the Lake Selfe sites were 30–50 m from *Nothofagus* forest, while the remaining four sites were at least 1000 m distant from *Nothofagus*, as well as from *Kunzea* or other mature *L. scoparium* stands.

Whole roots were collected from saplings after plants were excavated to a depth of 10–15 cm using a spade, whereas for more mature plants root samples were collected from a block of soil dug to a similar depth near the plant stem. Samples were placed in a plastic bag and stored in a refrigerator (7°C) on the day of collection, before further use. Roots were washed over a 2-mm sieve. The soil was soaked in water overnight to ease washing and sorting of roots. The washed roots were stored in 50% ethanol. The roots were cleared and stained according to Brundrett et al. (1996) with some modifications. The roots were cleared by autoclaving for 20 min at 121°C in a KOH (10% w/v) solution and were bleached in a 5% H_2O_2 solution for 3–4 h to remove phenolic compounds before they were stained in 0.05% trypan blue – lactoglycerol solution (3 lactic acid : 3 glycerol : 4 water) for 10 min. The stained roots were preserved in lactophenol to destain the plant material (while the fungal structures retained their blue colour) before mycorrhizal colonisation was determined.

Table 1. Site and vegetation characteristics, and distance from other indigenous ectomycorrhizal source. Standard deviations of plant diameters are shown, $n = 5$.

Site	Grid ref.	Alt. (m)	Associated vegetation and drainage	Life stage	Plant diameter (cm)	Plant height (m)	Distance from other indigenous EM source (m)
Lake Hill 1	K35 943602	670	*Agrostis* grassland, well drained	Saplings	<0.5	<0.5	>1000
Lake Hill 2	K35 943602	670	*Agrostis* grassland, well drained	Saplings	<0.5	<0.5	>1000
Lake Hill 3	K35 943602	670	Small closed stand, well drained	Mature plants	8.1 ± 2.4	2.3–3.0	>1000
Glenthorne	K34 843766	561	Large closed stand, poorly drained	Mature plants	2.8 ± 0.2	2.5–3.0	50
Lake Selfe 1	K34 904725	617	Mosses and sedges, poorly drained	Saplings	<0.5	<0.5	30
Lake Selfe 2	K34 904725	617	Large closed stand, poorly drained	Mature plants	3.9 ± 1.1	3.5	30
Lyndon	K35 026634	805	Grassland, flush site, poorly drained	Mature plants	3.3 ± 1.1	1.5–2.0	>1000

Mycorrhizal colonisation of roots was measured using the gridline intersect method (Giovannetti & Mosse 1980; Norris et al. 1994). Subsamples of roots (<2 mm diameter) were placed in a Petri dish with a 0.5-cm grid, and the mycorrhizal colonisation (arbuscular or ectomycorrhzal) was determined as a percentage of root length. Since ectomycorrhizas were not restricted to the root tips, the whole root was viewed for mycorrhizal colonisation (Jones et al. 1998; Püttsepp et al. 2004). An intersection was scored as arbuscular mycorrhizal if internal hyphae and/or vesicles were present and as ectomycorrhizal if a mantle was observed. Every subsample was counted three times by rearranging the roots in the Petri dish, and the average was used for further data analysis.

Soil samples to a depth of 15 cm were collected along with roots. Subsamples of soil free of roots and large organic debris were taken before washing and removal of roots and used for determining loss on ignition, soil water content (% water loss after drying at room temperature for 24 h), pH in water, and total nitrogen after Kjeldahl digestion. Approximate organic carbon concentrations were determined by multiplying loss on ignition values by 0.58. Total and inorganic phosphorus were determined in H_2SO_4 extracts of unignited and ignited (550°C) soil respectively using the Murphy–Riley method. Organic phosphorus was determined by difference.

Statistical analysis of mycorrhizal colonisation was made using analysis of variance followed by a Tukey's test to examine differences between sites. All data were analysed by multiple regression, and Pearson correlations were used to compare soil parameters with total mycorrhizal colonisation.

Results

Field soil water contents ranged from very low at all Lake Hill sites to high at the Lake Selfe 1 and Lyndon sites (Table 2), reflecting site drainage conditions. The latter soils also had very high organic carbon and high nitrogen levels and C/N ratios. Soil water content, organic carbon and nitrogen concentrations, and C/N ratios were all positively correlated with each other ($P < 0.05$ in all cases). Total phosphorus levels were in the medium to high range (Blakemore et al. 1987) with most phosphorus being in organic form. The soils ranged from being slightly acidic at Lake Selfe to strongly acidic at Glenthorne (Table 2).

Total mycorrhizal colonisation of *L. scoparium* ranged from 8 to 56% of root length (Table 3). EM colonisation ranged from 7% of root length on saplings from Lake Hill to 55% on mature plants at Glenthorne. There was no or very little (<2%) AM colonisation. The highest AM colonisation found in any one sample was 4%. Dual colonisation (AM and EM) was found in only three of the 35 samples. Although variance in EM colonisation was high, the Glenthorne and Lake Self 2 sites had significantly higher EM than the Lake Hill 1, Lake Hill 2 and Lyndon sites (Table 3). Mean EM colonisation at the Lake Hill 3 and Lake Selfe 1 sites was also high, but differences with other sites were mostly not significant. EM colonisation was higher in root samples from mature plants (41 ± 6.0%; mean ± SE) than from saplings (<0.5 m tall; 19 ± 4.5%), but the difference was only weakly significant (unpaired $t\text{-test}_{df = 5}$, $P = 0.08$.

Discussion

We showed consistently (very) low AM colonisation, whereas EM colonisation was highly variable between sites. Our results differ from those of Moyersoen and Fitter (1999) who found root samples of *L. scoparium* collected from dominantly AM forest environments in South Westland to be mostly colonised by AM fungi. They found up to 60% colonisation, whereas in our study colonisation was always lower than 4%.

Table 2. Soil water content (SWC), pH, organic carbon (C), total nitrogen (N), C:N ratio (C/N), and total (Pt), inorganic (Pi) and organic (Po) phosphorus of sampling sites.

Site	SWC (%)	pH	C (%)	N (%)	C/N	Pt (mg g^{-1})	Pi (mg g^{-1})	Po (mg g^{-1})
Lake Hill (1)	20	5.5	5.1	0.27	19.0	0.61	0.07	0.54
Lake Hill (2)	20	5.2	7.3	0.39	18.5	0.63	0.09	0.54
Lake Hill (3)	11	5.5	7.9	0.39	20.1	0.78	0.10	0.68
Glenthorne	33	4.8	9.5	0.46	20.7	0.63	0.09	0.54
Lake Selfe (1)	67	6.6	31.5	0.75	41.8	0.54	0.08	0.47
Lake Selfe (2)	47	6.3	10.1	0.51	19.8	0.86	0.09	0.77
Lyndon	63	5.3	23.1	0.88	26.2	0.85	0.08	0.77

Table 3. Ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) colonisation (as a percentage of root length) and EM as a percentage of total colonisation of *Leptospermum scoparium* roots (mean ± SD; $n = 5$). Within columns, values without same letter in column are significantly different (Tukey's test, $P < 0.05$).

Site	EM (%)	AM (%)	EM as % of total
Lake Hill 1	7 ± 5 [a]	1 ± 1	87
Lake Hill 2	15 ± 6 [ab]	2 ± 2	88
Lake Hill 3	49 ± 29 [bc]	1 ± 1	98
Glenthorne	52 ± 20 [c]	0	100
Lake Selfe 1	36 ± 22 [abc]	2 ± 1	95
Lake Selfe 2	55 ± 21 [c]	1 ± 1	98
Lyndon	11 ± 3 [a]	1 ± 2	85

Because their samples were either dual mycorrhizal or exclusively arbuscular mycorrhizal these authors claimed that *L. scoparium* is unable to maintain EM colonisation in the absence of an alternative EM host. If true, we have to conclude *L. scoparium* is unlikely to facilitate reforestation of *Nothofagus*. However, our results do not support that suggestion by Moyersoen and Fitter (1999). On the basis of our data we suggest *L. scoparium* appears to be facultatively AM, because all root samples examined were dominantly EM regardless of the mycorrhizal status of surrounding vegetation.

We suggest the differences between the study by Moyersoen and Fitter (1999) and our study are related to differences in soil and climatic conditions. These conditions would select for mycorrhizal associations with different functional attributes. In contrast to AM species, some EM fungi can mobilise N and P from organic sources through the production of extracellular proteinase and phosphatase enzymes, and hence obtain access to organic N and P sources unavailable to AM species (Read & Perez-Moreno 2003). At low elevations in temperate latitudes and in relatively basic soils where organic matter mineralisation rates and the supply of mineral N and P are high, AM species predominate. As temperature declines with increase in altitude, mineralisation of organic matter and consequently the supply of mineral nutrients declines, the pH decreases, and vegetation becomes dominated by EM species capable of accessing N and P from organic sources. With one exception the *L. scoparium* root samples taken by Moyersoen and Fitter (1999) were from a moist coastal transect where mineralisation and supply of mineral N and P should be high, allowing AM associations to dominate. On the most basic sites with ultramafic soils, EM associations were even completely lacking. In the present study root samples were collected from a distinctly upper montane environment (altitude 560–805 m a.s.l.), which would favour EM associations. Thus *L. scoparium* establishing in the study area would be favoured by associating with EM rather than AM fungi. Although AM associations were exclusively present or predominant in six of the 10 samples collected by Moyersoen and Fitter (1999), four samples were exclusively or dominantly ectomycorrhizal.

High EM and low AM colonisation in species that can have both forms of mycorrhizae, as shown for *L. scoparium* in this study, is consistent with previous studies performed on the large majority of dual-mycorrhizal host species (van der Heijden & Vosatka 1999; van der Heijden 2001; Neville et al. 2002; Hashimoto & Higuchi 2003). Eucalypts have been shown to be predominantly AM when young, with EM colonisation becoming more abundant with increasing age (Chen et al. 2000; Neville et al. 2002; Gange et al. 2005). This was not the case in the present study, where both saplings and mature plants were found to be predominantly EM. Our findings are also consistent with the hypothesis that most, if not all, EM plant species can occasionally be colonised by AM fungi, whose functional significance needs to be elucidated further. However, the studies by Baylis (1971), Cooper (1976), and Moyersoen and Fitter (1999) clearly point out that *L. scoparium* is functionally dual mycorrhizal and that under certain edaphic conditions it can survive in the presence of only one group of symbionts. Being able to form functional EM and AM mycorrhizas would contribute to the success of *L. scoparium* at being able to invade grassland in New Zealand. In view of the fact that *L. scoparium* is a highly variable taxon and that there is substantial genotypic variation for tolerance for soil acidity, soil fertility response, and root anatomy (Stephens et al. 2005), it may be worthwhile to explore whether there is any genetic basis for preferential association with AM or EM fungi. Khasa et al. (2002) also observed large variation in mycorrhizal colonisation between clones of hybrid *Populus*, with EM dominating in most clones, but with a proportion of clones with AM exceeding EM colonisation, and suggested that a genetic component was involved in determining the kind of mycorrhizal association.

Although all roots were dominantly EM, the degree of EM colonisation varied greatly between sample sites. Distance to an EM source and plant-age appeared to have influenced the degree of EM colonisation. Colonisation was greater in mature plants than in saplings. Among mature plants, colonisation was significantly lower in plants from the Lyndon site than in plants from the remaining sites. The Lyndon site was the most distant from other indigenous ectomycorrhizal sources; it also differed from other mature-plant sites in that the plants existed as discrete units among grassland and did not form a continuous canopy. Among saplings, colonisation was greater in plants from the Lake Selfe 1 site, which adjoined mature *Nothofagus* forest and a large mature *Leptospermum* stand (Lake Selfe 2 site), than in plants from the Lake Hill 1 and 2 sites, which were located among grassland, although near a small patch of mature *Leptospermum* (Lake Hill 3 site).

Although these differences among sapling sites were not significant, the results are similar to those of Moyersoen and Fitter (1999), whose study indicated distance from an EM source was important in determining whether roots of young plants (<2 m tall) were colonised by EM or AM. Lower EM colonisation in young plants may be a result of a lower chance of contact between EM fungi propagules and the host root system because of the small size of the root system, or the limited period of time available for colonisation. The relationship between EM colonisation and proximity of indigenous EM sources suggests *L. scoparium* may act as both receiver and donor of EM inoculum.

Several studies have shown most colonisation is in nutrient-stressed situations (Read et al. 1976), with decreasing mycorrhizal abundance when soils are enriched with nitrogen (Baum & Makeschin 2000). In our study the two sites with highest soil nitrogen concentrations, Lake Selfe 1 and Lyndon, had the lowest degree of mycorrhizal colonisation. Total nitrogen, however, may not provide a good index of nitrogen bioavailability, which is likely to be low in the wet, organic soils of these sites. The soils of both sites had high C/N ratios, indicating N mineralisation rates were likely to be low, and neither the species present nor the vigour of the vegetation at these sites suggests high nitrogen availability. Low mycorrhizal colonisation of the Lake Selfe 1 and Lyndon sites is more likely to be associated with plant age and distance from EM source (see above) than with high levels of total nitrogen in soil.

Conclusions

While this study has shown *L. scoparium* in the upper Rakaia catchment to be dominantly EM, the degree of root colonisation by EM may vary substantially between sites. Tentative results from this study indicate that older *L. scoparium* communities or young communities close to substantial indigenous EM sources may have a higher EM colonisation than young plants or more distant communities and therefore may better facilitate establishment and spread of *Nothofagus*. For *Leptospermum* to facilitate spread of *Nothofagus*, the two genera need to host the same mycorrhizal species. While *Leptospermum* and *Nothofagus* host more than 30 fungal genera in common (Orlovich & Cairney 2004), it has not been confirmed to what extent such sharing extends to the species level. The occurrence of long-distance spread of *Nothofagus solandri* into *L. scoparium* communities (Burrows & Lord 1993), however, indicates the possibility that these two species may host at least some mycorrhizal fungal species in common. Further study to determine the extent of sharing of EM species by *Nothofagus* and *Leptospermum* is required.

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