Mycorrhizal colonisation of exotic conifers in kānuka and mānuka shrublands

Murray Davis* and Simeon Smaill

Scion, PO Box 29 237, Christchurch, New Zealand
*Author for correspondence (Email: murray.davis@scionresearch.com)

Published online: 8 July 2009

Abstract: Mycorrhizal colonisation of Douglas-fir and Corsican pine seedlings in soil from kānuka and mānuka dominated shrublands in Canterbury was studied using a bait plant technique. Soil cores were collected from 10 sites of each shrubland, transferred to a glasshouse, and sown with seed of both tree species. Mycorrhizal colonisation was examined after 19 weeks' growth. Overall, seedlings of Douglas-fir were larger than those of Corsican pine, but the amount of Corsican pine seedlings that were colonised (56%) was about twice that of Douglas-fir (29%). Across the sites, 7–61% of Douglas-fir and 16–81% of Corsican pine seedlings had mycorrhizas. Colonisation of the two tree species was correlated in soil cores from kānuka stands, but not from mānuka stands. Colonisation was significantly greater in kānuka than mānuka stands in Douglas-fir, but not in Corsican pine. Kānuka stands were of a lower overall elevation than mānuka stands and had higher levels of soil available phosphorus. Both factors may have contributed to the greater mycorrhizal colonisation of Douglas-fir in kānuka stands. Differences in colonisation between sites could be partially explained by the proximity of sources of spores of mycorrhizal fungi. It is concluded that low numbers of mycorrhizal propagules may constrain mycorrhizal formation in environments distant from a spore source, but are ultimately unlikely to preclude it, or limit successful seedling establishment.

Keywords: *Kunzea ericoides*; *Leptospermum scoparium*; *Pinus nigra*; *Pseudotsuga menziesii*; tree spread

Introduction

A number of exotic conifer tree species have the potential to invade indigenous vegetation communities in New Zealand, threatening scenic, biological and hydrological values (Hunter & Douglas 1984; Ledgard 1988, 2001; Allen & Lee 1989). Hunter and Douglas (1984) noted that spread of exotic conifers is more prolific in short open grassland and open shrubland communities and that conifer seedling success is poor in closed-canopy undisturbed forest and shrubland communities. Forest and shrubland communities, if disturbed by natural or anthropogenic events, however, may become vulnerable to invasion by exotic tree species (Richardson et al. 1994). Pine species (*Pinus* sp.), European larch (*Larix decidua* Mill.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) are the most prominent invading species in New Zealand rangelands (Hunter & Douglas 1984; Ledgard 1988, 2003), and of these Douglas-fir is generally considered to be the more shade tolerant and therefore have most potential to invade forests and shrublands (Ledgard 2002).

Most vascular plants form mycorrhizal associations in which the fungus benefits from photosynthetically derived carbon compounds and the plant benefits from enhanced nutrient uptake (especially of nitrogen and phosphorus), enhanced moisture uptake, and improved resistance to fungal pathogens (Allen 1991). Formation of effective mycorrhizas is almost certainly a requirement for exotic conifer seedlings to establish and thrive in most indigenous vegetation communities (e.g. Smith & Read 1997; Read 1998; Read & Perez-Moreno 2003). All of the exotic conifers that spread into native landscapes in New Zealand form ectomycorrhizas and are likely to rely primarily on exotic fungal species to form the relationship (Orlovich & Cairney 2004), though some ectomycorrhizal fungi have broad host ranges (Molina & Trappe 1982; Smith & Read 1997; Johnson et al. 2005). Although the absence of appropriate mycorrhizal symbionts may have initially prevented the successful establishment of pines in some Southern Hemisphere countries, Richardson et al. (1994) suggest appropriate symbionts are now ubiquitous in the Southern Hemisphere and are unlikely to act as barriers to invasion. In New Zealand, this may hold for pines more than for Douglas-fir as there have been failures of Douglas-fir forest plantings in the South Island that have been caused by the lack of development of appropriate mycorrhizas (Gilmour 1958; Chu-Chou & Grace 1987).

Mānuka (*Leptospermum scoparium* J.R.Forst. & G.Forst.) is the most abundant New Zealand shrub, is an important seral species, and often forms closed stands

up to 8 m tall (Wardle 1991). Kānuka (*Kunzea ericoides* (A.Rich) Joy Thomps.) often co-dominates with mānuka in seral shrubland but grows somewhat taller. Mānuka is found on a wider range of soils, and on generally less fertile soils than kānuka (Wardle 1991). Kānuka and mānuka shrublands normally succeed to tall forest communities, but can also maintain themselves as more or less stable communities if they are in harsh environments, are frequently burnt, are some distance from forest seed sources, or if seedlings of other establishing species are browsed (Wardle 1991). The potential exists for kānuka and mānuka shrublands to be invaded by exotic conifer species if there is a seed source in the vicinity.

We tested the hypothesis that a lack of suitable mycorrhizal inoculum does not limit spread of Douglas-fir and pine species into kānuka and mānuka shrubland. We used the bait plant technique of Brundrett and Abbott (1995) to assess mycorrhizal formation on pine and Douglas-fir seedlings grown in soils collected from kānuka and mānuka stands throughout the Canterbury Region. Brundrett and Abbott (1995) suggested that bioassays that measure mycorrhizal formation by bait plants grown in undisturbed soil cores would be expected to provide a better estimate of inoculum potential than counting propagules such as spores. Corsican pine (*Pinus nigra* J.F.Arnold) was used as the pine comparison with Douglas-fir as it is one of the important pine species to spread in the region, and has a similar seed size and similar growth rate in the seedling stage to Douglas-fir (unpubl. data). Douglas-fir shares some, but not other species of mycorrhizal fungi with *Pinus* (e.g. Chu-Chou & Grace 1987, 1988).

Methods

Soil cores were collected from beneath 10 kānuka and 10 mānuka mature closed-canopy stands located in the Canterbury Region, central South Island, New Zealand (Fig. 1). The elevation of the sites was recorded, and the distance and direction to the nearest Douglas-fir and pine stands as potential mycorrhizal fungal spore sources were noted (Table 1). All mānuka and six of the kānuka sites were from inland montane locations, while the remaining kānuka sites were either from coastal or central plains locations (Fig. 1, Table 1). Three kānuka sites were of lower elevation than any mānuka site and the mean elevation of kānuka sites (471 m) was lower ($P < 0.01$) than that of mānuka sites (729 m).

Table 1. Location and elevation of sample sites and distance and direction to nearest Douglas-fir and pine stands as potential mycorrhizal fungal spore sources. Site numbers are as in Fig. 1.

Site	Location	Elevation (m)	Distance (m) and direction to nearest *Ps. menziesii*	Distance (m) and direction to nearest *Pinus* sp.
Kānuka sites				
1 Ellangowan	Coastal	605	1000 W	500 SE
2 Okuti Valley	Coastal	230	500 SW	200 NW
3 Eyrewell Forest	Central plains	210	1190 NE	250 ESE
4 Lake Hill	Montane	508	1500 W	1500 W
5 Mt Barker	Montane	630	300 W	20 W
6 Acheron River	Montane	700	2400 SW	2400 SW
7 Dans Stream	Montane	620	3800 S	9690 SW
8 Lewis River	Montane	630	4300 S	10800 SW
9 Gorge Stream	Montane	420	2100 NW	1400 NW
10 Kate Valley	Coastal	160	7400 NE	800 N
Mānuka sites				
11 Bealey	Montane	825	2700 SE	2700 SE
12 Cass	Montane	760	2500 NW	1500 W
13 Avoca	Montane	550	11070 NW	700 N
14 Lake Selfe	Montane	605	1430 NE	1430 NE
15 Craigieburn 1	Montane	826	150 W	150 W
16 Craigieburn 2	Montane	828	270 NW	270 NW
17 Craigieburn 3	Montane	820	680 NW	680 NW
18 Mt Torlesse E	Montane	656	1710 SE	1800 E
19 Mt Torlesse W	Montane	562	950 S	1040 NE
20 Jacks Pass	Montane	862	3000 NW	3000 NW

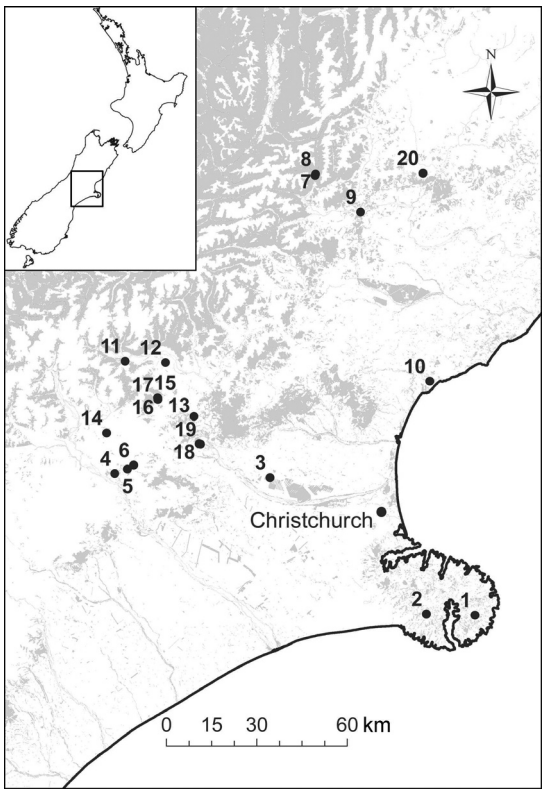

Figure 1. Location of kānuka (1–10) and mānuka sites (11–20) where soil cores were collected. Site numbers correspond to those in Table 1.

Twenty soil cores were collected at points spaced five or more metres apart at each site. The cores were collected by driving plastic tubes (105 mm long by 65-mm internal diameter) into the ground to a depth of approximately 95 mm. The cores were arranged in 20 polystyrene boxes on a glasshouse bench with one core from each site in each box. Each box formed one replicate. Seeds of Douglas-fir and Corsican pine were sterilised with 5% sodium hypochlorite and dried before sowing. Mean seed weights were 11.0 mg and 10.0 mg and mean laboratory germination percentages were 96% and 77% for Douglas-fir and Corsican pine respectively. Five seeds each of Douglas-fir and Corsican pine were sown in each pot and covered with inert plastic beads to a depth of about 10 mm to reduce the chance of cross-contamination of mycorrhizal propagules between pots. The pots were watered daily using an automated misting system. The position of the boxes on the greenhouse bench was randomised fortnightly. The seeds were sown in November 2006 and the trial was harvested in early April 2007, after a period of 19 weeks. No fertiliser was applied.

Seedlings were harvested by gently removing the soil cores from the tubes, then breaking up the cores by hand. In some cases, the soil cores were soaked in water to aid in the removal of the seedlings, and to minimise damage to the seedling root system. The harvested seedlings were placed in a shallow tray of water, and gently brushed to remove any soil adhering to the roots. The seedlings from each tube were then placed into plastic bags and refrigerated until further inspection could take place.

After all seedlings had been harvested, the root structure of each seedling was examined microscopically to detect mycorrhizal colonisation. Chu-Chou & Grace (1983) was used as a reference to confirm the presence and type of mycorrhizas in the Douglas-fir seedlings, and images identified by L. Grace were used to confirm mycorrhizal colonisation in the Corsican pine seedlings. These data were used to calculate mycorrhizal colonisation as the percentages of germinated seedlings infected with mycorrhizas in the soil cores collected from the different sites. The seedlings were then cut into stem and root sections, and the mean dry mass of these was determined after drying for 24 h at 70°C.

Soil was saved from five pots from each location and bulked, air dried and passed through a 2-mm sieve prior to analysis for total carbon and total nitrogen using a LECO CNS 2000 analyser, and bicarbonate extractable phosphorus (Olsen P) (Blakemore et al. 1987). The latter was used to provide a measure of plant available P and the C:N ratio was calculated to provide an index of available N.

Analysis of variance was carried out on the seedling germination, dry weight and mycorrhizal colonisation data to identify any significant variations at $P = 0.05$. Correlation analysis (Pearson's product moment correlation coefficient) was performed to identify statistically significant relationships between sites, seedling total dry weight, above- and below-ground allocation of biomass by the seedlings, and mycorrhizal colonisation.

Correlation analysis was also used to investigate the relationship between the distance and direction of the nearest stands of Douglas-fir and pine species as potential sources of mycorrhizal fungal spores, and the relative rate of mycorrhizal colonisation in the soil cores from the different sites. The prevailing wind direction of the Canterbury montane zone and foothills, and to a lesser extent of the plains to the east, is from the north-west (de Lisle 1969). It is a strong, turbulent föhn wind characterised by high temperatures and low humidities, conditions that promote dispersal of fungal spores (Bannon et al. in press). In contrast, winds from a southerly quarter, although also turbulent, are characterised by high humidities and reduced temperatures, conditions likely to reduce spore dispersal. Thus, sites downwind from a spore source to

the north-west are likely to receive greater numbers of spores than sites downwind from a source to the south. The correlation analysis investigating the relationship between colonisation and distance and direction to the nearest spore source was therefore carried out assigning an arbitrary multiplier to the distance between site and spore source to adjust for wind direction effects on spore dispersal. The multipliers used were 0.25 when spore source was north-west of the site, 1.0 when the spore source was to the south, south-west or south-east of the site, and 0.5 when the spore source was in any other direction.

All statistical analyses were carried out with R (Version 2.5.0, The R Foundation for Statistical Computing).

Results

Seedling germination

Seedling germination was greater in Douglas-fir (72%) than in Corsican pine (63%) ($P < 0.01$) and germination also varied significantly with site. Germination at Lake Selfe (34%) was substantially less than that at all other sites, where germination exceeded 50% and ranged up to 86% (data not presented). There was no interaction between site and tree species for germination.

Seedling biomass and mycorrhizal colonisation

Douglas-fir seedlings had greater mean root and shoot dry weights than Corsican pine seedlings. Seedlings of both species had greater dry weight in kānuka than mānuka soils, with the difference being more marked for Douglas-fir (Table 2). Across sites, shoot and whole-plant dry weights of the two species were significantly correlated ($r = 0.78$ and 0.62 respectively, $P < 0.01$), but root dry weights were not correlated to shoot or whole-plant weights.

Table 2. Mean (±SE) root, shoot and whole-plant weights (mg) of Douglas-fir and Corsican pine seedlings over all sites ($n = 20$), and within kānuka and mānuka sites ($n = 10$).

		Root	Shoot	Root + shoot
Douglas-fir	All sites	26 (1.6)	62 (3.9)	88 (2.3)
Corsican pine		23 (0.8)	53 (2.0)	75 (5.0)
	P	0.03	0.002	0.004
Douglas-fir	Mānuka sites	23 (1.8)	51 (2.9)	74 (3.6)
	Kānuka sites	29 (2.6)	73 (5.4)	102 (7.1)
	P	0.01	<0.001	<0.001
Corsican pine	Mānuka sites	23 (0.6)	49 (2.2)	71 (2.6)
	Kānuka sites	22 (1.5)	57 (2.9)	79 (3.6)
	P	0.67	0.003	0.03

Mean mycorrhizal colonisation of Corsican pine seedlings (56% of seedlings) was about twice that of Douglas-fir (29% of seedlings) ($P < 0.01$). Colonisation of Douglas-fir ranged between 7% and 61%, while in Corsican pine colonisation ranged between 16% and 81%. Colonisation of Douglas-fir seedlings was greater ($P < 0.01$) in soil from kānuka stands (38% of seedlings) than mānuka stands (19% of seedlings). Colonisation was also greater in Corsican pine seedlings in soil from kānuka (61% of seedlings) than mānuka (51%) stands, but this difference was not significant ($P = 0.27$). When kānuka and mānuka sites were considered together, colonisation of Douglas-fir seedlings was positively related to that of Corsican pine seedlings ($r = 0.67$, $P < 0.01$). When considered separately, colonisation of the two species was positively correlated for kānuka sites ($r = 0.79$, $P < 0.01$), but not for mānuka sites ($r = 0.46$ $P = 0.18$). Least colonisation in both species occurred in seedlings in soil from the montane mānuka site in the Rakaia catchment, which also had the lowest germination (Lake Selfe, Fig. 2). Less than 20% of Douglas-fir seedlings were infected in soil from five other sites, four of which were mānuka stands, while only Corsican pine seedlings in soil from Lake Selfe had less than 20% colonisation. In Douglas-fir greatest colonisation occurred in seedlings in soil from two kānuka sites on Banks Peninsula (Okuti Valley and Ellangowan), while in Corsican pine greatest colonisation occurred at a Waiau Valley site (Gorge Stream) and at Ellangowan (Fig. 2).

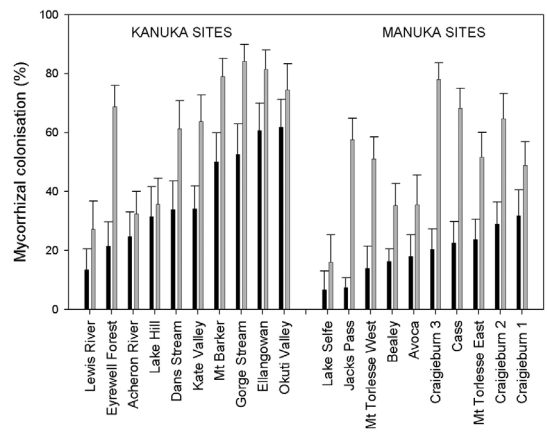

Figure 2. Mycorrhizal colonisation of Douglas-fir (shaded) and Corsican pine (open) seedlings in soil cores from mānuka and kānuka shrublands. Sites are arrayed in increasing order of colonisation of Douglas-fir seedlings. Bars show standard errors.

When kānuka and mānuka sites were considered together, mycorrhizal colonisation of both Douglas-fir and Corsican pine seedlings was positively correlated with seedling root weight and with whole seedling weight

in Douglas-fir (Table 3). When kānuka and mānuka sites were considered separately, seedling colonisation was positively correlated with seedling root weight for kānuka sites, but not for mānuka sites, while there were no significant relationships between colonisation and whole seedling weight for either Douglas-fir or Corsican pine (Table 3).

Table 3. Correlation between mycorrhizal colonisation and seedling root weight and whole seedling weight for kānuka and mānuka stands considered together or separately. Correlation coefficients (r) and P values for significance of the correlation are shown.

Tree species	Vegetation	Plant part	r	P
Douglas-fir	Kānuka + mānuka	Root	0.74	<0.01
		Whole seedling	0.63	<0.01
	Kānuka only	Root	0.76	0.01
		Whole seedling	0.38	0.28
	Mānuka only	Root	0.51	0.14
		Whole seedling	0.38	0.27
Corsican pine	Kānuka + mānuka	Root	0.52	0.02
		Whole seedling	0.18	0.44
	Kānuka only	Root	0.73	<0.01
		Whole seedling	0.34	0.15
	Mānuka only	Root	0.10	0.66
		Whole seedling	0.32	0.17

The analysis of the relationships between spore source location (given in Table 1) and mycorrhizal colonisation in the soil cores determined there were no statistically significant relationships between distance to spore source and mycorrhizal colonisation, but for kānuka stands the relationships approached significance for both Douglas-fir and Corsican pine (Table 4) and a trend of decreasing rate of colonisation with increasing distance was observed in all cases (Fig. 3a, c). All but one of the relationships were strengthened when distance to spore source was

Table 4. Correlation between the mycorrhizal colonisation of seedlings and distance or direction adjusted distance to the nearest stands of Douglas-fir (for Douglas-fir seedlings) or *Pinus* species (for Corsican pine seedlings). Correlation coefficients (r) and P values for significance of the correlations are shown. Asterisk denotes significance at $P < 0.05$.

Tree species	Vegetation	Distance		Direction adjusted distance	
		r	P	r	P
Douglas-fir	Kānuka	0.46	0.09	0.62	0.02*
	Mānuka	0.25	0.24	0.36	0.15
Corsican pine	Kānuka	0.53	0.06	0.53	0.06
	Mānuka	0.20	0.29	0.34	0.17

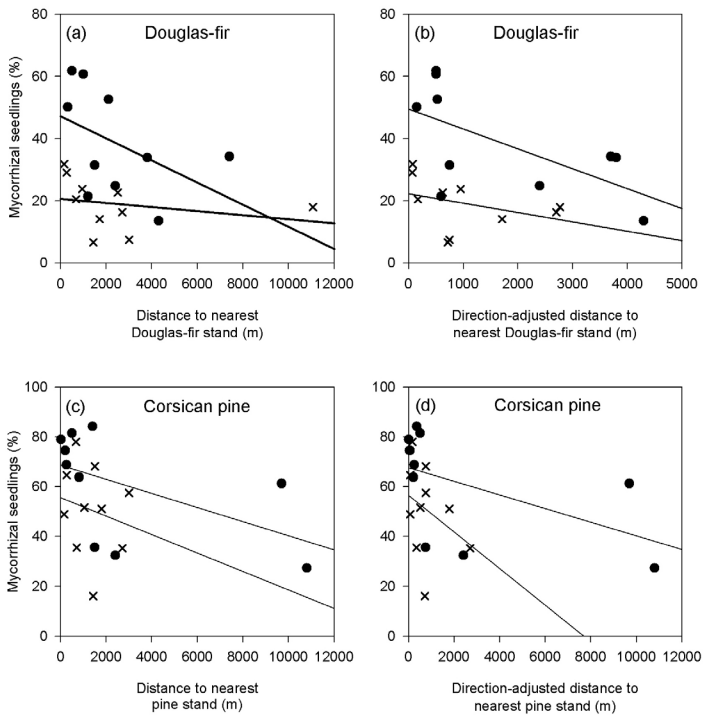

Figure 3. Correlation between the mycorrhizal colonisation of seedlings and distance (a, c) or direction-adjusted distance (b, d) to the nearest stands of Douglas-fir (for Douglas-fir seedlings) or *Pinus* species (for Corsican pine seedlings). Full lines and circles show seedlings in cores from kānuka stands, dotted lines and crosses show seedlings in cores from mānuka stands.

adjusted for direction, with that for kānuka stands and Douglas-fir becoming significant (Table 4) and the trends of decreasing rate of colonisation with increasing distance persisted (Fig. 3b, d).

Soil N and P availability

Soil C concentrations ranged between 3.8% and 8.5%, N concentrations between 0.24% and 0.71%, and C:N ratios between 11.8 and 18.2. Mean values for kānuka and mānuka stands did not differ significantly for these parameters (Table 5). Soil Olsen P values ranged more widely, between 2 and 43 mg kg^{-1}, and mean values were significantly higher under kānuka (18.4 mg kg^{-1}) than mānuka (7.2 mg kg^{-1}) stands ($P = 0.03$) (Table 5).

Table 5. Mean soil carbon, total nitrogen and Olsen phosphorus concentrations and C:N ratios.

Location	C (%)	N (%)	C:N ratio	Olsen P (mg kg^{-1})
Kānuka sites	5.94	0.43	14.0	18.4
SEM	0.591	0.049	0.57	4.38
Mānuka sites	5.56	0.37	15.2	7.2
SEM	0.411	0.033	0.86	1.71
P	0.58	0.33	0.12	0.03

Soil properties, seedling growth and mycorrhizal colonisation

Shoot and whole seedling weights of Corsican pine seedlings were positively correlated with soil Olsen phosphorus values, while shoot weights were negatively correlated with soil C:N (Table 6). Douglas-fir shoot, root and whole seedling weights were not significantly correlated with any of the soil properties measured, though the correlations between shoot weight and Olsen phosphorus (positive) and C:N (negative) approached significance ($P = 0.07$ and $P = 0.08$ respectively). Correlation coefficients between mycorrhizal colonisation and soil properties did not approach significance for either species.

Mycorrhizal fungal species

Mycorrhizal fungi resembling *Laccaria laccata* and *Thelephora terrestris* as shown in Chu-Chou & Grace (1983) were the most abundant fungal species present on the roots of Douglas-fir seedlings, with the latter perhaps being the more common of the two. A type resembling their Type 1 (*Rhizopogon* sp.) or Type 9 (*Boletus* sp.) was present at the three Craigieburn sites, but not elsewhere. There they were most abundant closest to a stand of Douglas-fir and declined in abundance with increasing distance from the stand. Mycorrhizas of *Tuber* species were occasionally observed.

Table 6. Correlation coefficients between seedling weight and mycorrhizal colonisation, and soil properties. Asterisks denote significance at $P < 0.05$.

Tree species	Plant part	C	N	C:N	Olsen P
Douglas-fir	Root	0.13	0.05	0.13	0.04
	Shoot	0.10	0.36	−0.57	0.60
	Root + shoot	0.12	0.29	−0.40	0.48
	Colonisation	−0.28	−0.19	−0.11	0.09
Corsican pine	Root	−0.27	−0.30	0.08	0.05
	Shoot	−0.02	0.32	−0.68*	0.71*
	Root + shoot	−0.11	0.17	−0.56	0.63*
	Colonisation	−0.12	−0.19	0.17	0.00

Mycorrhizas on Corsican pine seedling roots were insufficiently developed to be sure of identification of the fungal species present, but, based on comparisons with images identified by L. Grace, appeared to be either *Suillus luteus* or *Rhizopogon rubescens*.

Discussion

Variation in mycorrhizal colonisation

Overall, mycorrhizal colonisation of Corsican pine was about twice that of Douglas-fir, despite the fact that Douglas-fir seedlings were slightly larger than those of Corsican pine. Pines have been much more widely planted than Douglas-fir in the Canterbury Region, as elsewhere in New Zealand. Consequently, ambient levels of mycorrhizal fungal spores or other inocula capable of infecting pines should be greater than levels of inocula for Douglas-fir, giving greater colonisation in Corsican pine.

Kānuka and mānuka both form ectomycorrhizas and share many ectomycorrhizal fungal species (McKenzie et al. 2006). As noted previously, some ectomycorrhizal fungal species have broad host ranges (Molina & Trappe 1982; Smith & Read 1997; Johnson et al. 2005) and it is possible that kānuka and mānuka share some species in common with Douglas-fir and Corsican pine, though this is considered unlikely (Orlovich & Cairney 2004). If Douglas-fir and Corsican pine do not share associations with mānuka and kānuka, successful mycorrhizal colonisation of their seedlings in kānuka or mānuka soils will be highly dependent on the presence of spores of appropriate fungal species arising from outside spore sources. Most of the fungi genera observed on seedling roots in this study (*Laccaria, Suillus, Thelephora,* and perhaps *Boletus*) have above-ground fruiting bodies (mushrooms) from which spores are dispersed by wind, particularly from fruiting bodies around forest margins where wind turbulence is high (Allen 1991).

Correlation analysis indicated distance from and direction to existing stands, as potential mycorrhizal spore sources, explained small to moderate amounts of

variation between sites in mycorrhizal colonisation. Study of dispersal of spores of fungal pathogens has shown that once spores escape from a canopy their concentration in the air decreases rapidly with distance from the source due to dilution by wind shear and atmospheric turbulence, resulting in large deposition near the source and a tail that extends downwind (Aylor 2003). As expected, colonisation tended to be greater in soil from stands close to a spore source and in the direction of the prevailing northwest wind. Relationships were stronger for kānuka than mānuka sites, possibly because of the generally lower elevation of the former. In Canterbury mountain valleys and basins, airflow is strongly affected by localised topographic effects (Sturman 2008), which are likely to be greater at higher elevations.

The three Craigieburn mānuka sites form a sequence of increasing distance from Douglas-fir stands on the lower slope of Lyndon Hill immediately to the south-west. Pine species are also present as both planted and wilding stands over much of the hill, extending to the summit. Craigieburn sites 1, 2 and 3 are about 150, 270 and 680 m distant, respectively, from the Douglas-fir stand, and mycorrhizal colonisation in soil from these sites declined with increasing distance from the stand. With Corsican pine, however, the opposite occurred; colonisation increased with distance from the base of the hill, possibly because spores dispersed from the higher elevation pines on the hill travelled further and in greater quantity than from spore sources located at the base of the hill. Thus, higher elevation hillslopes may act as 'take-off' sites for spores in an analogous manner to that for tree seed as suggested by Ledgard (2001).

The opposite trends of decreasing (Douglas-fir) and increasing (Corsican pine) colonisation with distance from the base of Lyndon Hill resulted in large differences in mycorrhizal colonisation between the two species at the Craigieburn 3 site. Marked differences in colonisation between the two species also occurred in soil from three other sites – Eyrewell Forest, Jacks Pass and Cass (Fig. 2). The Eyrewell Forest site is located immediately to the west of Eyrewell Forest, which is planted almost entirely in *Pinus radiata*. Consequently, there would have been a ready source of spores for colonisation of Corsican pine, not only from the forest but also from pines that have been widely planted for shelter on Canterbury Plains farms. In contrast there were few Douglas-fir spore sources in the vicinity, the nearest being 1.2 km in a north-easterly direction. The second site, Jacks Pass, lies above and about 2 km to the north of Hanmer Forest. The forest is predominantly planted with radiata pine but also contains areas of Douglas-fir. Ledgard (unpubl. report) notes that wilding spread around Hanmer Forest is largely to the south-east, in the prevailing wind direction, and that wildings present in the Jacks Pass area are most likely from plantings around St James homestead, some 3 km to the north-west. Presumably spores in the soil from the mānuka stand would also most likely arise from the St James plantings, which are mostly pines, although a small number of Douglas-fir trees are also present, a difference that could explain the difference in mycorrhizal colonisation of the two species. At Cass, the third site, stands of both pines and Douglas-fir occur to the west; however, pine plantings were about 1 km closer to the sample site, explaining the difference in colonisation of the two species there.

The greater fertility of the kānuka soils may have contributed to the greater colonisation of Douglas-fir seedlings in those soils. Kānuka soils had higher available phosphorus levels than the mānuka soils, and the roots of Douglas-fir seedlings in kānuka soils were larger and may therefore have been more receptive to colonisation. This did not hold for Corsican pine seedlings, which, in turn, had only marginally and not significantly greater colonisation in soils from the kānuka sites. Distance and direction to a potential spore source do not appear to have contributed to the greater colonisation of Douglas-fir in soil from kānuka sites; the mean distance of the nearest Douglas-fir stand to mānuka and kānuka sites was 2446 and 2449 m respectively, and nearest spore sources were generally more to the north-west for mānuka than for kānuka sites (Table 1). As spore numbers in the atmosphere will decline with increasing elevation (Aylor 2003), the generally lower elevation of the kānuka sites may also have contributed to greater colonisation of Douglas-fir seedlings in soil from kānuka sites. Greater ambient levels in the atmosphere of mycorrhizal fungal spores capable of infecting pines than Douglas-fir may have contributed to the lack of influence of stand type (kānuka or mānuka) on colonisation of Corsican pine seedlings.

The mycorrhizal fungal genera on the roots of Corsican pine seedlings were identified as being similar to *Rhizopogon rubescens* or *Suillus luteus*, from images identified by L. Grace. *Rhizopogon rubescens* is a hypogeous species, fruiting below the surface of the soil. Spore dispersal of hypogeous species (as well as some mushroom-producing species) is facilitated by browsing animals (Allen 1991). In New Zealand, mice, possums, pigs and deer and perhaps some invertebrates are potential browsers of above- and below-ground fruiting bodies. Elsewhere, animal vectors have been observed to move mycorrhizal inocula up to 0.7 km from a source area (Allen 1991). Because of the distance from spore sources it seems likely that most of the spore inocula at the sites of the present study would have been wind dispersed from above-round fruiting bodies, indicating *Suillus luteus* to be the more likely fungal symbiont than *Rhizopogon rubescens*. *Suillus luteus* is commonly seen fruiting in association with pine stands of all ages in the Canterbury Region.

It is possible that some of the mycorrhizas observed on the seedling roots may have arisen from contaminant airborne spores settling on the soil surface of the pots while

the trial was in progress, as the glasshouse was vented to the outside atmosphere, rather than from spores contained in or on the cores when collected in the field. The trial was completed in early April, at a time when fruiting body production and wind dispersal of spores would have been occurring. Colonisation from ambient air spores is considered unimportant, however, as seedlings require 4 weeks or longer for ectomycorrhizal associations to become established (Brundrett et al. 1966), thus limiting the opportunity of mycorrhizas from these spores to have been present at the time of assessment. The occurrence of five- (Corsican pine) to eight-fold (Douglas-fir) variation in colonisation in cores from the different sites further suggests that mycorrhizal formation from such spores was unlikely to be of importance.

Does lack of mycorrhizal fungi constrain establishment of exotic conifers in kānuka and mānuka shrublands?

The low mycorrhizal colonisation of seedlings in soil cores collected from some of the kānuka and mānuka stands in this study suggests mycorrhizal formation could be a major constraint to seedling establishment, particularly of Douglas-fir seedlings. As both seed of exotic conifers and spore propagules are carried by air movement, however, sites receiving seed rain are, in practice, also likely to be exposed to spore rain of appropriate mycorrhial fungi from the same source if the fungi involved have above-ground fruiting bodies. Spore density will be greatest close to a source and decline with increasing distance from the source, thus the chances of successful mycorrhizal formation will also decline with increasing distance from the source.

Read (1998) suggested that the processes of invasion by host and fungus must occur with some simultaneity to ensure the development of the symbiosis that is a prerequisite for successful establishment in new environments. There is little information on longevity of spores in field environments, but dry spores are known to survive for years in laboratory conditions in a physiologically quiescent state (Brundrett et al. 1996), and wet spores have also been reported to last for up to 2 years with refrigeration (Castellano 1994). The fact that seedlings became mycorrhizal in the present study indicates spores are at least able to overwinter, as cores were collected in spring and spores of the genera observed in this study, with the exception of *Rhizopogon*, are dispersed from sporophores in autumn. Spores of ectomycorrhizal fungi are normally dependent on the presence of a host root to stimulate germination (Theodorou & Bowen 1973; Read 1998). Thus, spores arriving in a new environment may lie dormant and accumulate until germination is stimulated by roots of establishing seedlings, obviating the need for seed and spores to arrive together as suggested by Read (1998). The attributes of spore persistence and germination stimulation by a host root would favour mycorrhizal colonisation of seedlings in new environments distant from a spore source. Thus low numbers of mycorrhizal propagules may constrain mycorrhizal formation in environments distant from a spore source, but are unlikely to ultimately preclude it, or preclude successful seedling establishment.

Acknowledgements

Funding for this project was provided by the Department of Conservation (Contract No. 3820). We thank Nick Ledgard (Scion) and Clayson Howell (Department of Conservation) for helpful comments on the manuscript.

References

Allen MF 1991. The ecology of mycorrhizae. Cambridge University Press. 184 p.

Allen RB, Lee WG 1989. Seedling establishment microsites of exotic conifers in *Chionochloa rigida* tussock grassland, Otago, New Zealand. New Zealand Journal of Botany 27: 491–498.

Aylor DE 2003. Spread of plant disease on a continental scale: role of aerial dispersal of pathogens. Ecology 84: 1989–1997.

Bannon F, Gort G, van Leewen G, Holb I, Jeger J. Diurnal patterns in dispersal of *Monilinia fructigena* conidia in an apple orchard in relation to weather factors. Agricultural and Forest Meteorology, in press.

Blakemore LC, Searle PL, Daly BK 1987. Methods for chemical analysis of soils. NZ Soil Bureau Scientific Report No. 80. 103 p.

Brundrett MC, Abbott LK 1995. Mycorrhizal fungus propagules in the jarrah forest II. Spatial variability in inoculum levels. New Phytologist 131: 461–469.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N 1996. Working with mycorrhizas in foresty and agriculture. ACIAR Monograph 32. Canberra, Australian Centre for International Agricutural Research. 374 p.

Castellano M 1994. Current status of outplanting studies using ectomycorrhiza-inoculated forest trees. In: Pfleger F, Linderman B eds. Mycorrhizae in plant health. St Paul, The American Phytopathological Society. Pp. 261–281.

Chu-Chou M, Grace LJ 1983. Characterisation and identification of mycorrhizas of Douglas-fir in New Zealand. European Journal of Forest Pathology 13: 251–260.

Chu-Chou M, Grace LJ 1987. Mycorrhizal fungi of *Pseudotsuga menziesii* in the South Island of New Zealand. Soil Biology and Biochemistry 19: 243–246.

Chu-Chou M, Grace LJ 1988. Mycorrhizal fungi of

radiata pine in different forests of the North and South Islands in New Zealand. Soil Biology and Biochemistry 20: 883–886.

de Lisle JF 1969. The climate and weather. In: Knox GA ed. The natural history of Canterbury. 1st edn. Wellington, A. H. & A. W. Reed. Pp. 68–76.

Gilmour JW 1958. Chlorosis of Douglas-fir. New Zealand Journal of Forestry 7(5): 94–106.

Hunter GG, Douglas MH 1984. Spread of exotic conifers on South Island rangelands. New Zealand Journal of Forestry 29: 78–96.

Johnson D, IJdo M, Genney DR, Anderson IC, Alexander IJ 2005. How do plants regulate the function, community structure, and diversity of mycorrhizal fungi. Journal of Experimental Botany 56: 1751–1760.

Ledgard NJ 1988. The spread of introduced trees in New Zealand's rangelands — South Island high country experience. Review – Tussock Grasslands and Mountain Lands Institute 44: 1–8.

Ledgard N 2001. The spread of lodgepole pine (*Pinus contorta*, Dougl.) in New Zealand. Forest Ecology and Management 141: 43–57.

Ledgard N 2002. The spread of Douglas-fir into native forests. New Zealand Journal of Forestry 47(2): 36–38.

Ledgard NJ 2003. Wilding conifers – New Zealand history and research background. In: Hill RL, Zynebos SM, Bezar CM eds Managing wilding conifers in New Zealand: present and future. Proceedings of a workshop held in conjunction with the New Zealand Plant Protection Society. Christchurch, New Zealand Plant Protection Society Inc. Pp. 1–25.

McKenzie EHC, Johnston PR, Buchanan PK 2006. Checklist of fungi on teatree (*Kunzea* and *Leptospermum* species) in New Zealand. New Zealand Journal of Botany 44: 293–335.

Molina R, Trappe JM 1982. Patterns of ectomycorrhizal host specificity and potential among Pacific Northwest conifers and fungi. Forest Science 28: 423–458.

Orlovich DA, Cairney JWG 2004. Ectomycorrhizal fungi in New Zealand: current perspectives and future directions. New Zealand Journal of Botany 42: 721–738.

Read D 1998. The mycorrhizal status of *Pinus*. In: Richardson DM ed. Ecology and biogeography of *Pinus*. Cambridge University Press. Pp. 324–340.

Read DJ, Perez-Moreno J 2003. Mycorrhizas and nutirent cycling in ecosystems – a journey towards relevance? New Phytologist 157: 475–492.

Richardson DM, Williams PA, Hobbs RJ 1994. Pine invasions in the Southern Hemisphere: determinants of spread and invadability. Journal of Biogeography 21: 511–527.

Smith SE, Read DJ 1997. Mycorrhizal symbiosis. 2nd edn. San Diego, CA, USA, Academic Press. 605 p.

Sturman A 2008. Weather and climate. In: Winterbourne M, Knox G, Burrows C, Marsden I eds The natural history of Canterbury. 2nd edn. Christchurch, Canterbury University Press. Pp. 119–142.

Theodorou C, Bowen GD 1973. Inoculation of seeds and soil with basidiospores of mycorrhizal fungi. Soil Biology and Biochemistry 5: 765–771.

Wardle P 1991. Vegetation of New Zealand. Cambridge University Press. 672 p.

Editorial Board member: David Wardle
Received 6 November 2008; accepted 9 February 2009