Ectomycorrhiza of *Pinus radiata* (D. Don 1836) in New Zealand — an above- and below-ground assessment

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Abstract

Ectomycorrhizal (ECM) species identity, richness and diversity of the exotic plantation species *Pinus radiata* were investigated in a nursery and stands of different ages (2, 8, 15 and 26 years of age) in the North Island of New Zealand over the course of two years. ECM species were identified by morphology and molecular (restriction fragment length polymorphism (RFLP) and DNA sequencing) analysis. Eighteen ECM species were observed above-ground, 19 ECM species below-ground. *Inocybe sindonia, Lactarius rufus, Pseudotomentella* sp., *Pseudotomentella tristis, Rhizopogon pseudoroseolus, R. luteorubescens, Tomentella* sp. and *Wilcoxina mikolae* were recorded as ECM associates of *P. radiata* in New Zealand for the first time. Species richness and diversity of ECM fungi associated with *P. radiata* in New Zealand were low compared to native forests in the Northern Hemisphere but comparable to other exotic plantations in the Southern Hemisphere.

Key words: Ectomycorrhiza, *Pinus radiata*, plantation forest, RFLP, sequence analysis.

Introduction

Pinus radiata comprises 90% of New Zealand's plantation estates and is one of the most extensively domesticated forest species worldwide (Dilworth 2004). It was introduced to New Zealand via England and Australia in the 1850s as a shelter tree (Shepherd 1990) and since then established as a plantation tree because of its rapid growth induced by New Zealand's mild climate. *Pseudotsuga menziesii*, on the other hand, failed to establish in New Zealand initially in the late 1950s (Gilmour 1958). This establishment problem was caused by a lack of ectomycorrhizal (ECM) colonisation of the seedlings and demonstrated the need for ECM presence for successful introduction of an exotic tree species and establishment of a plantation forest.

Significant research on ectomycorrhizal colonisation was conducted by Chu-Chou & Grace in the late 1970s to 90s (Chu-Chou 1979; Chu-Chou & Grace 1983, 1987, 1990) and focused on the ECM fungal diversity in exotic plantations in New Zealand (*P. radiata, Ps. menziesii* and *Eucalyptus* spp.). Even though forestry is New Zealand's third largest export earner (NZFOA 2006), ECM associates of plantation species in New Zealand have not been a research focus since then. Chu-Chou & Grace's research was extensive; however, it was mainly restricted to above-ground ECM diversity because the methods necessary to identify below-ground ECM species were limited at that time. As Chu-Chou & Grace could not culture many ECM types from root tips, numerous ECM species remained unidentified. This present study explores the below-ground ECM communities more extensively than was previously possible using methods, such as molecular fingerprinting (restriction fragment length polymorphism, RFLP) and DNA sequencing.

The aim of this research was to investigate ECM species associated with *P. radiata* of different ages with molecular tools and to correlate above- and below-ground ECM communities. To investigate the ECM diversity of *P. radiata* in New Zealand, a combined approach of surveys of above-ground reproductive structures ('sporocarps'), morphotype analysis of ECM colonizing root tips ('morphotypes') and molecular methods was used to identify ECM of different age *P. radiata* stands over two years.

Material and Methods

Site description

The study was conducted at Te Ngae Nursery, in Rotorua and in Kaingaroa Forest, both located in the interior volcanic plateau of the North Island of New Zealand. The study sites were in a frost-prone area with evenly distributed and relatively high rainfall, a mean annual temperature of 10.8°C and a mean annual rainfall of 1475 mm. The soil type in the stands in Kaingaroa Forest is Kaingaroa loamy sand, whereas in the nursery it is a mix of loamy sand, Rotomahana mud and Kaharoa ash. The nursery stage and four stands of varying age (2, 8, 15 and 26 years of age in 2006) were investigated. The same sites were used for sporocarp and soil core assessments. All stands were monoculture *P. radiata* plantations in their third rotation and located within 2 km of each other. Soil conditions, slope and aspect were the same across all sites.

Experimental design

Both sporocarp and soil core sampling were carried out in

2005 and 2006. At all study sites in Kaingaroa Forest, a 100 m x 100 m plot was established approximately in the middle of each stand to avoid edge effects (stand size ranging from 56 –144 ha). Within each plot, sporocarps were surveyed along five permanent 100 m long, randomly positioned, parallel transects with the exception of the 15 year old site which had only two transects. At Te Ngae Nursery, the plot was 100 m × 60 m in 2005, and was increased to 100 m × 100 m in 2006.

Sampling of ectomycorrhizal sporocarps

Sporocarp surveys were conducted over two consecutive fruiting seasons (2005 and 2006) along all transects within each plot. Surveys were carried out at 2- to 3-weekly intervals from March to June each year. In 2005, presence/absence of sporocarps was recorded, and in 2006 abundance of sporocarps was also recorded. Specimens within two metres of the transect were recorded but decayed specimens were ignored and searches for hypogeous species were kept to a minimum to avoid disturbance. Specimens were identified based on macro-morphology. Voucher specimens were dried at 40°C using a commercial fruit dehydrator and placed in the National Forestry Mycological Herbarium (NZFRI-M; Scion, Rotorua, New Zealand). DNA was extracted from representative sporocarps for genetic comparisons with that from ectomycorrhizal roots (RFLP and DNA sequencing) and to aid in identification.

Sampling of ectomycorrhizal morphotypes

Soil core collections were undertaken in June 2005, December 2005 (no sampling in the nursery) and May/June 2006. In the nursery sites, the whole seedling was removed from the soil due to the small root system. In June 2005, a total of 21 cores were collected, 23 in December 2005 and 32 cores in May/June 2006. Soil cores were collected 60 mm from the tree base and each soil core was 50 mm in diameter and 400 mm in depth. Following collection, samples were placed in a plastic bag and stored at 4°C. Soil cores were processed within two weeks by soaking in distilled water overnight followed by gentle washing with tap water over a 2 mm sieve to clean the root tips. Colonised root tips were removed under a dissecting microscope (Zeiss, Jena, Germany) using forceps. ECM root tips were categorized into '*ad hoc* morphological groups' (Dickie & Reich 2005), a less detailed examination than a true 'morphotype' as defined by Agerer (1987). The categorization into morphological groups was based on mantle colour and texture, root branching pattern, root tip shape and the morphology of mycelial strands and emanating hyphae (Agerer 1987; Goodman *et al.* 2003). The presence of a Hartig net and mantle was confirmed microscopically for one representative of each morphological type. ECM colonised root tips were quantified, each ECM tip was counted as a mycorrhiza, as this approach takes branching intensity into account (Brundrett *et al.* 1996). Representatives from each morphological type from each soil core were chosen randomly for DNA extraction.

Molecular analysis

DNA was extracted from sporocarps and root tips using the plant DNA extraction kit REDExtract-N-Amp™ Plant PCR kit (Sigma, St. Louis, Missouri, USA) following the manufacturer's instructions. For sporocarp DNA extraction, a sample was removed from the central portion of the stipe to avoid surface contamination. For extraction of DNA from ECM root tips, the manufacturer's instructions were modified as follows: 50 µL of extraction buffer and 50 µL of dilution solution were added to a sample and mycorrhizal root tips were broken into pieces with a pipette tip when adding the extraction solution, ensuring the solution would soak into the fungal tissue (Avis *et al.* 2003).

The internal transcribed spacer (ITS) regions of the rDNA was amplified using the fungal specific primer combination of ITS1F and ITS4 (White *et al.* 1990; Gardes & Bruns 1993). PCR was performed using the PCR mix supplied with the REDExtract-N-Amp™ kit, which included *Taq* enzyme, hot start TaqStart antibody and dNTP's. The primer concentration was 0.5 µM. The genomic extract was diluted 1:10 for the PCR reaction. The thermocycler program had an initial denaturation step of 94°C for 85 s, followed by 14 cycles of 95°C for 35 s, 55°C for 55 s and 72°C for 45 s, 15 cycles of 94°C for 35 s, 53°C for 55 s and 72°C for 2 min, 10 cycles of 94°C for 35 s, 53°C for 55 s and 72°C for 3 min, followed by a final extension step of 72°C for 10 min using an Eppendorf Mastercycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany). PCR products were purified using the GenElute PCR Clean-Up Kit (Sigma). Only PCR products consisting of a single band were used for sequencing and RFLP analysis. Where putative basidiomycete mycorrhizae produced more than one ITS PCR product, the DNA was amplified with the basidiomycete primers ITS1F and ITS4B (Bruns & Gardes 1993), before using a nested re-amplification with ITS1F and ITS4 to allow subsequent comparison of RFLPs and sequences (Genney *et al.* 2006). RFLP patterns were generated with *Alu*I (Roche Applied Science, Penzberg, Germany), *Hinf*I (Roche Applied Science) and *Mbo*I (Invitrogen) using the supplied buffers for each enzyme and incubation at 37°C for 1 h. Restriction digest products were separated by electrophoresis on a 2% agarose gel at 85 V for 2 h. Restriction fragment patterns were measured using Bio-Rad Quantity One® Image Acquisition & Analysis Software (Bio-Rad Laboratories, Hercules CA, USA) and compared manually and using the spreadsheet-based freeware GERM (Good-Enough RFLP Matcher, Dickie *et al.* 2003).

A representative of the ITS region from each ECM morphotype was cloned using the pGEM-T® Easy Vector System (Promega Corporation, Madison, USA) and Stratagene (La Jolla, CA, USA) XL-1 Blue MRF'KAN electroporation competent cells. DNA was extracted from positive transformants by alkaline lysis (Sambrook *et al.* 1987). Inserts were checked by restriction digestion with *Eco*RI (Roche Applied Science) according to manufacturer's instructions. DNA from sporocarp and ECM material was sequenced using the BigDye® Terminator v3.1 & v1.1 Cycle Sequencing Kits (Applied Biosystems). DNA sequences were edited and aligned using Sequencher version 4.7 (GeneCodes Corp. Ann Arbor, MI, USA) and identities were determined by BLASTn searching of GenBank and UNITE (Koljalg *et al.* 2005) nucleotide databases. For identification, a minimum of 95% sequence identity to an ITS sequence of at least 450 bp from a known specimen in the GenBank database was required. Those samples with a 97–100% match to a known species were considered a match and named to the species level. Those sequences with 96% or lower identity to known sequences were named to the genus, family or order. Samples that had no ITS sequence match were referred to as unknown 1, 2 etc. (Ashkannejhad & Horton 2006).

Statistical analysis

Relative abundance was calculated as the number of

Table 1 ECM taxa/morphotypes observed above— and below ground from different aged *P. radiata* stands and a nursery (2005 and 2006) – accession number of sequences generated from this study from root tip (R) or sporocarps (S) and closest match to NCBI or UNITE database.

ECM taxa/morphotype	Source	Accession	Closest match	Match (NCBI/Unite)
Amanita muscaria (L.) Lam. (1783)	S	GQ267469	AB080983	98
Amanita muscaria	R	GQ267468	AB080983	98
Cenococcum geophilum Fr. (1829)	R	n/a	n/a	n/a
Chalciporus piperatus (Bull.) Bataille (1908)	S	GQ267470	AF335357	99
Hebeloma sp.	S	GQ267472	EF411103	98
Hebeloma sp.	R	GQ267471	EF411103	98
Inocybe lacera (Fr.) P. Kumm. (1871)	S	GQ267473	AY750157	99
*Inocybe sindonia** (Fr.) P. Karst. (1879)	S	GQ267474	UDB002392**	n/a
Inocybe sp.	S	GQ267476	DQ974812	88
Inocybe sp.	R	GQ267475	DQ974812	88
Laccaria proxima (Boud.) Pat. (1887)	S	GQ267477	DQ068958	99
*Lactarius rufus** (Scop.) Fr. (1838)	S	GQ267478	EF685089	100
Pseudotomentella sp.*	R	GQ267479	DQ377428	96
*Pseudotomentella tristis** (P. Karst.) M. J. Larsen (1971)	R	GQ267480	AJ889968	100
Rhizopogon luteolus Fr. (1817)	S	GQ267481	AF062936	97
*Rhizopogon luteorubescens** A.H. Sm. (1966)	R	GQ267482	AJ810038	99
*Rhizopogon pseudoroseolus** A. H. Sm. (1996)	S	GQ267484	AJ810042	99
Rhizopogon pseudoroseolus	R	GQ267483	AJ810042	99
Rhizopogon rubescens (Tul. & C. Tul.) Tul. & C. Tul. (1844)	S	GQ267485	AF158018	95
Rhizopogon rubescens	R	GQ267486	AF158018	95
Scleroderma bovista Fr. (1829)	S	GQ267487	AB099901	100
Suillus sp.	S	GQ267488	AY880932	99
Thelephora terrestris Ehrh. (1787)	S	GQ267490	U83486	98
Thelephora terrestris	R	GQ267489	U83486	98
Tomentella sp.*	R	GQ267491	DQ990851	95
Tricholoma sp.	S	GQ267492	AF458435	96
Tuber sp.	R	GQ267493	AY748861	99
*Wilcoxina mikolae** (Chin S. Yang & H.E. Wilcox) Chin S. Yang & Korf (1985)	R	GQ267499	DQ069000	98
unknown Basidiomycete	R	GQ267494	AB211143	98
unknown 2	R	n/a	n/a	n/a
unknown 8	R	GQ267496	AY641466	91
unknown 9	R	GQ267497	AM1096	99
unknown 10	R	GQ267498	AM901986	90
unknown 12	R	n/a	n/a	n/a

Primer pairs ITS1F and ITS4 were used for the PCR amplification of the ITS region; * new report as ECM associate of *P. radiata* in New Zealand; ** UNITE specific accession number, no % match applicable from this database; n/a = not applicable, ECM type failed to amplify or identification based on morphology only.

sporocarps or ECM species for each ECM taxon divided by the total number of sporocarps sampled or ECM root tips counted per study site and expressed as a percentage. The diversity measures (species richness, evenness, Shannon and Simpson diversity index) were calculated using the analysis package PRIMER (Plymouth Routines In Multivariate Ecological Research, 2002 PRIMER-e Ltd) and presented as the mean. Data from the two above- and three below-ground assessments were pooled for analysis.

Results

ECM above-ground—richness, abundance and diversity

A total of 18 ECM taxa were observed during the two survey periods. *Wilcoxina mikolae*, *Rhizopogon pseudoroseolus*, *Inocybe sindonia* and *Lactarius rufus* were observed in this study for the first time in New Zealand as ECM associates of *P. radiata* (Table 1). Total observed richness ranged across stands from 3 to 10 species, mean species richness ranged from 2.6 in the nursery to 6.8 in the oldest investigated site (Table 2). Shannon diversity was 1.8 overall and ranged from 0.4 in the nursery to 1.1 in the 15 and 26 year old sites, whereas Simpson diversity overall was 0.79 and ranged from 0.3 to 0.6, respectively, in the nursery, and 15 and 26 year old sites. A total of 4782 specimens were counted in 2006 and the highest number of species was found in the 26 year old stand (Table 3). Overall the most abundant species was *Lactarius rufus* (33%), followed by *Laccaria proxima*, *Inocybe sindonia* and *I. lacera* (22%, 18% and 14%, respectively) (Fig. 1).

ECM below-ground—richness, abundance and diversity

The three soil core assessments in 2005 and 2006 revealed the presence of 19 distinct ECM morphotypes (Table 1). Below-ground, *Wilcoxina mikolae*, *Rhizopogon pseudoroseolus*, *R. luteorubescens, Pseudotomentella* sp., *Pseudotomentella tristis* and *Tomentella* sp. were recorded as new ECM associates of *P. radiata* in New Zealand (Fig. 3). The observed richness ranged from 5 morphotypes in the nursery and 2 year old stand to 13 ECM morphotypes in the 15 and 26 year old stands. Mean species richness was lowest in the 2 year old site in Kaingaroa Forest (1.6) and highest in the 26 year old stand that was assessed (5.0) (Table 2). The overall Shannon diversity was 2.2 and ranged from 0.4 in the 2 year old site to 1.2 in the 26 year old site, Simpson diversity ranged from 0.3 in the 2 year old site to 0.6 in the 26 year old site, with the overall Simpson diversity being 0.8 (Table 2). A total of 33,964 ECM root tips were analysed; the number assessed from each stand across all assessment times is shown in Table 3. The most abundant ECM morphotype was *R. rubescens* (32%), followed by *Wilcoxina mikolae*, *Tuber* sp., *Amanita muscaria* and *Hebeloma* sp. (abundance ranging from 16% to 7%, Fig. 2). All remaining ECM morphotypes had an abundance of less than 4%. ECM morphotypes found in the nursery and the 2 year old stand (*R. rubescens*, *Hebeloma* sp., *Tuber* sp., *Wilcoxina mikolae* and Type unknown 2) were not present in the 8, 15 and 26 year old stands and vice versa.

Discussion

In this study, a total of 18 ECM sporocarps and 19 ECM morphotypes were observed in above- and below-ground assessments of *P. radiata* in a nursery and four plantations sites in Kaingaroa Forest, New Zealand. With the use of RFLP fingerprinting and DNA sequencing for species identification, ECM species that have not previously been reported as associates of *P. radiata* in New Zealand were identified and included: *Wilcoxina mikolae, Rhizopogon pseudoroseolus, Pseudotomentella* sp., *Pseudotomentella tristis, Tomentella* sp., *Inocybe sindonia* and *Lactarius rufus.*

The putative ECM taxa *Tricholoma pessundatum*, *Laccaria laccata* and *Hebeloma crustuliniforme* (Chu-Chou 1979; Chu-Chou & Grace 1988) were collected as sporocarps during this study, but sequence analysis of the collected material did not confirm the suggested species identities. Sequence analysis of reference material from the Landcare Research Herbarium (http://nzfungi.landcaresesearch.co.nz/html/mycology.asp) resolved the species identities for the *Laccaria* species to *Laccaria proxima*. It was not possible to clarify the species identity for the other two taxa and these are thus referred to as *Tricholoma* sp. and *Hebeloma* sp. Walbert (2008, 2010).

In the nursery, *Hebeloma* sp., *Rhizopogon rubescens* and *Wilcoxina mikolae* were found in high abundance both as sporocarps and colonised root tips, while *Tuber* sp. was only found on the root tips. All of these species are known nursery fungi and were not observed in the plantation sites apart from the outplanting stage (2 year old site) in Kaingaroa Forest. *Rhizopogon rubescens* has been reported to be a very effective, adaptable and beneficial associate of *P. radiata* in New Zealand and other exotic plantations in the nursery stage (Chu-Chou & Grace 1985; Karkouri *et al.* 2002; Duñabeitia *et al.* 2004). In both the present and prior studies of ECM fungi associated with *P. radiata* in New Zealand, *Hebeloma* sp. was only found in the nursery (Chu-Chou 1979). This is in contrast to reports from Western Australia where the fungus was found to be widely distributed in exotic plantations and associated with pines up to 60 years of age (Dunstan *et al.* 1998). Interestingly, *Thelephora terrestris*, a species frequently found in pine nurseries (Cairney & Chambers 1999) was not observed in the nursery investigated in this study but was present in the older plantation sites. This is similar to reports from plantations in Western Australia (Dunstan *et al.* 1998). Chu-Chou & Grace (1990) only

Table. 2 Above- and below-ground species richness (observed and mean), mean evenness and mean Shannon and Simpson diversity indices of ECM species of different aged *P. radiata* stands and nursery site (2005 and 2006; (± standard error SE).

Diversity measure	Above/ Below	Nursery (± SE)	2 year (±SE)	8 year (±SE)	15 year (±SE)	26 year (±SE)	Overall
n (above/ below)		(5/11)	(5/28)	(5/17)	(2/15)	(5/15)	(22/86)
Total observed richness	Above	3	4	10	6	10	18
	Below	5	5	12	13	13	19
Richness	Above	2.6 (± 0.4)	2.8 (± 0.2)	6.2 (± 0.4)	5.0 (± 0.0)	6.8 (± 0.6)	4.7
	Below	3.2 (± 0.3)	1.6 (± 0.2)	2.7 (± 0.4)	4.1 (± 0.4)	5.0 (± 0.5)	3.6
Evenness	Above	0.4 (± 0.1)	0.5 (± 0.1)	0.5 (± 0.1)	0.7 (± 0.0)	0.6 (± 0.0)	0.6
	Below	0.8 (± 0.1)	0.7 (± 0.1)	0.7 (± 0.1)	0.8 (± 0.1)	0.7 (± 0.0)	0.8
Shannon diversity index	Above	0.4 (± 0.3)	0.5 (± 0.1)	0.8 (± 0.3)	1.1 (± 0.1)	1.1 (± 0.2)	1.8
	Below	0.9 (± 0.1)	0.4 (± 0.1)	0.7 (± 0.1)	1.0 (± 0.1)	1.2 (± 0.1)	2.2
Simpson diversity index	Above	0.3 (± 0.2)	0.3 (± 0.1)	0.4 (± 0.2)	0.6 (± 0.0)	0.6 (± 0.1)	0.8
	Below	0.5 (± 0.1)	0.3 (± 0.0)	0.4 (± 0.1)	0.6 (± 0.0)	0.6 (± 0.0)	0.8

found this species under peaty soil conditions in New Zealand's nurseries and it is commonly found in containerised pine seedlings using peat soil (Minchin, Jones and Ridgway, personal communication 2008) indicating that *T. terrestris* might prefer this soil type. The species *Laccaria proxima* fruited most abundantly in the 8 year old site, but apart from the 15 years old site was present aboveground in all plantation stands, although it was not recorded in the nursery. *Laccaria* is commonly associated with pines and found in disturbed sites (Cairney & Chambers 1999). The high plasticity within the genus *Laccaria* makes it difficult to distinguish species morphologically. In this study it was confirmed using DNA sequence data that the species associated with *P. radiata* in New Zealand is *L. proxima*, and not *L. laccata*, as stated in previous studies (Chu-Chou 1979; Chu-Chou & Grace 1988). *Amanita muscaria* was found both above- and below ground from the 8 year old stand onwards. This species is of special interest for New Zealand and is considered a regulated pest by the Ministry of Agriculture and Forestry (MAF). *Amanita muscaria* may displace the native ECM fungal "flora" since it has been found associated with native *Nothofagus* spp. in the Nelson Lakes National Park and other locations in the northern half of the South Island (Johnston *et al.* 1998). Recently, *A. muscaria* sporocarps have been reported from *Nothofagus* spp. forests in remote locations in the Kaimanawa Ranges in the Central Plateau of the North Island (N. Singers, personal communication, 2008).

The genus *Inocybe* is a known ectomycorrhizal associate of conifers and hardwoods and it was observed fruiting abundantly in the plantation sites. The plantation sites contained *I. lacera*, *I. sindonia* and *Inocybe* sp., the latter was also found colonising root tips. In this study the species *Inocybe sindonia* was identified for the first time in New Zealand using direct sequencing. Of the rare ECM fungal types found belowground, the tomentelloid species *Pseudotomentella* sp., *Pseudotomentella tristis* and *Tomentella* sp. are noteworthy. This group of resupinate thelephoroid fungi has a world-wide distribution, but their ability to form ECM has only been realised recently (Kõljalg *et al.* 2000). This study is the first to find these resupinate species colonising root tips of *P. radiata* in New Zealand.

In comparison to native conifer forest systems of the Northern Hemisphere, ECM species richness above- and below-ground assessed in this study was low. Epigeous sporocarp studies from western North American conifer forests reported 70–263 species (Smith *et al.* 2002; Durall *et al.* 2006). Similarly, studies on the below-ground ECM on other native forest systems reported higher species richness, for example 69 ECM types were found in a *Ps. menziesii* forest in Canada (Goodmann & Trofymow 1998) and 61 ECM morphotypes in a *Quercus rubra* chronosequence in Germany (Gebhardt *et al.* 2007) and ECM sporocarp richness of other exotic plantations in the southern hemisphere have also reported low sporocarp richness (11 to 55) (Garrido 1986; Dunstan *et al.* 1998; Giachini *et al.* 2000, Barroetaveña *et al.* 2005). Our above- and below-ground diversity indices of ECM fungi in Kaingaroa forest were low compared to native systems, where indices can be close to the potential maximum value (Yamada & Katsuya 2006; Gebhardt *et*

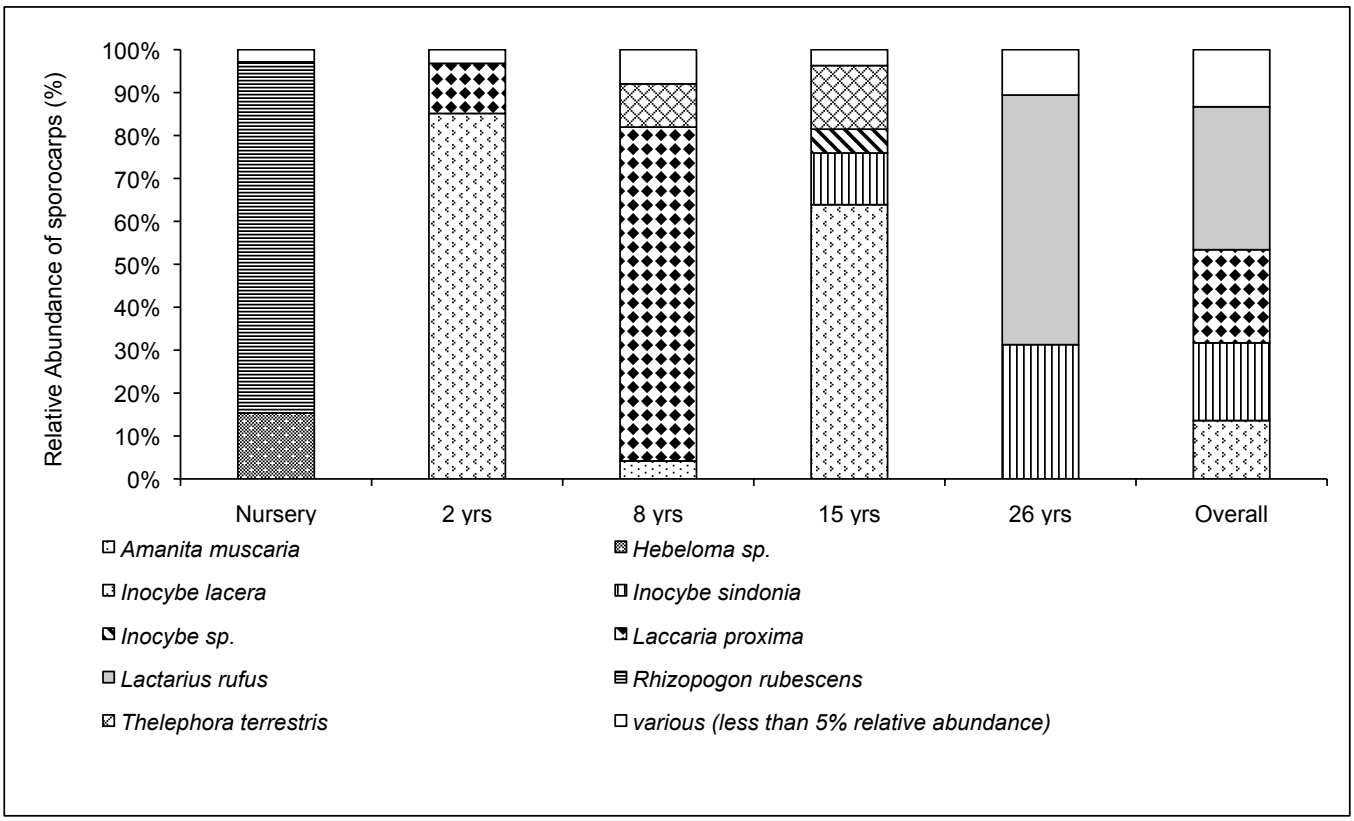

Fig. 1 Above ground abundance of the most frequent (>5% relative abundance) sporocarps from the *Pinus radiata* nursery and stands aged 2–26 years that were investigated.

al. 2007), but are similar to other studies conducted in exotic plantations (Giachini *et al.* 2004) or monoculture plantations (Korkama *et al.* 2006).

The low richness and diversity of ECM communities associated with *P. radiata* in New Zealand and other exotic plantations that have been studied are likely due to the recent introduction of the tree species and their exotic status. Barroetaveña *et al.*'s (2007) comparison of species richness of *P. ponderosa* and *Ps. menziesii* between native western North American forests and exotic plantations in Argentina demonstrated the relationship between ECM richness and the time elapsed since introduction of the host. *Pinus radiata* is native to California, USA and was introduced into New Zealand in the 1850s via England and Australia (Shepherd 1990). No specific ECM inoculation treatments on the imported seedlings are known; therefore, it is assumed that *P. radiata* has successfully established in New Zealand with a minimal number of ECM symbionts from California and could have the potential to associate with a wider suite of mycorrhizal species.

A review by Orlovich & Cairney (2004) listed 42 fungal ECM genera associated with native host species (*Nothofagus* spp., *Leptospermum scoparium* and *Kunzea ericoides*) with at least 226 species of fungi reported as ECM partners of *Nothofagus* spp. In this present study 18 ECM species were observed above-ground and 19 ECM species below-ground and none were known native ECM species. Furthermore there are no records of native ECM fungi in exotic *P. radiata* plantations in New Zealand, raising the question of which, if any, native fungi will become associated with *P. radiata* and what the ECM richness and diversity of this host will look like in 100 years time. Below-ground, six ECM morphotypes that were observed were unidentifiable using DNA sequence database searches. It is not known which species are 'hiding' in the assemblages of unknown ECM types found in this study. There is a chance that these could be native fungi as the availability of sequence data from native NZ ECM fungi is limited. It should be noted that *Cenoccocum geophilum*, frequently encountered belowground in the plantation sites, could potentially be a native ECM species. The fungus has been found to be associated with *Nothofagus solandri* var. *cliffortoides* in New Zealand (Mejstrik, 1972) and in intact *Nothofagus* forests distant from pine plantations (I. Dickie, personal communication 2008), which suggests that it may be native to New Zealand. In this study, the species was identified based on morphology only and not sequenced but is an area of research that warrants further work.

New Zealand's *P. radiata* plantations are monocultures — a factor further contributing to the low ECM species richness and diversity that was observed. Ishida *et al.* (2007) demonstrated the positive relationship between

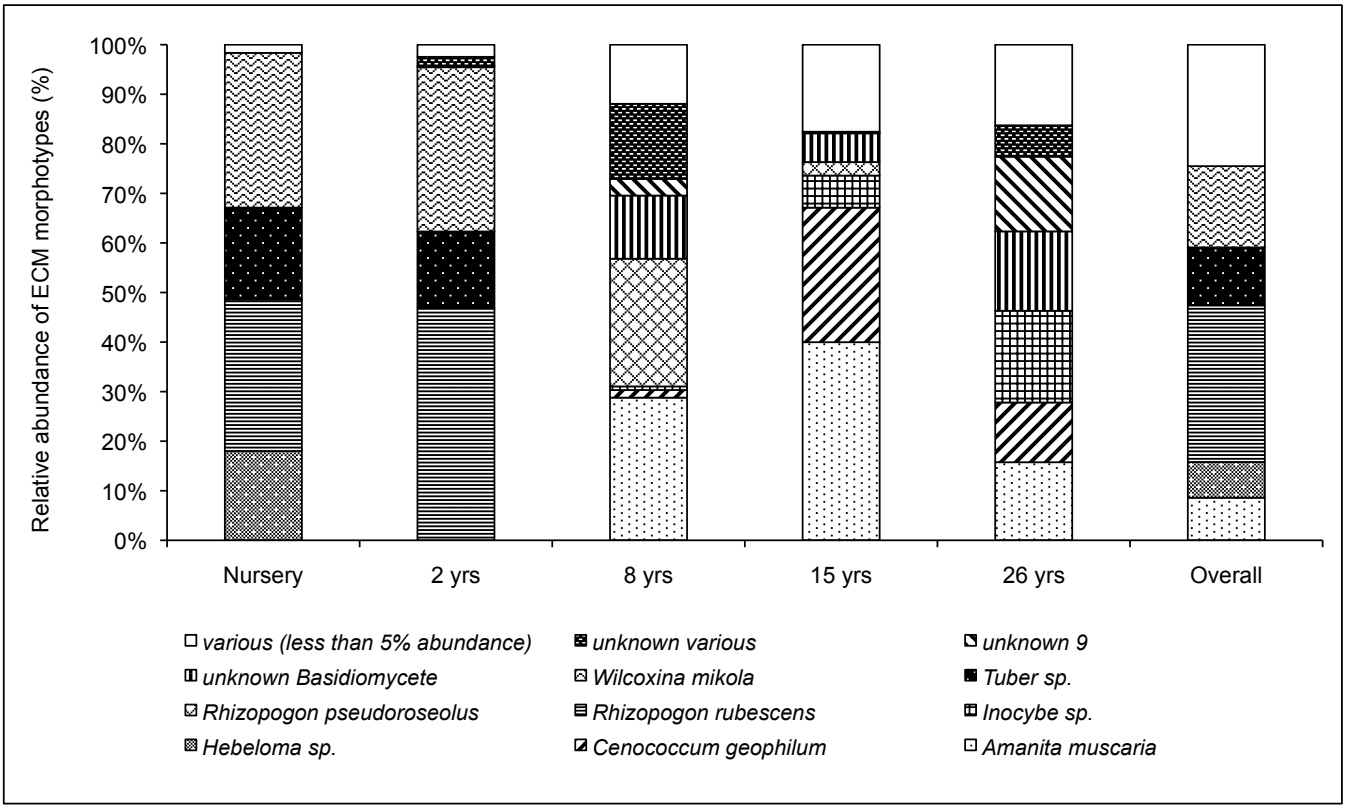

Fig. 2 Below ground abundance of the most frequent (>5% relative abundance) ECM morphotypes from the *Pinus radiata* nursery and stands aged 2–26 years that were investigated.

ECM species richness and the number of hosts present. Most native forests are made up of multiple mycorrhizal host species in a mixed age class structure, which is in contrast to plantation forests, which consist of the same plant species at the same age in each stand. This factor limits the ability of a monoculture plantation to achieve fungal diversity equal to ECM of native forests. This limitation in ECM richness and diversity is further increased in the plantation forests of New Zealand due to the short rotation of *P. radiata*. Because of the extremely favourable growth conditions in New Zealand, *P. radiata* is able to develop fast without a dormancy stage and can be harvested 25–30 years after planting. This artificially reduces the lifespan of the ECM host and alters the forest age-class distribution (Kranabetter *et al.* 2005).

In this study we assessed plantation sites of increasing age but the stands of different ages were not replicated; therefore, conclusions related to the effect of stand age on ECM communities could not be drawn. However, some observations of the diversity of the ECM communities within different aged stands were noted. The below-ground diversity measures of ECM dropped from the nursery to the 2 year old site, reflecting the effect of different environments between nursery and outplanting as well as the influence of clear cutting on the ECM communities. The nursery is an artificial environment, low diversity values are expected from this kind of setting (Chou-Chu & Grace 1988, 1990). Plants with an already low ECM richness were then planted on a site in Kaingaroa Forest which was clear felled two years prior to establishment. It is widely known that clearcutting affects ECM species richness and diversity negatively, since the dominant source of carbon is interrupted, followed by drastically changed environmental conditions due to changes in soil chemistry (Jones *et al.* 2003, Durall *et al.* 2006).

The difference in the values of the diversity measures between the nursery and 2 year old site compared to the older plantation stands (8, 15 and 26 years) was striking, as values were almost double in the oldest stand. In the harsh and disturbed environment of the nursery and young outplanting sites only a few ECM species are adapted to the conditions and able to colonise the host roots. The increase in species richness and diversity in the older stands is mainly due to the development of the root system of the host and increased root density (Kranabetter & Friesen 2002), combined with the establishment of an understorey in the older stands and the associated changes in soil chemistry leading to an environment that is suitable for a greater variety of ECM species.

This study was a snapshot in time of one biological system, ECM fungi associated with the exotic plantation host *P. radiata*, taken from two different perspectives, above-ground and below-ground. The study confirmed

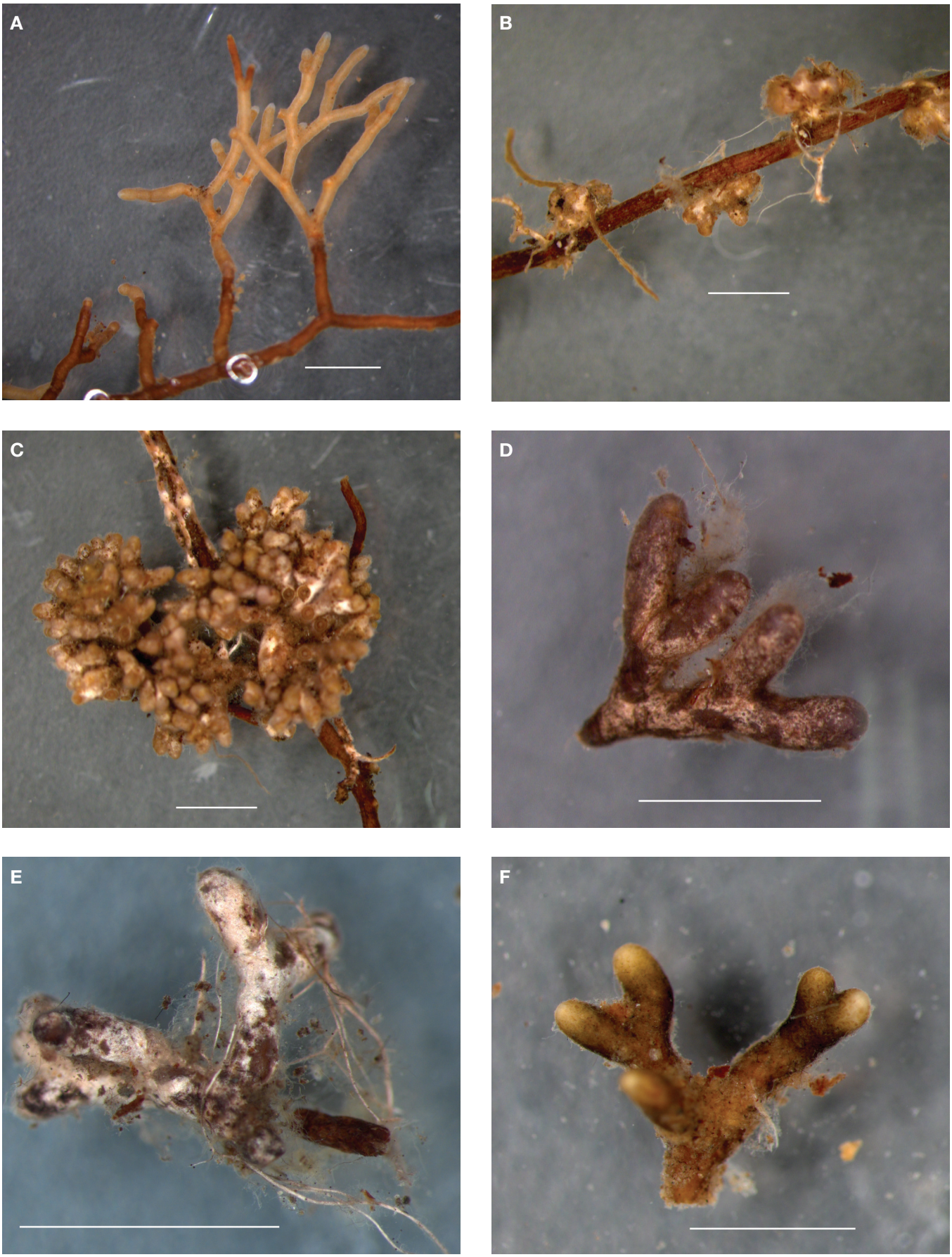

Fig. 3 ECM morphotypes of (**A**) *Wilcoxina mikolae*, (**B**) *Rhizopogon pseudoroseolus*, (**C**) *R. luteorubescens*, (**D**) *Pseudotomentella* sp., (**E**) *Pseudotomentella tristis* and (**F**) *Tomentella* sp., new ECM associates of *P. radiata* in New Zealand. Scale bar = 5 mm.

the low ECM species diversity of New Zealand's exotic plantations. However, with the application of molecular identification methods it was possible to identify more ECM species in the below-ground environment than have been previously been reported in New Zealand. With the increase in molecular studies on native ECM species in New Zealand it will be interesting to determine whether an ECM species exchange between native and introduced hosts is occurring. This study also confirmed the need for long term, frequent surveys to fully inventory the diversity of ECM fungi. However to determine which ECM fungal species are most important and beneficial to its host tree, the focus has to be on the abundance and community structure of ECM species colonising the root tips, as this is where nutrient exchange takes place and is of the greatest benefit to the host.

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