

Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire

J. BAAR¹*, T. R. HORTON², A. M. KRETZER³ AND T. D. BRUNS⁴

¹ Department of Aquatic Ecology and Environmental Biology, University of Nijmegen, 6525 ED Nijmegen, The Netherlands

² Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA

³ Department of Botany and Plant Pathology, Oregon State University, 2082 Cordley Hall, Corvallis, OR 97331, USA

⁴ Plant and Microbial Biology, 111 Koshland Hall, University of California, Berkeley, CA 94720, USA

Received 12 October 1998; accepted 27 March 1999

SUMMARY

Colonization of mycorrhizal fungi was studied in a *Pinus muricata* forest on the coast in California, USA, burned by a stand-replacing wildfire in October 1995. Naturally established field seedlings of *P. muricata* were harvested 1 yr after the fire. The species composition of the mycorrhizal fungi on these field seedlings was dominated by *Rhizopogon* species, *Wilcoxina mikolae* and *Tomentella sublimacina*. Bioassays, set up with soil collected immediately after the fire, were used to determine which mycorrhizal species had colonized the burned area from resistant propagules. The *P. muricata* seedlings in these bioassays were dominated by suilloid and ascomycetous fungi, the same fungi which dominated the mycorrhizal flora of seedlings in pre-fire bioassays derived from the same forest site, suggesting that resistant propagules were the primary inoculum source for naturally establishing seedlings. Drying of post-fire soil for 1 month raised the number of bioassay seedlings associated with *Rhizopogon olivaceotinctus*, while the number of bioassay seedlings associated with *Rhizopogon ochraceorubens* was reduced. Fire appeared to have either stimulated or provided a competitive advantage to *R. olivaceotinctus*, which increased in abundance on the post-fire bioassay and field seedlings. Soil collected from the burned area was diluted with sterile soil in three different concentrations, and the number and frequency of mycorrhizal taxa on bioassay seedlings decreased with increased dilution. Although precise quantification was not possible, propagules of the *Rhizopogon* species were much more abundant than those of *Tomentella* or *Wilcoxina* species. Differences between the mycorrhizal associates of bioassay seedlings, naturally regenerated seedlings, and different inoculum sources are discussed.

Key words: community structure, competition, inoculum potential, PCR identification, spore bank, succession.

INTRODUCTION

Closed-cone pine forests dominated by *Pinus muricata* D. Don (Bishop pine), *Pinus radiata* D. Don (Monterey pine) and *Pinus attenuata* Lemmon (Knobcone pine) are common communities on the California coast, USA (Vogl *et al.*, 1988). It is well known that plants in these communities have various fire survival and colonization strategies, e.g. closed-cone pine species require heat to open their cones to release their seeds (Raven, 1988; Vogl *et al.*, 1988).

*Author for correspondence (fax +31 24 3652134; e-mail jbaar@sci.kun.nl).

However, little is known about the survival and colonization of mycorrhizal fungi after fire. Given that pine species are obligately mycorrhizal, colonization of mycorrhizal fungi must be an important component of pine regeneration in burned areas.

To examine mycorrhizal post-fire colonization, we took advantage of a recent wildfire which burned approx. 5000 ha of Point Reyes National Seashore along the coast of California, USA, including several *P. muricata* stands. Pre-fire studies of these stands revealed that the mycorrhizal community of the mature *P. muricata* trees was dominated by species in the Russulaceae and Thelephoraceae, and to a

lesser extent by *Amanita* spp. (Gardes & Bruns, 1996; Horton & Bruns, 1998). Within the first 6 months following the fire, the post-fire mycorrhizal community of *P. muricata* was described by Horton *et al.* (1998). *Pinus muricata* seedlings which established within the formerly arbuscular-mycorrhizal (AM)-dominated shrub community were primarily colonized by *Rhizopogon ochraceo-rubens* Smith, *Rhizopogon subcaerulescens* Smith and *Suillus pungens* Thiers & Smith, while *P. muricata* seedlings established within the formerly forested areas were colonized by a more complex set of species that included the *Rhizopogon* species and several ascomycetous taxa, but also some of the pre-fire dominant species such as *Russula brevipes* Peck, and two *Amanita* species, a cantharelloid type and a theleporoid type (Horton *et al.*, 1998). However, the species composition of the mycorrhizal community of *P. muricata* over 6 months after the fire is still unknown, and may differ from that before the fire.

Several studies based on sporocarp surveys and morphological descriptions of the mycorrhizal roots report that severe forms of disturbance, such as wildfire, have an impact on the mycorrhizal community by reducing mycorrhizal populations and changing species composition (Parke *et al.*, 1984; Visser, 1995). For example, Visser (1995) noted dominance of *Coltricia perennis* (L: Fr.) Murr., *Thelephora* spp. and E-strain fungi in a 6-yr-old Jack pine (*Pinus banksiana* Lamb.) stand established after a wildfire in Canada, while *Inocybe* spp., *Lactarius* spp., *Russula* spp., *Suillus* spp. and *Tricholoma* spp. were abundant in older (>40 yr) stands.

Colonization of burned forest sites by mycorrhizal fungi has not been well studied, and the main inoculum source is still unknown. Colonization may occur from different sources of inoculum such as resident mycelia that survived the fire, or resident and dispersed spores (Visser, 1995). Horton *et al.* (1998) suggested that initial colonization of *P. muricata* by mycorrhizal fungi mainly occurred from resident mycelia and propagules. Excised mycorrhizal root tips can remain viable in the soil for up to 8 months (Ferrier & Alexander, 1985). Viable sclerotia and spores of mycorrhizal fungi were found in coniferous forests up to 2 yr after a wildfire (Miller *et al.*, 1994; Torres & Honrubia, 1997).

The main objectives of the present study were to determine how the mycorrhizal community of *P. muricata* was affected by a high-intensity wildfire, and to investigate whether resistant propagules were a major inoculum source of the mycorrhizal species colonizing naturally established seedlings after the fire. These goals were facilitated by the fact that the burned area was well studied prior to the fire, so that the mycorrhizal flora of the mature *P. muricata* trees was known (Gardes & Bruns, 1996; Horton & Bruns, 1998; Taylor & Bruns, in press). Molecular

characterization of the mycorrhizal fungi in the mature *P. muricata* forest provided a basis for comparison and facilitated the identification of unknown taxa by RFLP and sequence analyses (Gardes *et al.*, 1991; Gardes & Bruns, 1993; Bruns *et al.*, 1998). *Pinus muricata* seedlings were grown in post-fire soil removed from the study site as bioassays to investigate the mycorrhizal inoculum potential of the burned site. This bioassay approach was used previously by Brundrett *et al.* (1996a,b).

The following hypotheses were tested: (i) the major inoculum source of mycorrhizal species colonizing a *P. muricata* forest area burned by a high intensity wildfire consisted of resistant propagules; (ii) the inoculum potential of mycorrhizal fungi was reduced in dried post-fire soil; (iii) the inoculum potential of mycorrhizal fungi decreased with increased dilution of post-fire soil with sterile soil; (iv) the mycorrhizal formation on bioassay seedlings decreased with soil depth; and (v) the mycorrhizal species composition of *P. muricata* seedlings harvested from the burned area 1 yr after the wildfire reflected the species composition on bioassay seedlings.

MATERIALS AND METHODS

Fire and site characteristics

During 3–7 October 1995, a wildfire burned c. 5000 ha of forest and shrub community at Point Reyes National Seashore, situated on the coast of central California. This wildfire was a high-intensity crown fire within most of the *P. muricata* forest, and was the first large fire in the park in several decades. The wildfire killed the majority of the *P. muricata* trees, which were aged 35–45 yr at the time of the fire. Most *P. muricata* trees (including all trees at the site used in the present study) were killed directly by the fire, but the few which survived elsewhere were dead by the following spring as a result of an attack from the bark beetles *Dendroctonus valens* Leconte and *Ips plastographus maritimus* Lanier. The tree crowns at the study site were severely scorched but not entirely destroyed by the fire. This probably represented an intermediate intensity relative to other sites, where we observed that the crowns either remained partially green or were entirely burned off. The wildfire completely burned off the well developed 5–10 cm litter and humus layers at all places. The nearest surviving forest was approx. 0.3 km away. No direct measures of soil heating were made, because the fire was an unexpected event. However, from prior studies we would expect that the upper 2–5 cm of the mineral soil had reached temperatures sufficient to kill most organisms, but the mineral soil deeper than 5 cm probably did not experience excessive heating, because dry mineral soil is a good insulator (Whelan, 1995). In localized spots within our site and

elsewhere, stumps were burned out by the fire, and the resulting heat probably created killing temperatures at greater depths

Included in the burned area in Point Reyes National Seashore were four *P. muricata* forest sites where the mycorrhizal community before the fire has been described (Gardes & Bruns, 1996; Horton & Bruns, 1998; Horton *et al.*, 1998; Taylor & Bruns, in press). For the present study, one of these forest sites was selected, located at 38° 04' 10" N, 122° 50' 24" W. Prior to the fire it was a small forest patch of *P. muricata*, several hundred m² in extent, surrounded by a coastal shrub community dominated by *Baccharis pilularis* D.C., *Rhamnus californica* Eschsch., *Toxicodendron diversiloba* Torrey & A. Gray, *Rubus* spp., *Lupinus* spp. and *Ceanothus* spp. Some of these same shrubs, particularly *T. diversiloba* and the *Rubus* spp., were also common understorey components of the *P. muricata* forest, but do not form associations with ectomycorrhizal fungi. The forest patch has been studied prior to the fire by Taylor & Bruns (in press), and other sites studied by Gardes & Bruns (1996), Horton & Bruns (1998), and Horton *et al.* (1998) were located within 1 km. The climate is Mediterranean, but moderated by summer fog. Average annual air temperature ranged from 11 to 14°C, and average rainfall varied from 63 to 88 cm.

Field seedlings

Within 4 wk of the wildfire, *P. muricata* seedlings germinated from seeds released by the serotinous cones which had opened in the heat of the fire (Horton *et al.*, 1998). Within the first year after the fire, regenerating herbs and shrubs were removed from the burned forest site to reduce competition and to facilitate development of the *P. muricata* seedlings. We located and sampled the five 1-m-diameter circular plots originally established in the mature *P. muricata* forest prior to the fire by Taylor & Bruns (in press). The plots were located within the centre of the forest patch at least 5–7 m from each other. In January and February 1997, all seedlings (29 in total) were harvested from the plots. The root systems were harvested almost completely and mycorrhizal root tips examined to a soil depth of 25–30 cm. Average length and dry weight of the shoots of the seedlings were 14.3 ± 5.6 cm and 3.3 ± 2.8 g, respectively.

Bioassay seedlings

The study site was covered with tarpaulin within 3 d of the fire, to prevent post-fire arrival of wind-blown inoculum. Within the next 2 wk, prior to the fall rains and the fruiting period of mycorrhizal fungi, soil was collected from the five plots at the study site. Per plot, three 40-cm deep soil cores (10 cm diameter) which were spread evenly on the perimeter

of a circle (1 m diameter) were taken. Soil cores were divided into top and bottom parts. Top parts of the three soil cores originating from one plot were pooled as well as bottom parts. Half of the soil was used immediately for setting up bioassays; the remaining soil was left in enclosed paper bags to dry at room temperature (20°C) for 1 month and then used for setting up bioassays. This was done to kill active mycelium in the soil.

Field-collected soils were mixed with sterile soil and used to set up bioassays in three different concentrations (undiluted, 1:10 and 1:100) in order to reduce the number of rapidly growing fungal taxa which could overgrow less aggressive fungi. Sterile soil was collected from the burned forest outside the plots and steam sterilized (2 h at 121°C). In addition, all soils were mixed with sterile sand in equal parts before planting to allow better water percolation through the loamy soils collected from the field. After mixing with sand, the final dilutions of the test soils in the bioassays were 1:2, 1:20 and 1:200.

Soil mixtures were put into Ray Leach tubes (Stuewe & Sons, Inc.) and one *P. muricata* seed was planted per tube. *P. muricata* seeds that did not germinate or seedlings that died were replaced by newly planted *P. muricata* seeds. In a few cases, repeated planting resulted in repeated death of seedlings. Seedlings were grown at room temperature (20°C) for *c.* 1 yr and watered twice a week. They were located in the laboratory to avoid greenhouse contamination by *Thelephora* spp.

Each treatment was replicated five times, resulting in a total of 300 bioassay seedlings [five plots \times two depths \times two treatments (moist versus dry soil) \times three dilutions \times five replicates], and the experimental set-up was according to a randomized block design. To investigate mycorrhizal contamination from the laboratory, 25 seedlings were grown in entirely sterile soil and sand mixtures (dilution 1:2); these seedlings were considered as control seedlings. At the time of harvest, 15 bioassay seedlings and two control seedlings were dead.

Harvest of *P. muricata* seedlings and morphological examination of mycorrhizal root tips

Both bioassay and naturally regenerated field seedlings were harvested when approximately 1 yr old. Soil was carefully rinsed off the roots, and root systems were examined under a stereomicroscope. The presence of mycorrhizal infection was determined by a combination of the following characters: presence of either well-developed mantles or hyphae emanating from the surface of short roots; swelling of the short roots; and lack of root hairs. If the mycorrhizal status remained ambiguous, especially when a well-developed mantle was not present, cross sections were made and examined under a compound microscope for the presence of a Hartig net.

Morphological characters used to differentiate mycorrhizal morphotypes were: colour, shape, branching pattern, presence or absence of mantles, and rhizomorphs (Agerer, 1987–1997). From every seedling harvested from the field or from the bioassays, two or three representative samples of each morphotype observed on a single seedling were picked and freeze-dried for molecular analysis.

Molecular methods

For each morphotype, DNA was extracted from two or three individual root tips using hexadecyltrimethyl ammonium bromide extraction, and specific DNA regions were PCR amplified according to protocols described by Gardes & Bruns (1993). RFLP patterns were determined using PCR products of the ITS region of the rDNA, amplified with the primer pairs ITS1-F/ITS4-B or ITS1-F/ITS-4 and the restriction enzymes *AluI*, *DpnII* and *HinfI* to match unknown taxa to previously identified taxa (Gardes & Bruns, 1993). Three different regions were sequenced for identification of unmatched mycorrhizal morphotypes: (i) the two internal transcribed spacers (ITS) of the nuclear ribosomal repeat with the intercalated 5.8S rRNA gene; (ii) an approximately 400-bp fragment of the mitochondrial large subunit rDNA; (iii) an approx. 600-bp fragment at the 5' end of the nuclear large subunit rDNA. The primer pairs used were ITS1-F/ITS-4, ML-5/ML-6 and ITS1-F/Tw-14 for amplification of the corresponding regions. ITS-4, ITS-5, ML-5, ML-6, Ctb-6 and Tw-13 were used for sequencing of the PCR products (Gardes & Bruns, 1993; Bruns *et al.*, 1998; Taylor & Bruns, 1999). Cycle sequencing was done by the reaction termination method using fluorescence-labelled dideoxynucleotide triphosphates. The sequence reaction and the processing of the reaction products for electrophoresis were performed following the instructions for the sequencing kit (ABI PRISM[®] Dye Terminator Cycle Sequencing Core Kit, Perkin-Elmer Corporation). Electrophoresis and data collection were done on an ABI Model 377 DNA Sequencer (Perkin-Elmer). DNA Sequencing Analysis (version 2.12) and Sequence Navigator (version 1.0.1) were used for processing the raw data.

Molecular typing and identification

Restriction digests with the enzymes *AluI*, *DpnII* and *HinfI* were used to differentiate morphotypes into different RFLP types. RFLP patterns of at least two samples of each morphotype were determined. In total, RFLP patterns of over 500 samples were examined. Identical patterns for the three enzyme digests were generally our criterion for a species, but two exceptions to this rule were made. For *R. olivaceotinctus*, two morphologically indistinguishable morphotypes were grouped that shared identical

HinfI and *DpnII* patterns, but differed in their *AluI* patterns by the presence or absence of one additional restriction site. The grouping is justified by partial sequence analysis of the mitochondrial large subunit rDNA and on a match of one of the RFLP types to an *R. olivaceotinctus* fruiting body. Similarly, we grouped three RFLP types that differed only by their *AluI* patterns into a morphotype we call *Phialophora*-like.

The RFLP patterns were used to identify fungal symbionts by matching RFLP patterns produced from mycorrhizal root tips to those produced from identified fruit bodies. In case no RFLP match between a morphotype and fruiting fungi from the study area was found, sequence analysis and phylogenetic grouping were used to narrow the search or to place the unknown fungal symbiont into a phylogenetically meaningful group. Unknown fungal symbionts were classified as either ascomycetes or basidiomycetes by the following two characters: (i) ability (most basidiomycetes) or inability (ascomycetes and some basidiomycetes) of the ITS region to be amplified with the basidiomycete-specific primer pair ITS1-F/ITS4-B (Gardes & Bruns, 1993); or (ii) phylogenetic grouping of the 5.8S rDNA sequence using a published database (Cullings & Vogler, 1998) – the latter method was used mostly to confirm the identity of presumed ascomycetes.

The classification of unknown basidiomycetes was further narrowed down by determining sequences of the ML5/ML6 region of the mitochondrial large subunit rDNA and use of a previously published database of sequences from that region (Table 1; Bruns *et al.*, 1998). For unknown ascomycetes, on the other hand, the 5' end of the nuclear large subunit rDNA was sequenced and subjected to phylogenetic analysis with homologous ascomycete sequences from GenBank (Taylor & Bruns, 1999; Table 1). Sequence analyses were done using either an incomplete heuristic search (parsimony criterion) or a neighbour-joining analysis in PAUP 3.3.1. (Swofford, 1993) and in test versions of PAUP 4.

Statistical analysis

Data were based on the presence or absence of morphotypes on the seedlings, and the average score per treatment was calculated. Although presence-absence data are scores, we considered the data as ordinal and tested them for normal distribution by Bartlett test (Sokal & Rohlf, 1995). No homogeneity of variance was found, and therefore differences between treatments were tested with the Kruskal-Wallis and Mann-Whitney *U*-tests (Siegel & Castellan, 1988). The tests could be applied only for the average total number of mycorrhizal taxa observed on the bioassay seedlings and for the mycorrhizal taxa *R. olivaceotinctus*, *R. ochraceorubens*, *R. sub-*

Table 1. Identification of mycorrhizal fungi found on 1-year-old *P. muricata* seedlings harvested from bioassays and from the field

Identified fungal symbionts	Method of identification
<i>Hebeloma</i> sp.	ML5/6 sequence, RFLP
<i>Rhizopogon ochraceorubens</i>	RFLP
<i>Rhizopogon olivaceotinctus</i> *	ML5/6 sequence, RFLP
<i>Rhizopogon subcaerulescens</i> (species group)†	RFLP
<i>Tomentella sublilacina</i>	ML5/6 sequence, RFLP
tricholomatoid	ML5/6 sequence
<i>Tuber californicum</i>	Partial 28S rDNA, RFLP
<i>Tuber</i> sp.	Partial 28S rDNA, RFLP
<i>Wilcoxina mikolae</i> and <i>W. mikolae</i> -like	Partial 28S rDNA, RFLP
<i>Phialophora</i> -like‡	5.8S + partial 28S rDNA

*Two RFLP types were found that had identical *Hinf*I patterns, but differed in their *Alu*I patterns by the apparent presence or absence of one additional restriction site. As they are apparently genetically very closely related and as we were unable to differentiate them morphologically, they were lumped for further analyses. Only one of the RFLP types matched RFLP patterns produced from voucher collections of *R. olivaceotinctus*.

†The species group includes closely related taxa such as *R. ellenae* that are known from other studies to be difficult to differentiate by RFLP analysis.

‡Three RFLP types of this ascomycetous type were found which were identical in *Hinf*I patterns and differed in *Alu*I patterns by the presence or absence of one to two additional restriction sites. Analysis of partial 28S rDNA sequences placed the three morphotypes in the broader vicinity of a *Phialophora* isolate.

caerulescens and the *Phialophora*-like fungi. The remaining taxa occurred too infrequently to apply statistical tests.

RESULTS

By grouping the mycorrhizal root tips into morphotypes which were subsequently analysed by molecular analysis, we were able to identify 88% of the morphotypes on the 1-yr-old *P. muricata* seedlings naturally established after the fire, and 94.2% of the morphotypes on the bioassay seedlings. In total, 11 mycorrhizal taxa were observed on the field and bioassay seedlings. Seven fungi were identified to species group, four others were identified to genus (2), family (1) or phylum (1) (Table 1).

The mycorrhizal taxa were identified by RFLP analysis, by determining the sequences of the ML5/ML6 region of the mitochondrial subunit rDNA and by partial sequence analysis of the 28S rRNA gene (Table 1). *Rhizopogon ochraceorubens* and *R. subcaerulescens* were identified by matching the RFLP patterns produced from fruit bodies. The taxa *R. olivaceotinctus* and *Tomentella sublilacina* (Ellis & Holw.) were identified by determining the sequences of the ML5/ML6 region of the mitochondrial subunit rDNA which were submitted to GenBank (AF 127114 for *R. olivaceotinctus* and AF 127113 for *T. sublilacina*) and by matching the RFLP patterns produced by fruit bodies. The partial sequences of the 28S rRNA gene was determined for the *Phialophora*-like fungi (GenBank: AF 127115–8), *Tuber californicum* (GenBank: AF 127120), *Tuber* sp. (GenBank: AF127121) and *Wilcoxina mikolae*

(Yang & Wilcox) (GenBank: AF 127119). The identification of the *Tuber* and *Wilcoxina* species was confirmed with RFLP analysis by Egger (personal communication). The remaining taxa, *Hebeloma* sp. and the tricholomatoid fungus, were identified by the use of the ectomycorrhizal basidiomycete database of Bruns *et al.* (1998). The identification of *Hebeloma* sp. was based on a ML5/ML6 sequence which resembled the ML5/ML6 sequence of *Hebeloma crustuliniforme* (Bruns *et al.*, 1998) and an RFLP-pattern that matched *Hebeloma* sp. The identification of the tricholomatoid fungus was based on the position of the ML5/ML6 sequence in the ectomycorrhizal basidiomycete database by Bruns *et al.* (1998) close to *Tricholoma* spp.

There were seven identified mycorrhizal taxa on the field seedlings, and *Rhizopogon* species and ascomycetous fungi dominated. *Rhizopogon olivaceotinctus* was the most abundant species (30.6%) followed by *W. mikolae* (18.4%) (Fig. 1). Other species observed at the field seedlings were *Hebeloma* sp., *R. subcaerulescens*, *T. sublilacina* and a species belonging to the Tricholomataceae. The average number of mycorrhizal taxa per field seedling was 1.76.

Mycorrhizas were formed on 86% of the 308 seedlings harvested from the bioassays. From the 23 control seedlings harvested, two seedlings had formed mycorrhizal root tips belonging either to a unique RFLP type or a very rare RFLP type (found on only three seedlings). These types were consequently excluded from all analyses.

On the bioassay seedlings, eight mycorrhizal taxa were observed. The three *Rhizopogon* species dom-

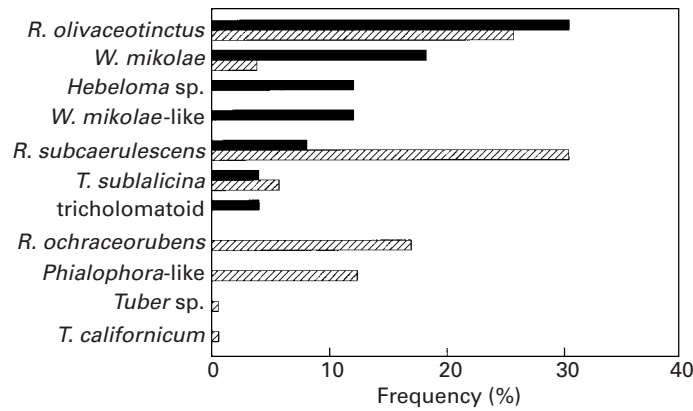


Fig. 1. Frequency of ectomycorrhizal fungi (percentage) on the bioassay and field seedlings based on presence and absence data. Details of each type of seedling appear under the headings ‘Bioassay seedlings’ and ‘Field seedlings’ in the Materials and Methods section. Solid bars, field seedlings; shaded bars, bioassay seedlings.

Table 2. ITS–RFLP band sizes of mycorrhizal fungi on bioassay (b) and field (f) seedlings

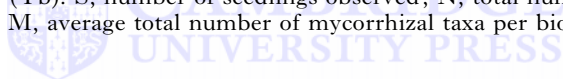
Taxa	Seedlings	AluI	DpnII	HinfI
<i>Hebeloma</i> sp.	f	340/257/214	560/258	398/356/145
<i>Rhizopogon ochraceorubens</i>	b	453/345/106/87	343/293/257	355/254/164/140/77
<i>Rhizopogon olivaceotinctus</i>	b/f	545/264	298/247/230	258/174/158/135/105
		292/264/234	298/247/230	258/174/158/135/105
<i>Rhizopogon subcaerulescens</i>	b/f	471/334/87	295/240	252/133/75
<i>Tomentella sublilicina</i>	b/f	458/111/86	374/227/196	366/223/126/107
tricholomatoid	f	291/270/208	560/258	398/356/145
<i>Tuber californicum</i>	b	613/93	323/240	389/188/157
<i>Wilcoxina mikolae</i>	b/f	645	222/95	295/193/167
<i>Wilcoxina mikolae</i> -like	f	660	329/223	365/108
<i>Tuber</i> spp.	b	586	nd	270/233/109
<i>Phialophora</i> -like	b	400/111	nd	345/273
		506	nd	345/273
		600	nd	345/273

nd, not determined. RFLP patterns of basidiomycetes: primers ITS1-F and ITS4-B; RFLP patterns of ascomycetes: primers ITS1-F and ITS4.

Table 3. Average scores (based on presence or absence) of mycorrhizal taxa on the bioassay seedlings per treatment

Treatment	Dilution	Depth	S	N	M	Rs	Rl	Ro	Ph	Ts	Wm	Tc	Tb
Dried	1:2	Top	23	7	1.56	0.39	0.52	0.17	0.09	0.09	0.13	0	0.04
Dried	1:20	Top	23	4	0.96	0.26	0.48	0.09	0.04	0	0	0	0
Dried	1:200	Top	23	3	0.39	0	0.26	0.04	0.04	0	0	0	0
Dried	1:2	Bottom	23	7	1.91	0.52	0.48	0.35	0.09	0.17	0.26	0.04	0
Dried	1:20	Bottom	23	5	1.39	0.61	0.39	0.13	0.17	0	0.04	0	0
Dried	1:200	Bottom	23	4	0.61	0.26	0.17	0.09	0.04	0	0	0	0
Moist	1:2	Top	24	7	1.71	0.38	0.42	0.17	0.25	0.08	0.13	0	0.42
Moist	1:20	Top	25	4	0.84	0.44	0.32	0.08	0.04	0	0	0	0
Moist	1:200	Top	24	4	0.54	0.29	0.13	0.54	0.13	0	0	0	0
Moist	1:2	Bottom	24	7	1.8	0.36	0.2	0.48	0.52	0.12	0.04	0.04	0
Moist	1:20	Bottom	25	5	1.8	0.52	0.36	0.24	0.2	0.32	0	0	0
Moist	1:200	Bottom	25	5	1	0.4	0.08	0.08	0.16	0.04	0	0	0
Control	1:2	Top	13	1	0	0	0	0	0	0	0	0	0
Control	1:2	Bottom	10	0	0	0	0	0	0	0	0	0	0

Mycorrhizal taxa: *Rhizopogon subcaerulescens* (Rs), *Rhizopogon olivaceotinctus* (Rl), *Rhizopogon ochraceorubens* (Ro), *Phialophora*-like fungi (Ph), *Tomentella sublilicina* (Ts), *Wilcoxina mikolae* (Wm), *Tuber californicum* (Tc), *Tuber* spp. (Tb). S, number of seedlings observed; N, total number of mycorrhizal taxa on the bioassay seedlings per treatment; M, average total number of mycorrhizal taxa per bioassay seedling per treatment.



inated and accounted for 73.4% of the species composition (Fig. 1). Ascomycetous fungi were the next abundant group on the bioassay seedlings (17.6%): taxa in this group were *W. mikolae*, *Tuber* sp. and the *Phialophora*-like fungi (Fig. 1). The telephoroid fungus *T. sublilacina* occurred at a low level (5.8%).

The species composition of the bioassay seedlings overlapped that of the field seedlings (Table 2 and Fig. 1). *Rhizopogon olivaceotinctus*, *R. subcaerulescens*, *T. sublilacina* and the ascomycete *W. mikolae* were observed on both field and bioassay seedlings (Fig. 1). The average number of mycorrhizal taxa per bioassay seedling in the least diluted soil (1:2) was in the range 1.56 to 1.91 (Table 3) and was about equal to the average number of mycorrhizal taxa per field seedling (1.76).

Drying and diluting of soil significantly affected mycorrhizal formation on the bioassay seedlings (Table 4). The number of bioassay seedlings associated with *R. olivaceotinctus* was significantly ($P < 0.05$, Mann-Whitney *U*-test) higher in the dried soil than in the moist soil, while the number of seedlings associated with *R. ochraceorubens* and the *Phialophora*-like fungi was significantly lower ($P < 0.05$) (Table 3). The number of mycorrhizal species on the bioassay seedlings was highest in the least diluted (1:2) soil and the average total number of mycorrhizal taxa per seedling was significantly lowest in the most diluted soil ($P < 0.05$) (Table 3). Mycorrhizal formation of the three *Rhizopogon* species in the dried soil was reduced significantly by dilution ($P < 0.05$).

The average total number of mycorrhizal taxa per bioassay seedling was significantly more abundant in the bottom layers of the collected soils than in the top layers ($P < 0.001$). Specifically, mycorrhizal formation by *R. subcaerulescens* and the *Phialophora*-like fungi was larger in the bottom than in the top soil layers ($P < 0.05$, Table 3).

DISCUSSION

Five of the seven most abundant colonizers of the field seedlings appear to have survived the fire primarily as resistant propagules. Taxa in this category include *R. olivaceotinctus*, *R. subcaerulescens*, *Wilcoxina* sp. and *T. sublilacina*, which were found on the bioassay seedlings grown in soil collected immediately after the fire (Fig. 1). These fungi were also found on bioassay seedlings grown in soil collected from the plots in the mature *P. muricata* forest at the study site prior to the fire, but only *T. sublilacina* was also observed on the roots of the mature trees within the same cores from which the pre-fire bioassay soil was derived (Taylor & Bruns, 1999). This suggests that viable propagules of these taxa were present in the mineral soil in the mature forest. Dormant spores of *Rhizopogon* spp. are

known to be present in forest soils (Schoenberger & Perry, 1982; Pilz & Perry, 1984; Danielson, 1991; Miller *et al.*, 1993) and spores of *Alpova diplophloeus* are thought to persist in the soil for many years (Miller *et al.*, 1994). E-strain fungi such as *W. mikolae* form thick-walled chlamydospores which can persist in the mineral soil and survive fire (Danielson, 1982; Visser, 1995; Torres & Honrubia, 1997). Fire survival of spores and other resistant propagules in the mineral soil was expected, because the high temperatures of forest fires usually extend less than 5 cm into the mineral soil (Whelan, 1995).

Survival of mycelium via colonized roots was highly unlikely for the *Rhizopogon* and *Wilcoxina* species because these taxa were not abundant colonizers of the pre-fire mature *P. muricata* forest, either at our study site or elsewhere (Gardes & Bruns, 1996; Horton & Bruns, 1998; Taylor & Bruns, 1999). The species could have been present on the roots of the pre-fire mature trees, but missed because of their highly clumped distributions; spatial heterogeneity is well documented for ectomycorrhizal communities (Gardes & Bruns, 1996; Dahlberg *et al.*, 1997). Nevertheless, even if clumps of mycorrhizal mycelia were present they must have been rare within the plots at our study site, as none of the *Rhizopogon* or *Wilcoxina* species was found in any of the 15 cores (10 cm diameter; 3.14 dm³ volume) removed from the plots prior to the fire (Taylor & Bruns, 1999). After the fire, *R. subcaerulescens* and *R. ochraceorubens* were uniformly observed on bioassay seedlings grown in soil sampled from all five plots, and *R. subcaerulescens* was uniformly present on post-fire field seedlings which established in the formerly shrub-dominated area (Horton *et al.*, 1998) where no mycelial inoculum would be present. The uniform presence of both *Rhizopogon* species strongly suggests that spores were the primary inoculum source, and were both widespread and abundant.

We cannot make a strong statement about the type of inoculum of *T. sublilacina*, because this species was a common fungus in the pre-fire *P. muricata* forest at all sites (Gardes & Bruns, 1996, Horton & Bruns, 1998, Taylor & Bruns, 1999). Mycelial transfer from dying roots of the fire-killed mature trees to the roots of newly establishing seedlings might have been possible, and a while after the fire, wind-borne dispersal of spores from unburned sites might also have occurred. However, *T. sublilacina* was observed on pre-fire and post-fire bioassay seedlings grown in dried soil, suggesting that desiccation-resistant propagules were present both before and after the fire. These propagules appear to be sufficient to account for the levels of colonization observed on the naturally regenerating seedlings (Fig. 1). Furthermore, *T. sublilacina* is not known to produce either sclerotia or even mycelial cords; thus the most likely resistant inocula are spores.

Wind-borne spores were probably the primary inoculum source for *Hebeloma* sp. and tricholomoid sp. 1. These species were not observed on the pre-fire mature *P. muricata* trees (Gardes & Bruns, 1996), and they were absent on the post-fire bioassay seedlings (Fig. 1). *Hebeloma* species are known as early colonizers which produce abundant wind-borne spores (Deacon & Fleming, 1992). In a population structure study, Gryta *et al.* (1997) observed small, short-life-span genets of *Hebeloma cylindrosporum*, suggesting colonization by spores.

Wind dispersal of spores after the fire might also have been significant for colonization by *Wilcoxina* sp., which appeared to be more common on post-fire field seedlings than on the post-fire bioassay seedlings. Although we did not observe fruiting, *Wilcoxina* spp. form fruit bodies after fires (Egger *et al.*, 1991; Egger, 1995), which we may have missed. *Wilcoxina* spp. are also common colonizers of nursery seedlings (Danielson, 1991; Egger, 1995), a setting where these ascomycetous fungi have presumably been dispersed in by wind.

For the other species, we cannot eliminate the possibility of recent dispersal. However, since the results of the post-fire bioassays suggest that resident inoculum was abundant in the mineral soil (Fig. 1), it is not necessary to invoke dispersal in order to explain the observed pattern. Furthermore, for taxa with rodent-dispersed spores, such as *R. olivaceotintus* and *R. subcaerulescens*, dispersal into the site within a year after the fire seems unlikely for several reasons. Firstly, no fruiting of any mycorrhizal fungi was observed within the burned area until January 1997. Secondly, dispersal from the unburned forest, approx. 1 km away, was possible but should have resulted in a patchy distribution. However, *R. subcaerulescens* was very common at all sites studied and a patchy distribution was not observed (Horton *et al.*, 1998).

Rhizopogon ochraceorubens and the *Phialophora*-like taxa were abundantly present on the post-fire bioassay seedlings, but absent from our sample of field seedlings. A similar, though less extreme pattern was seen with *R. subcaerulescens*, which was over-represented on the bioassay seedlings relative to the field seedlings (Fig. 1). This suggests that inoculum for these taxa was abundant, but for some reason was not as effective in colonizing seedlings in the field as in the bioassays. Spatial patchiness of inoculum is not a likely explanation for the lack of *R. ochraceorubens* or lower levels of *R. subcaerulescens* on the field seedlings, because they were found uniformly across all plots in the post-fire bioassays and were nearly uniform in the bioassays from the same plots prior to the fire (*R. ochraceorubens* was absent from a single plot; Taylor & Bruns, 1999). We also know that these *Rhizopogon* species were common on post-fire seedlings at other sites. In fact, both *Rhizopogon* species were found to be the almost

Table 4. Overall significant effects of the different treatments

Treatment	M	Rs	Rl	Ro	Ph
Drying	ns	ns	*(+)	*(-)	**(-)
Diluting	***(-)	**(-)	***(-)	*(-)	**(-)
Depth	***(+)	**(+)	ns	ns	*(+)

Average total number of mycorrhizal taxa per bioassay seedling per treatment (M), *Rhizopogon subcaerulescens* (Rs), *Rhizopogon olivaceotinctus* (Rl), *Rhizopogon ochraceorubens* (Ro), *Phialophora*-like fungi (Ph). Kruskal-Wallis test: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; significantly increased effect; -, significantly decreased effect.

universal and exclusive associates of seedlings that established in the former shrub-dominated community, but at forested sites their frequency and dominance was lower (Horton *et al.*, 1998). This pattern led Horton and colleagues (1998) to suggest that competitive interactions limited the abundance of the *Rhizopogon* species in forested sites. The results of our study fit this explanation well, as we demonstrate that inoculum for the *Rhizopogon* species was abundant in the same areas where *P. muricata* seedlings established without these mycorrhizal species.

Interestingly, *R. olivaceotintus* was the only species that exhibited a significant increase in colonization in dried versus undried post-fire soil (Table 4), suggesting that its spores either were activated by desiccation or were more resistant to drying than competing species. Fire stimulation is known for saprobic, 'fire-place' or phoenicoid fungi (El-Aybad & Webster, 1968; Wicklow & Zak, 1979; Carpenter & Trappe, 1985). Both heat and smoke stimulation of spores or seeds are recorded in the literature for plant and fungal species (Wicklow, 1988; Brown & van Staden, 1997; Enright *et al.*, 1997).

Total mycorrhizal inoculum increased with depth in the post-fire bioassays and, more specifically, the mycorrhizal formation of *R. subcaerulescens* was larger in the bottom than in the top soil layers of the bioassays (Tables 3 and 4). This relates to an observation by the first author that the biomass of the mycorrhizal root tips of the field seedlings at the second 5-cm root increment was four times higher than that of the top 5-cm root increment.

The soil-dilution series, mixing post-fire soil with sterile soil in different concentrations, were originally set up to estimate the inoculum potential of individual species by the most-probable number method. Unfortunately the results for the most frequent species in our study fitted expected most-probable number distributions so poorly that we do not believe that it provides a valid quantification of their inoculum potential. The most-probable number method was originally developed for liquid systems where mixing is more thorough than in soil,

and competitive interactions among species may also affect the presence of species, especially at the lower dilutions. These problems may be the cause of the high variability that we observed. Nevertheless, independently of precise quantification, the amount of inoculum of the *Rhizopogon* spp. and the *Phialophora*-like fungi was higher than that of *T. sublilacina* and *Wilcoxina* sp. in the soil-dilution series with post-fire soil. Similar results were found for soil-dilution series with pre-fire soil originating from the study site (Taylor & Bruns, 1999).

The absence of *Lactarius rufus* (Scop.: Fr.) Fr., *R. brevipes* and *Amanita* species on the 1-yr-old post-fire field seedlings was unexpected for two reasons. Firstly, these taxa, together with *T. sublilacina*, were the dominant species associated with the mature *P. muricata* prior to the fire (Taylor & Bruns, 1999). Secondly, in an earlier study, Horton *et al.* (1998) found *Russula* and *Amanita* species on 5-month-old seedlings at a burned site 1 km away from the study site. Therefore we know that colonization by Amanitaceae and Russulaceae occurred immediately after the fire. The difference between the post-fire mycorrhizal species composition at the sites could be a result of timing differences in our sampling, since the earlier study by Horton *et al.* (1998) examined younger seedlings, or it could be the result of spatial patchiness with respect to survival of the resident species. We favour the spatial patchiness hypothesis, because our ongoing work at other sites seems to support this hypothesis, and because the natural patchiness of fire intensity and fuel consumption could provide a mechanism for sporadic survival of mycelial inoculum.

Eighteen months after the fire, the mycorrhizal community of naturally established *P. muricata* seedlings was dominated by *Rhizopogon* and *Wilcoxina* species which were present as resistant inoculum in the soil prior to the fire. Over time it is likely that the former dominants, such as *Amanita*, *Russula* and *Tomentella* species, will dominate again, either by re-invading from new spore inoculum or by mycelial expansion from surviving patches. Judging from prior studies on mature stands we would expect this process to be complete by 35 yr (Gardes & Bruns, 1996; Horton & Bruns, 1998). However, it may occur much more rapidly and there may be other taxa that are dominant at intervening times. Long-term studies of the fungal community and population genetic structure of the dominant species will be needed to resolve these remaining issues.

ACKNOWLEDGEMENTS

The research was funded by NSF grant # DEB 9628852. We thank Dr D. L. Taylor for his cooperation in this study, M. Bidartondo for his work on the estimation of propagules, and V. Valinluck for her contribution to the

laboratory work. We are grateful to Dr K. Egger for identifying the *Wilcoxina* sp. We thank Dr M. Gardes for valuable comments on an earlier version of this paper.

REFERENCES

- Agerer R. 1987–1997.** *Colour atlas of ectomycorrhizae*. Schwäbische Gmünd: Einhorn Verlag.
- Brown NAC, van Staden J. 1997.** Smoke as a germination cue: a review. *Plant Growth Regulation* **22**: 115–124.
- Brundrett MC, Ashwath N, Jasper DA. 1996a.** Mycorrhizas in the Kakadu region of tropical Australia. I. Propagules of mycorrhizal fungi and soil properties in natural habitats. *Plant and Soil* **184**: 159–171.
- Brundrett MC, Ashwath N, Jasper DA. 1996b.** Mycorrhizas in the Kakadu region of tropical Australia. II. Propagules of mycorrhizal fungi in disturbed habitats. *Plant and Soil* **184**: 173–184.
- Bruns TD, Szaro TM, Gardes M, Cullings KW, Pan JJ, Taylor DL, Horton TR, Kretzer AM, Garbelotto M, Li Y. 1998.** A sequence database for the identification of ectomycorrhizal fungi by sequence analysis. *Molecular Ecology* **7**: 257–272.
- Carpenter SE, Trappe JM. 1985.** Phoenicoid fungi: a proposed term for fungi that fruit after heat treatment of substrates. *Mycotaxon* **23**, 203–206.
- Cullings KW, Vogler DR. 1998.** A 5.8S nuclear ribosomal RNA gene sequence database: applications to ecology and evolution. *Molecular Ecology* **7**: 189–196.
- Dahlberg A, Jonsson L, Nylund J-E. 1997.** Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. *Canadian Journal of Botany* **75**: 1223–1335.
- Danielson RM. 1982.** Taxonomic affinities and criteria for identification of the common ectomycorrhizal symbionts of pines. *Canadian Journal of Botany* **60**: 7–18.
- Danielson RM. 1991.** Temporal changes and effects of amendments on the occurrence of sheathing (ecto-)mycorrhizas of conifers growing in oil sands tailings and coal spoil. *Agriculture, Ecosystems and Environment* **35**: 261–281.
- Deacon JW, Fleming LV. 1992.** Interactions of ectomycorrhizal fungi. In: Allen MF, ed. *Mycorrhizal functioning, an integrative plant–fungal process*. New York, USA: Chapman & Hall, 249–300.
- Egger, KN. 1995.** Substrate hydrolysis patterns of post-fire ascomycetes (Pezizales). *Mycologia* **78**: 771–780.
- Egger KN, Danielson RM, Fortin JA. 1991.** Taxonomy and population structure of E-strain mycorrhizal fungi inferred from ribosomal and mitochondrial DNA polymorphisms. *Mycological Research* **95**: 866–872.
- El-Aybad MSH, Webster J. 1968.** Studies on pyrophilous discomycetes. I. Comparative physiological studies. *Transactions of the British Mycological Society* **51**: 353–367.
- Enright NJ, Goldblum D, Ata P, Ashton DH. 1997.** The independent effects of heat, smoke and ash on emergence of seedlings from the soil seed bank of a healthy Eucalyptus woodlands in Grampians (Gariwerd) National Park, Western Victoria. *Australian Journal of Ecology* **22**: 81–88.
- Ferrier RC, Alexander IJ. 1985.** Persistence under field conditions of excised roots and mycorrhizas of spruce. In: Fitter A, Atkinson D, Read DJ, Usher MB, eds. *Ecological interactions in soil*. Oxford, UK: Blackwell Scientific Publications, 175–179.
- Gardes M, Bruns TD. 1993.** ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.
- Gardes M, Bruns TD. 1996.** Community structure of ECM fungi in a *Pinus muricata* forest: above- and below-ground views. *Canadian Journal of Botany* **74**: 1572–1583.
- Gardes M, White TJ, Fortin JA, Bruns TD, Taylor JW. 1991.** Identification of indigenous and introduced symbiotic in ectomycorrhizae by amplification of the nuclear and mitochondrial ribosomal DNA. *Canadian Journal of Botany* **69**: 180–190.
- Gryta H, Debaud J-C, Effosse A, Gay G, Marmeisse R. 1997.** Fine-scale structure of populations of the ectomycorrhizal

- fungus *Hebeloma cylindrosporum* in coastal sand dune forest ecosystems. *Molecular Ecology* **6**: 353–364.
- Horton TR, Bruns TD. 1998.** Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*). *New Phytologist* **139**: 331–339.
- Horton TR, Cázaras E, Bruns TD. 1998.** Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. *Mycorrhiza* **7**: 11–18.
- Miller SL, Torres P, McLean T. 1993.** Basidiospore viability and germination in ectomycorrhizal and saprotrophic basidiomycetes. *Mycological Research* **97**: 141–149.
- Miller SL, Torres P, McLean T. 1994.** Persistence of basidiospores and sclerotia of ectomycorrhizal fungi and *Morchella* in soil. *Mycologia* **86**: 89–95.
- Parke JL, Linderman RG, Trappe JM. 1984.** Inoculum potential of ectomycorrhizal fungi of forest soils in southwest Oregon and northern California. *Forest Science* **30**: 300–304.
- Pilz DP, Perry DA. 1984.** Impact of clearcutting and slash burning on ectomycorrhizal associations of Douglas fir seedlings. *Canadian Journal of Forest Research* **14**: 94–100.
- Raven PH. 1988.** The California flora. In: Barbour MG, Major J, eds. *Terrestrial vegetation of California*. San Francisco, CA, USA: California Native Plant Society Press, 109–137.
- Siegel S, Castellan NJ. 1988.** *Nonparametric statistics for the behavioral sciences*. New York, USA: McGraw-Hill.
- Schoenberger MM, Perry DA. 1982.** The effect of soil disturbance on growth and ectomycorrhizae of Douglas-fir and western hemlock seedlings: a greenhouse bioassay. *Canadian Journal of Forest Research* **12**: 343–353.
- Sokal RR, Rohlf FJ. 1995.** *Biometry*. New York, USA: W. H. Freeman.
- Swofford, DL. 1993.** *PAUP: Phylogenetic analysis using parsimony, version 3.1*. Champaign, IL, USA: Illinois Natural History Survey.
- Taylor DL, Bruns TD. 1999.** Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. *Molecular Ecology*. (In press.)
- Torres P, Honrubia M. 1997.** Changes and effects of a natural fire on ectomycorrhizal inoculum potential of soil in a *Pinus halepensis* forest. *Forest Ecology and Management* **96**: 189–196.
- Visser S. 1995.** Ectomycorrhizal fungal succession in jack pine stands following wildfire. *New Phytologist* **129**: 389–401.
- Vogl RJ, Armstrong WP, White KL, Cole KL. 1988.** The close-cone pines and cypress. In: Barbour MG, Major J, eds. *Terrestrial vegetation of California*. San Francisco, CA, USA: California Native Plant Society Press, 295–358.
- Whelan RJ. 1995.** *The ecology of fire*. Cambridge, UK: Cambridge University Press.
- Wicklow DT. 1988.** Parallels in the development of post-fire fungal and herb communities. *Proceedings of the Royal Society of Edinburgh* **94B**: 87–95.
- Wicklow DT, Zak JC. 1979.** Ascospore germination of carbonicolous ascomycetes in fungistatic soils: an ecological interpretation. *Mycologia* **71**: 238–242.