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# Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire

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**Abstract** We followed the colonization frequency of ectomycorrhizal (EM), vesicular-arbuscular mycorrhizal (VAM), and dark septate (DS) fungi in 1- to 5-month-old bishop pine seedlings reestablishing after a wildfire. Seedlings were collected on a monthly basis at either a VAM-dominated chaparral scrub site or an EM-dominated forest site, both of which were burned. In both vegetation types, fully developed EM were observed from the third month after germination. EM fungi observed on the seedlings from the scrub site were limited to *Rhizopogon subcaerulescens, R. ochraceorubens* and *Suillus pungens.* Seedlings from the forest were colonized by a greater variety of EM fungi including *Amanita* spp., *Russula brevipes* and a member of the Cantharellaceae. VAM structures (vesicles, arbuscules or hyphal coils) were observed in the seedling root systems beginning 1 month after germination at the scrub site and 3 months after germination at the forest site. Seedlings from the scrub site consistently had more frequent VAM fungal colonization than those from the forest site through the fifth month after germination. DS fungi were observed in most seedlings from both the scrub and forest sites beginning in the first month post-germination. We propose that these fungi survived as a resident inoculum in the soils and did not disperse into the sites after the fire.

**Key words** *Pinus muricata* · Vesicular-arbuscular mycorrhizae · Ectomycorrhizae · Dark septate fungi · Fire · PCR

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## Introduction

A number of studies have been conducted which were designed to elucidate the ecology of ectomycorrhizal (EM) fungi in bishop pine stands at Point Reyes National Seashore, California (Gardes and Bruns 1996; Bonello and Bruns 1998; Horton and Bruns 1998). In October 1995, a wildfire burned all of the research sites involved in those studies. Fungal symbionts are necessary components of most plant communities and can help stabilize these communities through natural disturbances (Perry et al. 1989, 1992). To date, investigations of post-fire recovery of EM fungal species have commenced at least 1 year after a fire event (Danielson 1984; Pilz and Perry 1984; Visser 1995), making it unclear whether viable EM fungal inoculum remained at the site or re-colonized from neighboring intact forests. In this study, we follow the occurrence of EM fungi on bishop pine seedlings in the first 5 months of growth after a stand-replacing fire. In addition to EM colonization, we followed the occurrence of vesicular-arbuscular and dark-septate fungi in the seedling root systems.

All members of the Pinaceae have been considered obligate ectomycorrhizal hosts which do not associate with vesicular-arbuscular mycorrhizal (VAM) fungi. However, Golubinskaya (1967), Dowgiallo and Rambelli (1972), Malloch and Malloch (1981), and Cázares and Trappe (1993) all reported the presence of vesicles in the Pinaceae. Arbuscules were reported in the roots of *Pseudotsuga menziesii* and *Tsuga heterophylla* seedlings grown in a greenhouse soil bioassay in soils collected from the Oregon Coast Range (Cázares and Smith 1996). The occurrence of VAM in conifer hosts may be a function of VAM inoculum potential in soils. Cázares and Trappe (1993) reported that more seedlings of *Abies lasiocarpa, T. heterophylla* and *T. mertensiana* were colonized by VAM fungi when collected among grasses and forbs (typical VAM plants) than under EM forest canopies. VAM colonization could also

be influenced by lower levels of EM fungus inoculum because the EM mantle is known to prevent pathogens and other fungi from entering roots (Zak 1964; Marx 1971). Whether these kinds of inoculum effects are present immediately after a fire is unknown.

Dark-septate (DS) fungi colonize roots of many plant species (Haselwandter and Read 1982a; Stoyke and Currah 1991; Cázares 1992; O'Dell et al. 1993; Ahlich and Sieber 1996). These fungi have melanized hyphae and were first described as *Mycelium radicis atrovirens* and probably represent a diverse group of fungi (Smith and Read 1997). They colonize roots, forming occasional microsclerotia and sometimes a weak Hartig net in conifers (O'Dell et al. 1993; Ahlich and Sieber 1996). The functional role of these fungi in the host plant has been identified along a continuum from positive to negative, depending on the symbionts and the conditions of the experiment (Smith and Read 1997). Cázares (1992) reported the presence of DS fungi on many plants colonizing the forefront of a retreating glacier. Since the plants were establishing in a severely stressful habitat, the fungi were thought to be neutral or positive at this early stage of primary plant succession. Visser (1995) reported that the occurrence of *Mycelium radicis atrovirens* in *Pinus banksiana* Lamb. roots was similar in forests 6 and 121 years after fire. We are unaware of any literature on the occurrence of DS fungi in pine roots immediately after fire.

We hypothesized that the pre-fire mycorrhizal condition of a site (EM-dominated versus VAM-dominated) would influence the fungal colonization of seedlings through inoculum that survived the fire. To test this, we followed fungal colonization of naturally establishing bishop pine (*Pinus muricata* D. Don) seedlings collected from either a burned bishop pine forest (EM plants dominant) or a burned coastal scrub stand (VAM plants only). The seedlings were harvested sequentially during the first 5 months after germination. We identified EM fungi on the seedlings using molecular techniques and compare these data to pre-fire data from the area. We also recorded the frequency of seedlings colonized by VAM and DS fungi.

## Materials and methods

### Study area

The research location is within the Point Reyes National Seashore along the coast of central California. The local Mediterranean climate is moderated by summer fog. The average annual air temperature is 11–14 °C and the average rainfall is 63–88 cm. Peak precipitation occurs from January through March, but fog drip provides moisture during the summer. A large wildfire burned 4942 hectares in and around the park on October 3–7 1995. Immediately after the fire, serotinous cones of bishop pine released large numbers of seed, and by January seedlings began to appear. Short roots were absent in late January but were present when the seedlings were 1 month old. We followed fungal colonization of the seedlings from late February through late June.

Seedlings were collected at two locations. While we did not measure the exact position of these locations within the fire zone, they are both at least 1 km from an unburned forest edge. The first location (scrub site) is at longitude 122°51′3″W, latitude 38°03′16″N and 550 m above sea level. There were no ectomycorrhizal plants in the scrub prior to the fire, nor any evidence of ever being occupied by bishop pine trees. The site is typical for northern coastal scrub (Barbour and Major 1995) dominated by *Baccharis pilularis* DC, and containing species of *Rhamnus, Rubus* and *Toxicodendron,* all of which vigorously resprouted or germinated after the fire. This site also developed a dense community of post-fire herbs and forbs. The scrub site is adjacent to a bishop pine stand reported in Gardes and Bruns (1996) and Bonello et al. (1998), but the seedlings in this forest where left undisturbed for a long-term study on EM fungal post-fire recovery. For the forest site, we chose a stand about 0.6 km from the scrub site at longitude 122°51′18″, latitude 38°03′39″N, and 680 m above sea level. All bishop pine trees at this site died in the fire. In addition, this site contained some VAM plants which either germinated or resprouted after the fire, including species of *Rubus, Marah, Rhamnus, Toxicodendron, Ceanothus, Lotus, Umbellularia* and several genera of grasses.

### Sampling

Sampling was limited because of the low number of seedlings available at the scrub site. In January, 10 newly germinated seedlings were harvested at the forest site and were found free of fungal colonization. Then, once a month from February through June, 10 seedlings were collected at each site for a total of 100 seedlings overall. The forest site contained approximately $22 \pm 2.6$ (mean ± one SE) seedlings per $m^2$. Seedlings at this site were collected every 5 m along two parallel 20-m transects separated by about 10 m. The scrub site contained $<1$ seedling per $m^2$. The scrub transects were 25 and 50 m from a bishop pine stand. Seedlings at this site were collected along two parallel 75-m transects at approximately 15-m intervals. The collection strategy in the scrub was designed to avoid direct interactions with roots from the adjacent forest as much as possible and mature bishop pine roots were never observed at the scrub site. At each sampling date, seedlings were harvested at the same points along the transects, avoiding disturbances caused by previous harvests. To harvest seedlings, a 10-cm-diameter soil corer was placed over a healthy seedling and driven down 40 cm. Soil was carefully removed from the seedlings in the field and the rest was rinsed off in the laboratory. Seedlings were shipped to Corvallis, Ore. for VAM and DS analysis within 2 days of harvest. Beginning in April, ectomycorrhizal development had progressed to a point where clear morphotypes were present; these were removed from the seedlings for EM fungal identification as described below.

### Assessing fungal colonization

We felt all root tips that did not have a well-formed mantle were good candidates for VAM/DS colonization (Chilvers et al. 1987). Since we wanted to document VAM and DS colonization in the seedlings, we needed to save as many tips as possible for microscopic examination of stained roots. As a consequence, we did not directly sample for ectendomycorrhizae (E-strain fungi) in this study. It may be difficult to separate ectendomycorrhizae from ectomycorrhizae by external morphology alone. Ectendomycorrhizal fungi can form thick mantles on some hosts, including pine (Smith and Read 1997). Further, young ectomycorrhizas might have a poorly developed mantle, and ectendomycorrhizal fungi might not penetrate host cells during early stages of development. Using gross morphological differences to sort young root tips may be inadequate and one would have to destructively sample tips for sectioning, culturing, and/or molecular typing to confidently separate ectendomycorrhizae.

Fully developed ectomycorrhizae with thick mantles were removed from each seedling and sorted by morphological type (morphotype) using a dissecting microscope. Criteria for sorting included color, mantle structure, branching pattern and characteristics of rhizomorphs following Agerer (1987–1996). One ectomycorrhizal root tip from each morphotype was sectioned to confirm the presence of a Hartig net. All EM were lyophilized in preparation for molecular analyses and storage. EM were processed to the lyophilization step within 1 week after removal from the field. Although the abundance of EM varied among seedlings, we could not determine whether this was the result of hyphal spread after initial colonization or multiple colonization events. Rather than record the abundance or frequency of root tips colonized, we recorded only the presence or absence of fungal species on the seedlings as a measure of EM inoculum.

After removal of EM, the entire remaining root systems of seedlings from each plot were examined for VAM and DS fungi. Root systems were washed with running tap water and the roots cleared and then stained in a solution of 0.05% trypan-blue in lactoglycerol following a procedure modified from Phillips and Hayman (1970) (Cázares and Trappe 1993). VAM and DS colonization was observed in stained roots by stereo- and compound microscopy. VAM colonization was evaluated as the presence of vesicles, arbuscules, or coils for each seedling. DS colonization was recorded when darkly pigmented and septate hyphae was present. DS microsclerotia in the roots were counted; an aggregate of darkly pigmented, closely packed cells was considered a single microsclerotium (Fig. 2). In many cases, putative EM or VAM hyphae were observed in absence of a mantle and Hartig net (EM), vesicles, arbuscules or coils (VAM); these were not counted to avoid overestimating colonization by these fungi.

### Molecular identification of EM

For each seedling one or, when available, two root tips of each morphotype were selected for molecular identification of the fungus. We used polymerase chain reaction (PCR) methods to identify fungal symbionts to species or family group. DNA extraction, PCR amplifications and restriction fragment length polymorphism (RFLP) protocols follow Gardes and Bruns (1993). We identified fungi from EM by comparing RFLP patterns to those from voucher specimens of sporocarps; samples which displayed identical RFLP patterns, including sub-molar bands, were considered to belong to the same RFLP type. The ITS region of the internal transcribed spacer (ITS) of the nuclear ribosomal repeat was amplified from either EM or sporocarps using either the fungal specific primer pair ITS1f and ITS4 (Gardes et al. 1991) or the basidiomycete specific primer pair ITS1f and ITS4b (Gardes and Bruns 1993). Fungal ITS-RFLP patterns were produced using restriction enzymes *Hinf*I, *Alu*I and *Mbo*I. RFLP band sizes were estimated using GelReader version 2.0.5 (National Center for Supercomputing Applications, University of Illinois); this yields about a 3% (up to 10%) error in size estimates of ITS-RFLP bands of the same sample run on different gels.

If the species of fungus remained unknown because no match to a voucher specimen was found, we sequenced one of two regions for phylogenetic analysis. Basidiomycete family placement was conducted using a working version of the mitochondrial large subunit rRNA gene database (Bruns et al. 1998) which has been used in several other studies (Cullings et al. 1996; Gardes and Bruns 1996; Taylor and Bruns 1997; Horton and Bruns 1998). Determination of the fungal division (i.e. Zygomycota, Ascomycota, or Basidiomycota) was accomplished with a database of the 5.8S nuclear rRNA gene (Cullings and Vogler, unpublished data; PCR amplification with the primer pair ITS1f and ITS4). DNA sequences were produced with the cyclic reaction termination method using fluorescence-labeled dideoxyribonucleotide triphosphates and following the instructions provided with the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin-Elmer Corporation). DNA Sequencing Analysis (version 2.01) and SeqEd (version 1.03) were used to process raw data. Identification was based on phylogenetic analysis using PAUP 3.1.1 (Swofford 1993) and the heuristic search option or the test versions of PAUP 4 and the neighbor joining option. Where no ITS-RFLP match was found for basidiomycetes identified to family, we labeled the fungus by its family group name after replacing the ending with oid followed by an RFLP pattern number (i.e. the first unknown member of the Amanitaceae becomes Amanitoid 1, the fifth unknown member of the Thelephoraceae becomes Thelephoroid 5).

## Results

In the absence of EM, seedlings from both sites had similar root lengths and numbers of short roots. Fully developed EM first appeared in April on three and four seedlings at the forest and scrub sites, respectively (Fig. 1). EM colonization increased to 7 of 10 seedlings in the forest and 10 of 10 seedlings in the scrub in May and June. The frequency of seedlings colonized by EM

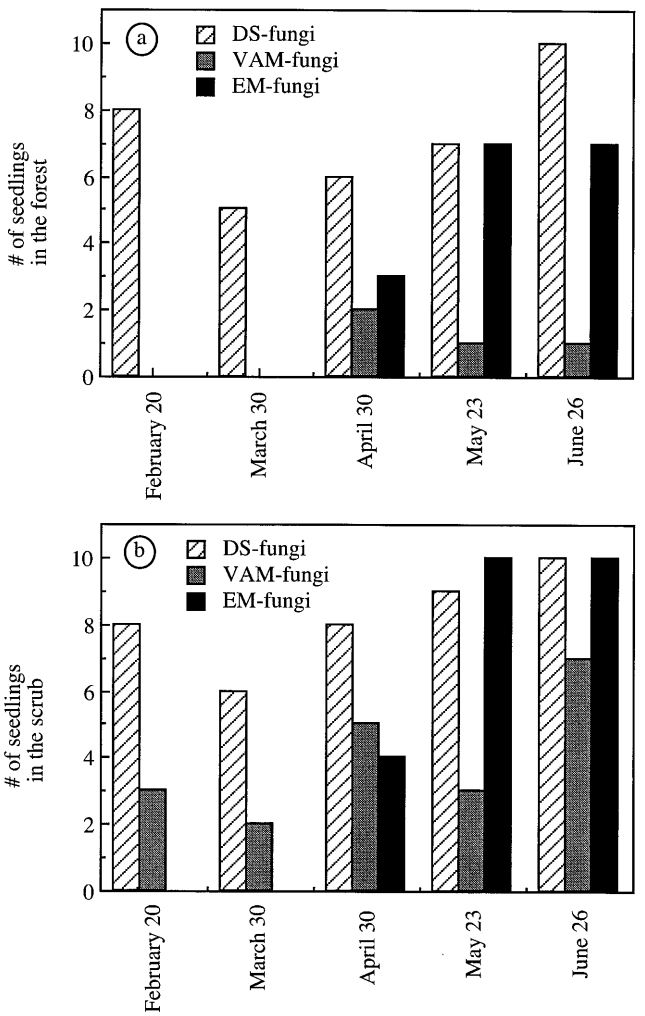


**Fig. 1a,b** Dark septate (DS), vesicular-arbuscular mycorrhizal (VAM) and ectomycorrhizal (EM) fungal colonization of post-fire bishop pine seedlings. **a** Seedlings collected from forest site. **b** Seedlings collected from scrub site ($n = 10$ seedlings/month at each site)

fungi was similar at both sites for each harvest date. In total, 14 EM types were observed on the seedlings (Table 1). There was a marked difference between the scrub and forest sites in the number of fungus species present on the seedlings and their frequencies (Table 2). Three related taxa colonized seedlings at the scrub site: *Rhizopogon subcaerulescens, R. ochraceorubens* in high frequency (six seedlings each in the June harvest) and, to a lesser extent, *Suillus pungens.* In contrast to the scrub site, 13 EM species colonized seedlings in the forest site in low to moderate frequency including the two *Rhizopogon* spp. above, three *Amanita* spp., a cantharelloid sp., *Russula* brevipes and *Tuber californicum.* By June, many seedlings from both sites had more than one EM associate (Table 2). *Rhizopogon* species appeared to cause a proliferation of its own EM and, since *Rhizopogon* was common at the scrub site, the scrub seedlings had more colonized root tips than the forest seedlings when EM were present.

Vesicles first appeared in February and April in the seedlings collected from the scrub and forest sites, respectively. Some short roots with VAM structures had root hairs. There were consistently fewer seedlings colonized by VAM in the forest than in the scrub (Fig. 1). In total, 24 seedlings had vesicles, five seedlings had hyphal coils and/or arbuscules and one seedling had *Scutellospora*-like auxiliary cells (Table 2).

Most seedlings were colonized by DS fungi in every harvest period and at both sites (Fig. 1). All DS hyphae were pigmented along their entire lengths. In some cases, the roots were sparsely colonized by DS hyphae and in others they were densely covered. No appressoria were observed in any of the seedlings. In May, one seedling from the forest had a DS microsclerotium (Fig. 2) and in June microsclerotia were common in four and eight seedlings harvested at the forest and scrub sites, respectively (Table 2). Microsclerotia varied in size and individual microsclerotia sometimes appeared fused into aggregates.

Eighteen seedlings were colonized by all three fungal types: VAM, DS and EM fungi (Table 2). Several EM types were observed repeatedly at some collection locations (cantharelloid 1 at forest 3; *Tuber californicum* at forest 5; rflp 6 at forest 8 and 9; *R. subcaerulescens* at scrub 1, 2 and 8; *R. ochraceorubens* at scrub 4, 5, 7, 9 and 10). Similarly, VAM colonization of seedlings was often observed on two or more collection dates at a specific location (Table 2).

## Discussion

### EM fungi

Most of the fungi colonizing the seedlings at the forest site were known components of the pre-fire bishop pine community based on EM, soil bioassays and sporocarp records (Gardes and Bruns 1996; Bruns et al. unpublished data). Post-fire windblown inoculum was not the main contributor, otherwise a similar mix of species likely would have been found on the scrub and forest seedlings. The primary mode of colonization for many EM fungi in intact forest ecosystems is from preexisting mycelial networks rather than from spores (Deacon and Flemming 1992). This may be true even after fire (Molina and Trappe 1982; Amaranthus and Perry 1989). Excised EM can remain viable in soils for up to 8 months (Harvey et al. 1980; Ferrier and Alexander 1985). In our study, the fire intensity varied and the abundance of surviving root systems was probably patchy, but seedlings began germinating 4 months after the fire. In addition to EM and mycelial networks, resident spores and sclerotia may have also acted as inoculum for the EM fungi at the forest site.

**Table 1** ITS-RFLP band sizes for EM fungi on post-fire bishop pine seedlings

| Fungal taxa | *Alu*I | *Hinf*I | *Mbo*I |
|---|---|---|---|
| [a] *Rhizopogon subcaerulescens* | 471/[b](423)/334/87 | 252/133/75 | 99/247/240 |
| *Rhizopogon ochraceorubens* | 453/345/106/87 | 355/254/164/140/77 | 343/293/257 |
| *Suillus pungens* | 671/97/83 | 235/221/133/76 | 284/238 |
| [c] *Amanita gemmata/pantherina* | 403/242/100/82 | 374/256/120 | 347/240 |
| *Amanita pachycolea* | 495/287/185/94 | 355/308/141 | 509/222 |
| Amanitoid 1 | 547/198/84 | 402/349 | 537/216 |
| *Russula brevipes* | 526/282/149/123/85 | 418/386/134 | 259/204/98 |
| Cantharelloid 1 | 523/256/135/105/86 | 308/184/135 | 310/296/220 |
| Thelephoroid 5 | 387/149/88 | 399/136 | 389/240/204 |
| [d,e] rflp 6 Basidiomycete | 556/126 | 379/342 | 229/220/172 |
| [d] *Cenococcum geophilum* | 694/398 | 609/180/122/108/79 | 396/317/146/122 |
| [d] *Tuber californicum* | 613/93 | 389/188/157 | 323/240 |
| [d,f] rflp 9 Ascomycete | 631/95 | 254/194/165 | 371/248 |
| [d,f] rflp 10 Ascomycete | 565/99 | 339 | 407/200 |

[a] ITS-RFLP region amplified with primer pair ITS1f and ITS4b unless otherwise noted
[b] ITS-RFLP bands in parantheses are submolar
[c] ITS-RFLP patterns do not differentiate *A. gemmata* and *A. pantherina*
[d] ITS-RFLP region amplified with primer pair ITS1f and ITS4
[e] Clamp connections observed in the mantle
[f] Phylogenetic analysis of the 5.8s nuclear rRNA gene suggests this is an *Ascomycete*

**Table 2** Fungal colonization of seedlings at each location along the forest and scrub transects. At each transect point, one seedling was harvested each month. Voucher sporocarp collection numbers are given for all named species. All voucher collections are located at the University of California Berkeley Herbarium except hs1543, which is at the H. D. Thiers Herbarium, San Francisco State University. Colonization for each type of fungus is shown separately for all seedlings: e.g., the seedling collected in May at Forest Transect point 2 had DS hyphae, 18 VAM vesicles and Cantherelloid EM (+ root colonized by DS hyphae, ++ root heavily colonized by DS hyphae, *m* <100 DS microsclerotia in root, *mm* >100 DS microsclerotia in root, numbers under VAM-fungi denote the number of structures in root, *v* vesicle, *a* plant cell with arbuscule, *c* plant cell with hyphal coil, *C1* Cantherelloid 1, *Ro Rhizopogon ochraceorubens* A. H. Smith, trh 188, *Ap Amanita pachycolea* Stuntz, tdb 1508, *Rb Russula brevipes* Peck, trh 115, *T5* Thelephoroid 5, *r9* rflp 9, *Tc Tuber californicum* Harkness, hs 1543, *Rs Rhizopogon subcaerulescens* A. H. Smith, tdb 939, *A1* Amanitoid 1, *Ag Amanita gemmata* (Fr.) Bertillon, trh 123, *r6* rflp 6, *Cg Cenococcum geophilum* Fr., *r10* rflp 10, *Sp Suillus pungens* Thiers and Smith, tdb 2222)

| Transect point | DS fungi | | | | | VAM fungi | | | | | EM fungi | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Feb | Mar | Apr | May | Jun | Feb | Mar | Apr | May | Jun | Apr | May | Jun |
| Forest | | | | | | | | | | | | | |
| 1 | | + | + | + | ++ | | | | | | | | |
| 2 | + | + | + | + | mm | | | 42v | 18v | | | C1 | |
| 3 | + | + | + | + | ++ | | | 36a/12c | | | | | Ro, Ap, Rb, Cl |
| 4 | + | | + | | m | | | | | | C1 | C1 | C1, T5, r9 |
| 5 | + | | | + | ++ | | | | | | | Tc | Tc |
| 6 | + | | | | ++ | | | | | | | Rs | Rb |
| 7 | + | + | ++ | | m | | | | | | A1 | Rs | Ro, Ag, C1, r6, Cg, r9 |
| 8 | + | | | m | mm | | | | | 2v | | r6, r9 | r6 |
| 9 | | | | + | ++ | | | | | | r6 | r10 | r6 |
| 10 | + | + | + | + | ++ | | | | | | | | |
| Scrub | | | | | | | | | | | | | |
| 1 | + | | + | + | m | 3v | | | | 2v | | Rs | Rs |
| 2 | + | + | + | + | mm | | | >150v | | 2v | Sp | Rs | Rs |
| 3 | | + | + | | ++ | | 65v | 60v/6c | | >150v | Rs | Sp | Ro |
| 4 | + | | | + | mm | | | | | | | Ro | Ro, Sp |
| 5 | + | + | + | + | ++ | | | | | 50c | | Sp | Rs |
| 6 | + | + | | + | m | | | | | | | Rs, Ro | Ro |
| 7 | + | + | + | + | m | | | 40v/20a | >150 v | 6v | Ro | Ro | Rs |
| 8 | | | + | ++ | mm | | | 8v | | 12v/3c | | Rs | Rs, Ro |
| 9 | + | | + | + | m | 2v | 8v | 10v | >100v | | | Ro | Rs, Ro |
| 10 | + | + | + | + | mm | 1v | | | >100v | 25v | Ro | Ro | Ro |

**Fig. 2** DS hypha and a microsclerotium in a bishop pine root; *bar* 25 μm

Although the scrub was dominated by resprouting VAM plants, inoculum of two pine-specific *Rhizopogon* spp. (Molina and Trappe 1994) was common in the soil. These two species were not abundant EM types (EM biomass) in the pre-fire forest community (Gardes and Bruns 1996; Horton and Bruns 1998). Root systems from the adjacent forest stand can be ruled out as a major contributor of inoculum since (1) *Rhizopogon* occurred at all points along the transects and (2) inoculum of other local bishop pine symbionts was apparently absent in the scrub. Spores of *Rhizopogon* are dispersed by mammals through feces or remain at a site after sporocarp deliquescence (Maser et al. 1978; Johnson 1996; Miller et al. 1994), both of which would produce point sources of inoculum. However, the uniform distribution of *Rhizopogon* along the scrub transects suggests that *Rhizonogon* spores were dispersed and homogenized in the soils by wind or water (Johnson 1996) after deliquescence or rodent dispersal.

The different number of seedlings colonized by *Rhizopogon* at the two sites was probably not related to *Rhizopogon* inoculum levels. Forest soils (pre-fire and post-fire) and scrub soils (post-fire only) have all yielded high levels of *Rhizopogon* EM on bishop pine seedlings in bioassays (Bruns et al. unpublished data). It is possible that a dormancy mechanism in *Rhizopogon* spores (Theodorou and Bowen 1973; Lamb and Richards 1974; Torres and Honrubia 1994) allows this fungus to survive removal from the field and subsequent laboratory treatments or, in this case, survive the conditions of the scrub site. Spores of *Russula, Cortinarius,* and *Amanita* are not as resistant as those of *Rhi-*

*zopogon* (Torres and Honrubia 1994), which may account for their absence in most soil bioassays and at the scrub site. However, while suggesting why *Rhizopogon* was particularly common at the scrub site, this does not account for its lower frequency at the forest site. This difference may result from an inability of *Rhizopogon* to compete well when EM fungi such as *Russula* and *Amanita* spp. are present. Competition was higher at the forest site, where more species were present to interact with *Rhizopogon,* than at the scrub site. A contributing factor could be that microorganisms associated with the soils differentially influenced the ectomycorrhizal fungi at the two sites (Garbaye and Bowen 1987; Garbaye 1994).

## VAM fungi

In total, 24 of 100 bishop pine seedlings had vesicles, and five had arbuscules or coils. Francis and Read (1995) suggested that the presence of vesicles in otherwise EM hosts indicates a pathogenic interaction. However, the VAM fungal colonization apparently was not recognized as pathogenic by the seedlings. In addition, hyphal coils and arbuscules are thought to be sites of nutrient transfer between the symbionts and, while these structures were observed in only some of our seedlings, they are ephemeral and can be difficult to observe.

Lapeyerie and Chilvers (1985) hypothesized that VAM may be important in otherwise EM hosts during seedling establishment in nutrient-poor sites. They report that *Eucalyptus* seedling growth in phosphorus-limited soils was enhanced by VAM fungi, which were then succeeded by EM fungi after 6 months. Indeed, Smith et al. (1998) reported that *Pseudotsuga menziesii* seedlings inoculated with the VAM fungus *Glomus intraradices* Schenck & Smith had higher foliar phosphorus levels than the uninoculated controls. However, our seedlings were probably not nutrient- or even water-limited. Soils in the first 6 months after a fire typically have high levels of available nitrogen and phosphorus (St. John and Rundel 1976; Debano and Conrad 1978; Marion et al. 1991; Vázquez et al. 1993), and the precipitation was 23% above normal during the spring following the fire in our study (Pt. Reyes National Seashore, National Weather Service Station #042303). The functional role of VAM fungi in this otherwise EM host remains unclear.

## DS fungi

Most seedlings were colonized by DS fungi in the first month and this frequency was maintained throughout the study. Like the VAM fungi, we do not know the functional role of the DS fungi. DS fungi as a group have been shown to have both positive and negative impacts on seedling growth (Haselwandter and Read 1982b; Wang and Wilcox 1985; Wilcox and Wang 1987; O'Dell et al. 1993; Fernando and Currah 1996; Jumpponen et al. 1998) and individual species may function along a mutualistic-parasitic continuum (Johnson et al. 1997). Little work has been conducted in the field, except to report the presence of these fungi in roots of various plant species. Our results show that DS fungi will colonize young bishop pine seedlings shortly after wildfire.

## Conclusions

It appears that inoculum levels and interactions with other soil fungi had a greater effect on colonization frequencies after fire than did host responses. The availability of pine short roots not fully colonized by EM fungi might have allowed VAM and DS fungal colonization. This is consistent with results reported by Chilvers et al. (1987) where VAM and DS fungi colonized seedlings when non-EM *Eucalyptus* root tips were encountered. The number of seedlings colonized by VAM was higher at the scrub site than the forest site, as predicted by the abundance of resprouting VAM hosts at the scrub site. Inoculum of DS fungi was apparently common at both sites. Once EM fully developed on the roots, a greater number of EM species were present at the forest site than the scrub site. This was likely related to the fact that the forest site had a complex EM community prior to the fire. We have no EM data from the scrub site prior to the fire except that bishop pine trees were absent directly at the site. However, the dominance of *Rhizopogon* at the scrub site is likely a function of spore inoculum and a lack of direct interactions with other EM species.

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