Ectomycorrhizal ecology under primary succession on coastal sand dunes: interactions involving *Pinus contorta*, suilloid fungi and deer

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Summary

- Ectomycorrhizal fungi (EMF) are critical for pine establishment under primary succession. The species of EMF supporting primary successional pine seedlings on coastal sand dunes and mechanisms for their establishment were investigated.
- Fungi were identified from ectomycorrhizal roots using molecular techniques. Field seedlings were collected from forested and nonforested zones. Laboratory seedlings were grown in soils collected from the same zones, and in sterile soils inoculated with fresh and 1-yr-old dry deer fecal pellets.
- Suilloid fungi were frequently observed on all seedlings. A diverse group of fungi was available to seedlings in forested zones. A less diverse group of fungi was available to field seedlings in nonforested zones and all laboratory bioassay seedlings. Deer fecal inoculant yielded an average of two EMF per seedling. Both *Suillus* and *Rhizopogon* species dominated seedlings inoculated with fresh deer feces, but only *Rhizopogon* species dominated seedlings inoculated with 1-yr-old feces.
- Suilloid fungi are dispersed by deer, produce resistant spore banks and are the principle fungi supporting seedlings on the sand dunes.

Key words: dispersal, ectomycorrhizal ecology, mycophagy, primary succession, *Rhizopogon*, spore bank, *Suillus*.

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Introduction

Sand dunes are excellent candidates for studies on primary succession (Cowles, 1899; Read, 1989). We investigated primary succession of a pine forest on coastal dunes near Florence, OR, USA. Owing to the introduction of the vigorous European beach grass *Ammophila arenaria* to the Oregon dunes *c.* 40 yr ago, much of what were formally shifting dunes have become, or are becoming, stabilized. The stabilization of the dunes allows *Pinus contorta* var. *contorta* to establish in isolated areas with no recent history of vegetation (Weidemann *et al.*, 1999; Pickart & Sawyer, 1998).

Pickett *et al.* (1987) suggest that plant establishment in different successional stages is a function of site and species availability, in conjunction with species performance in the new environment. We use this model to gain a better understanding of the role of ectomycorrhizal fungi (EMF) in the establishment of the pines at the Oregon dunes. Stabilized dunes provide new habitat for pines and their associated ectomycorrhizal fungi. Pine seeds are available via abundant wind dispersed seeds from the surrounding forests. Species performance of the plants is tested upon arrival due to the exposed conditions of the site and requirement for mycorrhizal fungi, adding a mutualistic component to species interactions under species performance in Pickett *et al.* (1987).

Plants establishing during primary succession have no mycorrhizal networks available but still depend on fungal inoculum from neighboring forests. Recolonization of EMF in the blast zone of the Mt St Helens (WA, USA) eruption was attributed to spore dispersal both by wind and animals

(Allen *et al.*, 1984; Allen, 1987; Allen *et al.*, 1992). Cázares & Trappe (1994) found spores of mycorrhizal fungi in a variety of mammal feces near a glacier forefront. Terwilliger and Pastor (1999) reported that red backed voles were the dispersal agent for black spruce mycorrhizal fungi in meadows formed in abandoned beaver ponds. Jumpponen (2003) attributed a dormant ectomycorrhizal (EM) fungus spore bank in forefront soils of a glacier to wind dispersed spores.

The purpose of this study was to identify EMF on seedlings in a primary successional ecosystem on the Oregon coastal dunes, and to identify mechanisms contributing to their establishment. We hypothesized that the fungi supporting the isolated seedlings represent a subset of species fruiting in the area with spores that are adapted for dispersal to and survival in the primary successional zones. Molecular methods were used to identify EMF on seedlings. The species composition on seedlings growing on the sand dunes was compared with EMF on seedlings from bordering pine forests and sporocarp records from the research area. We used soil bioassays to assess the inoculum potential of field soils and deer fecal bioassays to assess spore dispersal and resistance to desiccation.

Materials and Methods

Study site

The study ecosystem is located in the Oregon Dunes National Recreation Area 43°5′ N, 124°1′ W in the Siuslaw National Forest, Oregon, USA. An average of 178 cm of precipitation falls per year, with the majority in winter (Wiedemann *et al.*, 1999). Temperatures average 16°C in the summer months and 4°C in the winter months (Wiedemann *et al.*, 1999). Although seasonally dry in the summer, the lack of precipitation in the summer is mitigated by ocean fog. We identified four sites that each contained three zones: mature forest to the east (approx. 100 yr old; D. Segotta, pers. comm.), centrally located sand dunes that we called isolated areas, and a younger deflation plain forest (approx. 40 yr old; D. Segotta, pers. comm.) neighboring the ocean on the west (Fig. 1) ($n = 4$ for each zone). The deflation plain forms where wind has blown loose sand off to expose the water table. Wind direction varies by season with north-north-west and south-south-west winds prevailing in the summer and winter, respectively (Wiedemann *et al.*, 1999). The spring and autumn seasons are transitional and winter winds alternate with summer winds. Distances from the forest zones to isolated areas on the sand dunes average 334 m and range from 127 to 598 m. The four sites are along four positions on a north–south axis running along approx. 7 km of the coastline.

The mature forest edge and the deflation plain forest are dominated primarily by *Pinus contorta* var. *contorta*. Other EM hosts include *Picea sitchensis* Bong. Carr., *Pseudotsuga menziesii* Mirb. Franco, and *Tsuga heterophylla* Raf. Sarg. Shrub species occurring on the edges of the forests include the EM *Salix hookeriana* Barr., *Arctostaphylos uva-ursi* (L.) Spreng., *Arctostaphylos columbiana* Piper, and the arbuscular mycorrhizal (AM) *Cytisus scoparius* (L.) Link. By contrast, because of constantly shifting sands, the isolated dune zones either lack vegetation or are sparsely colonized by AM *Ammophila arenaria*, *Lupinus* spp. and *C. scoparius*. Pine seedlings establishing in the isolated areas are predominantly located in moderately stabilized locations partly created by *A. arenaria*.

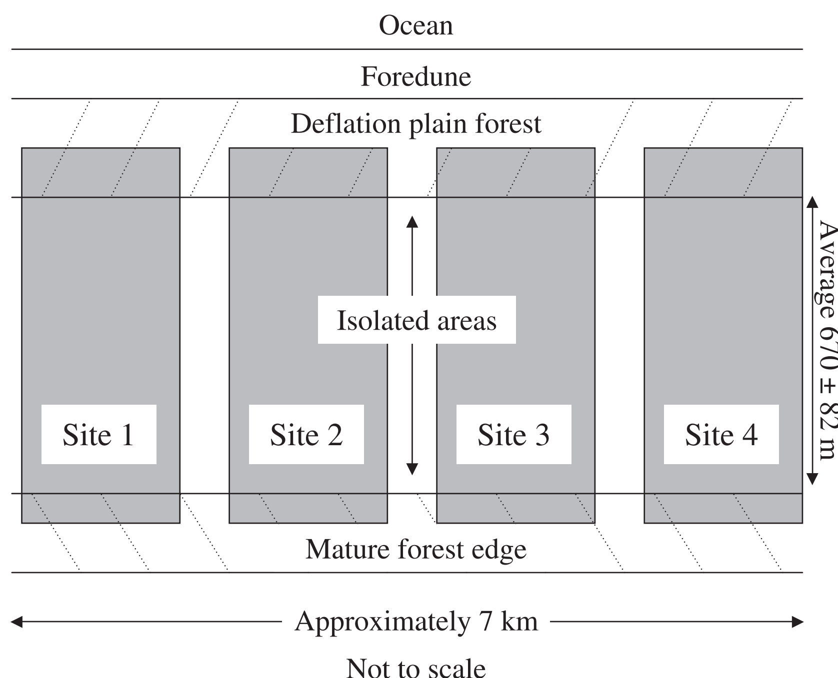

Fig. 1 Schematic diagram of the research area. Each study site, represented by the gray boxes, includes three zones: deflation plain forest, mature forest edge and the isolated dune areas. The entire distance from site 1 to site 4 is approx. 7 km and the distance between the mature forest edge and deflation plain edge averages 670 ± 82 m (mean ± 1SE). The distance between isolated seedling collections sites and any forest edges averaged 334 ± 74 m.

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The soils consist entirely of sand. The mature forest and deflation plain forest have a shallow A-horizon of < 5 cm, except in seasonally flooded areas of the deflation plain forests where the A-horizon was up to 10 cm.

Mycorrhizas on field seedlings

One to three seedlings up to 5 yr old were collected per zone (mature forest edge, isolated areas and deflation plain forest edge) per sample site in three seasons following a randomized complete block design with the four sample sites representing blocks. Seedlings from the isolated areas were at least 120 m from any forest edges. An average of five seedlings were collected per zone in each site. Eighteen seedlings were collected in the mature forest and isolated areas, and 25 were collected in the deflation plain. Seedlings were carefully extracted from the soil, transported to laboratory within 24 h, and kept moist and refrigerated at 4°C until processed. We aged the seedlings by counting the rings on cross-sections of the stems at ground level under ×100 magnification with a compound microscope. Winter and summer seedlings were collected in 2000 and the autumn seedlings were collected in 2002. Sampling was opportunistic because of the limited number of seedlings in the isolated areas.

Within 1 wk, seedling roots were carefully washed of rhizospheric soil using a 0.5 mm mesh screen (No. 35 USA standard testing sieve) and cold tap water. All live root tips of each seedling were removed and sorted into morphological groups (morphotypes) using a dissecting microscope generally following Agerer (1987–2002). Root tips with ambiguous mycorrhizal characters were also sorted and processed, including those with root hairs. Morphotypes were differentiated by characteristics such as color, branching pattern, and distinct features of the extramatrical hyphae and rhizomorphs). No attempt was made to match morphotypes between seedlings. Morphotypes were freeze-dried and stored at −20°C.

Soil bioassay

Soils were collected from each zone in the four sample sites during the peak fruiting season in the autumn of 2002. Soils were brought back to SUNY-ESF and bioassays were set up within 3 wk of collection. Each soil sample consisted of three pooled soil cores taken at random locations within a zone in each site (5 cm diameter × 15 cm deep). The soil samples were mixed with autoclaved peat and vermiculite (1 : 1 : 1). *Pinus contorta* seeds were surface-sterilized with 30% hydrogen peroxide for 10 min, washed in distilled water for 1 min, and a single seed was planted into Ray Leach tubes (Stuewe & Sons, Inc., Corvallis, OR, USA) filled with a soil mix. Ten seedlings were grown for each soil sample. Three seedlings were grown individually in a 1 : 1 : 1 soil mix with autoclaved field soil, peat and vermiculite as controls. The seedlings were randomized in the racks and grown for 6 months at room temperature with fluorescent grow lights (14 h daylength; 90 µmol m^{-2} s^{-1}). The root tips were harvested and processed as described above. Owing to problems with a soil pathogen and insect infestation, many of the seedlings died before harvest. Of the 40 seedlings planted in soil from each zone (4 zones × 10 seedlings per sample site), 25 survived in the mature forest soil (5–7 seedlings per site) 28 survived in the isolated area soil (6–8 seedlings per site), and 26 survived in the deflation plain soil (5–7 seedlings per site).

Deer fecal bioassays

Deer were rarely seen during the study, but tracks and fecal piles were commonly observed. Twenty-one fecal samples of black-tailed deer (*Odocoileus hemionus* Rafinesque) were collected opportunistically throughout the four sites in the autumn of 2000 during the peak fruiting season. Each fecal pellet in the sample was divided in two by halving individual pellets with a razor blade, and sorting them into two portions. One portion was used immediately as inoculant for a bioassay and the other portion was stored dry at room temperature for 1 yr to assess spore survival. Before use as inoculum, the portions were blended separately with 25 ml of deionized water to make an inoculant slurry. The majority of the spores were smooth and brown and morphologically identified as *Suillus* (asymmetrical) or *Rhizopogon* (symmetrical). A few spores of *Laccaria*, *Russula* and *Cortinarius* were observed in some fecal samples. Spore concentrations were quantified with a hemocytometer. We chose seven fresh fecal samples with the highest spore concentrations (portions that contained at least 10^8 spores) to use for the paired fresh-dry inoculant bioassay. We made a dilution series of fresh fecal–water mixtures containing spore concentrations of 10^8 to 10^2 spores ml^{-1} of water following Lamb and Richards (1978).

Pinus contorta seeds were surface-sterilized with 30% hydrogen peroxide for 10 min, rinsed with sterile water, and planted into individual tubes containing a sterile mixture of sand, peat and vermiculite (1 : 1 : 1). We inoculated seedlings 3 wk after they had germinated, when they had developed their first lateral roots. Three seedlings were inoculated with 10 ml of an inoculation solution for each fecal sample (3 seedlings × 7 spore concentrations × 7 fecal samples = 147 seedlings). Three seedlings were inoculated with autoclaved fecal slurries at full strength and 20 seedlings were inoculated with only deionized water. These last two groups were used as controls. Two weeks later, an additional 10 ml of the same inoculation treatments were added to each seedling. The seedlings were grown and the root tips were harvested and processed as described earlier.

The same procedure was conducted 1 yr later for the dry fecal samples with one change. Because seedlings in the fresh fecal treatment did not become mycorrhizal at low spore concentrations, only the two dilutions with the highest spore concentrations were made for the dry treatment. To investigate possible effects of increased sample size on the diversity of

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Table 1 Identification of ectomycorrhizal fungi (EMF) from root tips or sporocarps based on GenBank queries with nuclear rDNA ITS region

Sample label	Sequence length	GenBank accession #	GenBank score	Base pairs matched	Consensus identification
Ash58b	809	AY880943	1421	752/762	*Rhizopogon fuscorubens* AH Smith
Ashi2	786	AY880944	1451	747/752	*Rhizopogon occidentalis* Zeller & Dodge
Trh542	759	AY880930	1598	799/802	*Rhizopogon salebrosus* group[a]
Dps29.3b	770	AY880945	1119	624/626	*Rhizopogon evadens* (Vitt.) M. Lange
Ash111	645	AY880938	1249	633/634	*Suillus pseudobrevipes* Smith & Thiers
Trh664[b]	642	AY880941	1222	636/642	*Suillus brevipes* (Peck) Kunze
Ash17	671	AY880932	1076	626/650	Unknown *Suillus* species[c]
Ash109b	464	AY880937	902	457/458	*Suillus tomentosus* (Kauffman) Singer
S147a	640	AY880939	1255	633/633	*Suillus umbonatus* Dick & Snell
Trh686[b]	644	AY880940	1240	638/644	*Suillus neoalbidipes* (Peck) Singer
Ashf2	520	AY880937	1021	515/520	*Cenococcum geophilum* Fr.
Ashd2	608	AY880947	805	419/422	*Cantherallales*
Ash5051a	485	AY880948	978	478/480	*Cortinarius* species
Ash50844b4	750	AY880933	1063	667/709	*Laccaria laccata* (Scop.:Fr.) Cooke
Ashd15	556	AY880935	1003	546/556	*Phialocephela fortinii* Wang & Wilcox[d]
th5079	677	AY880930	1172	639/651	*Russula pectinatoides* Peck
Ash5096b	650	AY880934	1181	621/626	*Thelephora americana* Lloyd[e]
Ash36	653	AY880929	1253	640/641	*Tomentella sublilacina* (Ellis & Holw.) Wakef.
Ashd25	649	AY880928	1110	611/627	*Thelephoraceae*
AshT73.1	641	AY880927	959	593/629	*Thelephoraceae*
Ash5a	584	AY880942	959	524/532	*Wilcoxina mikolae* (Yang & Wilcox) Yang & Korf
Ash5141	530	AY880946	930	514/424	Ascomycete/ericoid root endophyte

Species name most similar to our sequences are given. Higher taxonomic names are given when the query did not yield a sequence with a similarity of 97% or above for a species or if our sequence was most similar to an unknown sequence in GenBank and less similar to known species. Sequences were obtained from root tip vouchers unless indicated otherwise.

[a]The *Rhizopogon salebrosus* group includes several related taxa including *R. subcaerulescens* (Bruns *et al.*, 2002).

[b]Sequence generated from a sporocarp voucher.

[c]The ITS sequence of this *Suillus* species is identical to an unknown species found in coastal *Pinus muricata* in California (L. Grubisha, pers. comm.; Kretzer *et al.*, 1996).

[d]*Phialocephala fortinii* may be pathogenic (Wilcox & Wang, 1987) or mutualistic (Jumpponen & Trappe, 1998).

[e]*Thelephora americana* and *T. terrestris* are very similar morphologically. We identified sporocarps in our area as *T. americana* based on their smaller spores compared to those of *T. terrestris*.

EMF on root tips, eight extra dried fecal samples with high spore concentrations were used to inoculate seedlings.

Molecular identification of fungi

DNA extractions, polymerase chain reaction (PCR) amplifications and restriction fragment length polymorphism (RFLP) generation followed White *et al.* (1990) and Gardes & Bruns (1996). We used either internal transcribed spacer (ITS)-1f and ITS-4 or ITS-1f and ITS4b as primers to amplify the fungal nuclear rDNA ITS region. DNA from PCR products and RFLPs were run in 3% agarose gels, stained with ethidium bromide, and digitally photographed (EpiChemi II Darkroom; UVP Laboratory Products, Upland, CA, USA). RFLP patterns were generated with *Hin*fI, *Alu*I, and *Dpn*II, and band sizes were measured using the LABWORKS computer software (UVP Laboratory Products, Upland, California, USA). Band sizes were calibrated using a 100 base pair ladder (Promega; Fisher Scientific, Pittsburgh, PA, USA). We compared RFLP patterns from EM root tips with each other and with those from voucher sporocarps of over 100 species collected in the area (T. R. Horton unpublished). A species name was assigned to a mycorrhizal root tip sample when the ITS-RFLP patterns from the root tips matched the sporocarp patterns with all three enzymes when possible (in a few cases, a restriction pattern was not obtained with *Alu*I). Studies from this research site and in Scandinavia suggest matching RFLP patterns with two to three enzymes is a good approximation for species matching (Kårén *et al.*, 1997; Horton, 2002).

We generated sequences for the nuclear rDNA ITS region for the main EMF in the study, including *Suillus neoalbidipes*, whose sporocarps (but not mycorrhizas) were collected in the study area (Table 1). To determine the identity of unknown sequences, a minimum of 95% sequence overlap to an existing ITS sequence of at least 450 bp in the GenBank database was required. Those samples with 97–100% similarity with existing species were considered a match and were named to the species level. Those sequences with 96% or lower similarity to existing sequences were designated names to the genus, family, order, or in one case, class (putative ericoid ascomycete).

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Not all unknown patterns sufficiently amplified to allow sequencing. Most of these were rare types and we did not pursue their identity any further. We called unknown patterns by their sample number in the study (e.g. 5152 1f/4, RFLP S186A, RFLP Pat2, etc.).

Data analyses

We used the absolute frequency of each EMF species on the seedlings in each zone for the field and soil bioassay seedlings. For example, in the field seedling survey *Rhizopogon fuscorubens* A. H. Sm. was found on none of four seedlings in the mature forest site one, and one of six seedlings mature forest site two, two of six seedlings mature forest site three, and zero of three seedlings mature forest site four. The average absolute frequency for this fungus was then ((0/4) + (1/6) + (2/6) + (0/3))/4 = 12.5% for the mature forest seedlings.

We initially calculated the absolute frequency of seedlings with mycorrhizas in the deer fecal bioassays based on the three seedlings per dilution for each fecal sample. The average frequency of seedlings with mycorrhizal roots was 0, 0, 0, 0, 0.33, 0.857 and 0.857 when inoculated with 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 spores from fresh fecal samples, respectively. Similar results have been reported elsewhere using spore slurries made from sporocarps (Lamb & Richards, 1978; Castellano *et al.*, 1985). The data from the top three spore concentration treatments (i.e. those that yielded mycorrhizal seedlings) were analysed using a generalized linear model based on a binomial distribution and a logit link (PROC GENMOD in SAS version 8, SAS Institute, Cary, NC, USA). The model included variables representing the seven blocks (fecal samples). A statistically significant spore concentration effect was present (likelihood ratio test, 2 df, $P < 0.0001$), but this effect was clearly attributable to the difference between concentrations 10^6 and 10^7 and 10^6 and 10^8. Seedlings inoculated with 10^2–10^5 spore concentrations did not produce mycorrhizal seedlings and were therefore eliminated from further analyses. Concentrations of 10^7 and 10^8 spores yielded exactly the same proportion of successes over the seven blocks, so the test of whether a concentration of 10^7 differed from a concentration of 10^8 was clearly not significant. Because no significant differences were found between the top two spore concentrations, data from these spore concentrations were pooled to make a total of six seedlings for each fecal sample used in paired nonparametric *t*-tests.

We tested the null hypothesis that there was no difference in the frequency of mycorrhizal seedlings in the paired fresh and dry treatments using the Wilcoxon signed-rank test. The same test was used to test the null hypotheses that there was no difference between the frequency of seedlings colonized by hypogeous or epigeous fungi in the paired fresh and dry fecal treatments. These latter analyses considered only suilloid fungi (Bruns *et al.*, 1998).

We calculated the average Simpson's diversity on field seedlings collected from each zone across the four sites and report species richness for each deer fecal sample. To test for differences in diversity on field seedlings between zones we used a randomized complete block model ANOVA, using the average number of mycorrhizal species occurring on individual seedlings in each zone and the sample sites for blocks. We used Jackknife estimates (Burnham & Overton, 1979) to compare the expected species richness between zones or bioassay treatments. The estimates provided by the jackknife equations are not intended to indicate the true species richness. Rather, they provide a useful tool to compare zones and fecal pellets for their EMF species availability to seedlings. Burnham & Overton (1979) found a minimum mean square error was usually achieved in the first three orders of the jackknife estimator, and so these orders were used here. The most appropriate of the three orders for each part of the study was chosen by testing the null hypothesis that there is no difference between the expected values following Burnham & Overton (1979).

Results

There were 44 fungal RFLP types recovered from root tips in the field seedling survey, soil bioassay and fecal sample bioassay (Table 2, Table 3). Of these, we identified 17 to species or genus by matching root tip RFLP patterns with known sporocarp RFLP patterns (T. R. Horton, unpublished; multiple sclerotia in the case of *Cenococcum geophilum* Fr.). Sequencing the unmatched RFLP types allowed for the identification of four more types to species, one type to genus, two to family, one to order and one to class (Ascomycetes with affinity to ericoid associates). Eighteen RFLP types were not identified, all of which occurred at low frequencies limited to one study and zone. A *Scleroderma* RFLP type was observed on seedlings in the dry fecal pellet bioassay, occurring on one control seedling, and 4 inoculated seedlings. This RFLP type was not included in the diversity estimations.

Mycorrhizas on field seedlings

All field seedlings were colonized by at least one EM fungus. We observed 25 and 21 RFLP types on the seedlings harvested from the edge of the mature forest and deflation plain forest, respectively (Table 3). The mature forest and deflation plain forest seedlings averaged 2.2 ± 0.24 (mean ± 1 SE) and 2.39 ± 0.42 RFLP types per seedling, respectively. Many typical EMF were found on the root tips of the seedlings growing in the proximity of mature pines, including Basidiomycetes in Boletaceae, Cortinariaceae, Russulaceae, Thelephoraceae, Tricholomataceae and Ascomycetes such as *C. geophilum* and *Wilcoxina mikolae*. Many RFLP types in the forest zones occurred with an average frequency of less than 5%. The relatively high beta diversity of EMF in the forest zones contrasts with the 10 RFLP types found on seedlings from in the isolated areas. Seven of the 10 RFLP types were suilloid

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Table 2 Internal transcribed spacer (ITS)-restriction fragment length polymorphism (RFLP) patterns of the most common ectomycorrhizal fungi (EMF) recovered from root tips in at least two parts of the study

EM root tip sample	Sporocarp voucher	*Hin*fI	*Alu*I	*Dpn*II	Species
S17A	TRH552	327/223/148/141	413/203/105	346/239/154	*Rhizopogon fuscorubens*
F29	TRH551	334/222/164/143	438/285/112	344/254/156	*Rhizopogon occidentalis*
S200A	TRH542	244/231/142	445/276	257/245/164	*Rhizopogon salebrosus* group
DPS29.3A	TRH553	431/218/149	401/249	249/241/148	*Rhizopogon evadens*
S19B	–	226/191/128	594/106	239/169/148	*Suillus pseudobrevipes*
S28A	TRH663	224/192/131	597/108	239/151	*Suillus brevipes*
S66A	TRH559	233/222/147	600/110	238/150	*Suillus tomentosus*
S147A	TRH662	228/210/146	559/158	240/146	*Suillus umbonatus*
ASH17	–	227/131/85	585/105	232/160/141	Unknown *Suillus* sp.[a]
S24A	–	118/109/90	370/187	293/145	*Cenococcum geophilum*[b]
5084	TRH1288	380/310	400/130/100	400/130/125	*Laccaria laccata*[c]
S34A	–	313/258	n.d.[d]	295/194	*Phialocephela fortinii*
S15A	TRH940	341/229/96	563	376/225	*Thelephora americana*
S215A	TRH598	377/330	480/111	253/236/172	*Tricholoma ustale*
S18A	–	285/187/168	613	218/99	*Wilcoxina mikolae*

The primer pairs ITS1-F and ITS-4 were used for PCR amplification of the ITS region.
[a]Phylogenetic analysis of the ITS sequence from *Suillus* sp. Ash17 using a database for the genus published by Kretzer *et al*. (1996) suggests that this is a unique and possibly unidentified species.
[b]ITS-RFLP patterns from root tips samples matched those from several *C. geophilum* microscelortia collected at the field sties.
[c]A number of RFLP patterns were observed from *L. laccata* sporocarps and root tips; the most frequently observed pattern is shown.
[d]Not determined.

fungi belonging to the closely related genera *Suillus* and *Rhizopogon*. Seedlings from the isolated areas were colonized by an average of 1.39 ± 0.12 RFLP types per seedling.

Mycorrhizas on bioassay seedlings

A total of 78 seedlings survived in the soil bioassay and no contamination was detected on the control seedlings. The mature forest soil yielded 24 mycorrhizal seedlings and one nonmycorrhizal seedling. The deflation plain soil yielded 26 mycorrhizal seedlings. The isolated area soils yielded 22 mycorrhizal seedlings and five nonmycorrhizal seedlings. Eighteen RFLP types were found in soils collected from the two forests, nine of which were *Suillus* or *Rhizopogon* spp. (Table 3). Ten RFLP types were found in the isolated dune soils, six of which were *Suillus* or *Rhizopogon* spp. *Rhizopogon* spp. were more commonly observed in the forest soil bioassays than in the isolated area bioassay.

Ten RFLP types were detected on the fecal bioassay seedlings (Table 3). Eight RFLP types occurred on the fresh treatment seedlings, and seven RFLP types occurred on the dry treatment seedlings. Five RFLP types occurred in both the fresh and dry treatments. Seven of the 10 RFLP types were *Suillus* or *Rhizopogon* species (pooled data from fresh and dry treatments). Other EMF were rare types with a frequency of less than 5% and included *Thelephora americana* Lloyd, another member of the Thelephoraceae, and one unidentified species. The additional eight dry fecal samples yielded one extra species, *Inocybe lacera*. Letting the fecal pellets dry for 1 yr reduced the total number of mycorrhizal seedlings from 90% in the fresh treatment to 62% in the dry treatment. This difference was marginally significant (df = 6, $P = 0.09$). The decrease in mycorrhizal seedlings in the dry treatment paralleled a drop in the frequency of occurrence of the epigeous genus *Suillus* ($P < 0.05$, $n = 7$, Fig. 2). At the same time, the difference in performance of hypogeous genus *Rhizopogon* in the fresh and dry treatments was not significant ($P = 0.45$, $n = 7$).

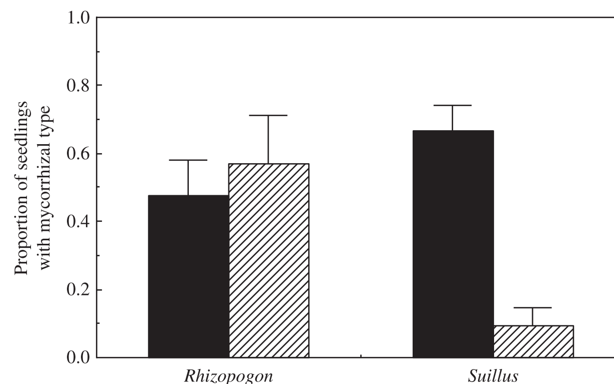

Fig. 2 Mean frequency of *Rhizopogon* spp. vs *Suillus* spp. recovered from paired fresh (closed bars) and dry (hatched bars) deer fecal bioassays. (Bars represent the mean ± 1SE, $n = 7$) *Rhizopogon* inoculant survived the drying treatment ($P = 0.45$, df = 6) while *Suillus* showed a marked decrease in mycorrhizal success upon drying ($P < 0.05$, df = 6).

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Table 3 Mean percentage of fungal types on seedlings harvested from the field, bioassays using soil as inoculum, and bioassays using deer fecal pellets as inoculum

	Field seedlings (*n* = 4)			Soil bioassay seedlings (*n* = 4)			Deer pellet bioassay seedlings (*n* = 7)	
	Forest	Deflation	Isolated	Forest	Deflation	Isolated	Fresh	Dry
Rhizopogon fuscorubens	13 (11)	33 (20)	5 (6)	30 (26)	47 (28)	8 (6)	26 (14)	36 (18)
R. occidentalis	25 (22)	11 (10)	29 (18)	18 (14)	14 (4)	0	24 (14)	24 (16)
R. salebrosus group	0	4 (5)	0	19 (13)	0	0	0	5 (4)
R. evadens	0	4 (5)	5 (6)	4 (4)	0	0	0	5 (5)
Suillus pseudobrevipes	4 (5)	0	0	10 (12)	4 (5)	7 (8)	0	0
S. brevipes	13 (14)	34 (26)	20 (18)	5 (6)	7 (4)	26 (19)	29 (16)	7 (6)
Suillus species	4 (5)	0	11 (9)	13 (14)	4 (5)	6 (7)	0	0
S. tomentosus	25 (22)	3 (4)	30 (21)	23 (17)	9 (7)	29 (17)	5 (5)	0
S. umbonatus	0	0	5 (6)	11 (10)	9 (7)	24 (15)	40 (20)	2 (3)
Boletus subtomentosus	4 (5)	0	0	0	0	0	0	0
Cenococcum geophilum	21 (17)	18 (18)	11 (9)	21 (17)	34 (23)	0	0	0
Cantharellales	0	11 (10)	0	0	0	0	0	0
Cortinarius sp.	8 (10)	4 (5)	0	0	0	0	0	0
Inocybe lacera	0	3 (4)	0	0	0	0	0	0
Leccinum manzanitae	0	3 (4)	0	0	0	0	0	0
Laccaria sp.	4 (5)	8 (7)	0	0	0	4 (4)	0	0
Lactarius rufus	0	15 (10)	0	0	5 (6)	0	0	0
Phialocephala fortinii	0	18 (13)	0	0	10 (12)	0	0	0
Russula pectinatoides	4 (5)	0	0	0	0	0	0	0
Thelephora americana	27 (19)	15 (10)	18 (12)	0	5 (6)	0	2 (3)	2 (3)
Tomentella sublilicina	0	0	0	19 (16)	0	0	7 (6)	0
Tricholoma ustale	6 (7)	13 (12)	0	21 (25)	0	0	0	0
Thelephoraceae D25	4 (5)	22 (15)	0	4 (4)	0	0	0	0
Thelephoraceae T73.1	0	0	0	0	0	0	0	2 (3)
Wilcoxina mikolae	6 (7)	5 (6)	0	27 (25)	54 (30)	4 (5)	0	0
Ericoid root endophyte 5141	0	5 (6)	5 (5)	0	0	0	0	0
5019 1f/4	0	3 (4)	0	0	0	0	0	0
5023 1f/4b	0	3 (4)	0	0	0	0	0	0
5050 1f/4b	8 (10)	0	0	0	0	0	0	0
5058 1f/4b	8 (10)	0	0	0	0	0	0	0
5059 1f/4b	8 (10)	0	0	0	0	0	0	0
5115 1f/4b	4 (5)	0	0	0	0	0	0	0
5116 1f/4	8 (7)	0	0	0	0	0	0	0
5120 1f/4b	4 (5)	0	0	0	0	0	0	0
5121 1f/4b	4 (5)	0	0	0	0	0	0	0
5124 1f/4	4 (5)	0	0	0	0	0	0	0
5152 1f/4	4 (5)	0	0	0	0	0	0	0
5202 1f/4	4 (5)	0	0	0	0	0	0	0
RFLP2	0	0	0	14 (16)	0	0	0	0
RFLP7	0	0	0	0	5 (6)	0	0	0
RFLP8	0	0	0	0	5 (6)	0	0	0
RFLP S186A	0	0	0	0	4 (4)	0	0	0
RFLP S212	0	0	0	0	0	4 (4)	0	0
RFLP Pat2	0	0	0	0	0	0	5 (4)	0

Data are shown as mean (± 1 SE) percentage of seedlings colonized by the fungi for each habitat type (field and soil bioassay) or fecal pellet treatment. Forest, mature forest edge; deflation, deflation plain forest edge; isolated, isolated area; fresh, fresh fecal pellets; dry, fecal pellets dried for 1 yr.

Diversity estimates

Although isolated seedlings tended to have a lower diversity of fungi on them compared with seedlings from the two forest zones, there were no significant differences between blocks ($F = 0.38$, $P = 0.77$) or zones ($F = 1.47$, $P = 0.30$). Simpson's diversity on seedlings inoculated with deer fecal pellets was similar to the diversity on field seedlings. According to Jackknife estimates, at least 30 RFLP types were available to field seedlings in the proximity of mature and deflation plain forests (Table 4). By contrast, the expected pool of EMF fungi available to the isolated dune seedlings was below 15 RFLP types

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Table 4 Simpson's and Jackknife estimates of diversity

Field seedlings (n = 4)	Simpson's diversity per seedling Mean (SE)	Jackknife order	Observed # of species	Expected # of species
Mature forest	1.9 (0.34)	$k = 2$	25	45
Deflation forest	2.1 (0.33)	$k = 2$	21	34
Isolated dunes	1.1 (0.12)	$k = 1$	10	13
Field soil bioassay seedlings (n = 4)				
Mature forest	nd	$k = 1$	15	20
Deflation forest	nd	$k = 2$	15	24
Isolated dunes	nd	$k = 1$	10	14
Fecal pellet bioassay seedlings (n = 7)	Species richness per fecal sample Mean (SE)			
Fresh treatment only	2.2 (0.19)	$k = 1$	8	11
Dry treatment only	1.5 (0.29)	$k = 1$	7	10
Fresh + Dry	nd	$k = 1$	11	14
Dry only with extra fecal pellets (n = 13)	1.6 (0.15)	$k = 1$	8	9

The jackknife equations and order used was determined by following Burnham & Overton (1979). nd, not determined.

based on the field or soil bioassay data. Similarly, the expected number of RFLP types available to seedlings inoculated with fecal pellets was also below 15.

Discussion

The number of RFLP types observed in the mature edge and deflation plain forests (pooled seedling data) is typical for conifer forests (Horton & Bruns, 2001; Taylor, 2002; Cline *et al.*, 2005). The seedlings collected from the forest zones averaged about two RFLP types per seedling compared with one on the isolated area seedlings. While suilloid fungi dominated the seedlings collected from the forest zones, many infrequently encountered RFLP types also colonized these seedlings. Field seedlings growing in the two forested zones have more EMF species available to them compared with seedlings in the isolated areas because (1) seedlings can interact with existing mycelial networks, (2) more fungi fruit and produce a localized spore rain and (3) habitat conditions are more conducive for vegetative growth when mature trees are present. Whether spores or hyphal interactions act as primary inoculum for uncolonized root tips in forest settings remains unclear. Some EMF appear to establish new individuals annually through spore inoculum (Redecker *et al.*, 2001). However, many EMF form below-ground mycelial networks (Newman, 1988; Simard *et al.*, 1997; Agerer, 2001) that likely involve perennial thalli, and these networks are thought to be an important source of mycorrhizal inoculation in established ectomycorrhizal communities (Horton *et al.*, 1999; Horton *et al.*, 2005).

The isolated seedlings on open dunes had fewer RFLP types available to them than those growing in proximity to the forests. Seven of the 10 RFLP types observed on seedlings from the isolated areas were suilloid fungi. The hundreds of meters between the isolated seedlings on the dunes and the forest zones prevented seedling access to the mycelial networks and local spore dispersal. Further, the exposed conditions in the isolated areas were likely inhospitable for many EMF, even if they did become established with a seedling. These results suggest that suilloid fungi are uniquely adapted for long distance dispersal to, and survival in, the isolated areas.

Spore dispersal

Dispersal of spores by wind produces a scattered and diffuse spore rain over long distances (Allen *et al.*, 1992). For example, Allen (1987) reported an average of one *Thelephora* spore was captured in unvegetated areas of the Mt St Helens blast zone per 24 trap hours. Even with a diffuse spore rain, viable spores can accumulate as a resistant spore bank. Despite over 100 epigeous (and presumably wind dispersed) species fruiting in the forest edges, most were not frequently encountered on field or bioassay seedlings. We did not quantify sporocarp production in our sampling sites. However, observational evidence suggests that species of *Suillus* and *Rhizopogon* were among the abundant fruiters in the forest zones, particularly *S. brevipes*, *S. umbonatus*, *R. occidentalis*, *R. evadens* and *R. fuscorubens.* The jackknife estimates suggest that our root tip sampling was fairly complete in the isolated areas for species richness. Epigeous basidiomycetes such as those found in the isolated areas on seedlings or in soils (*Suillus*, *Thelephora* and *Laccaria*) are thought to be wind dispersed, and were not observed fruiting in the isolated areas during the study. However, the presence of *Rhizopogon* in the isolated areas points to spore dispersal via animal vectors playing an important role in moving spores to the isolated areas in this case.

Rhizopogon spp. are truffle-like fungi whose sporocarps are produced below ground or erumpent at the soil surface and have lost the ability to propel their spores into the air. While

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many sporocarps may decay, leaving their spores below ground (Miller *et al.*, 1994), long-distance dispersal is thought to be primarily via mammals (Luoma *et al.*, 2003). Previous work on mycophagy and EMF has focused almost exclusively on hypogeous fungi and small mammals (Luoma *et al.*, 2003). Hypogeous fungi produce odors that attract rodents and marsupials that dig up the sporocarps, consume them and disperse their spores when they defecate (Thiers, 1984; Johnson, 1996; Luoma *et al.*, 2003). Spores in feces are viable and have been shown to successfully yield mycorrhizas on seedlings (Kotter & Farentinos, 1984; Claridge *et al.*, 1992; Colgan & Claridge, 2002). Small mammals on the Oregon dunes ecosystem, particularly the Townsend chipmunk (*Tamias townsendii*) and the deer mouse (*Peromyscus maniculatus*), preferentially eat *Rhizopogon* sporocarps (Ashkannejhad, 2003). However, the distance out to the isolated seedling areas reaches up to 600 m from the established forest and is sparsely vegetated. Limited cover from predators and a lack of food in these areas makes dispersal across them risky for small mammals (T. Manning, pers. comm.). To investigate mammal dispersal from the forests to the isolated areas, we focused on deer.

Deer and elk are known to eat epigeous and hypogeous fungi (Launchbaugh & Urness, 1992), and many epigeous fungi produce odors that likely attract mammals. Mycophagous mule deer and mountain goats were observed traversing primary successional glacial forefronts where small mammals did not venture (Cázares & Trappe, 1994; Jumpponen *et al.*, 1999), and elk were presumed to carry arbuscular mycorrhizal inoculum across the Mt St Helens blast zone (Allen, 1987). In our study, the same fungal species being dispersed by deer were found on the isolated seedlings. To our knowledge, this is the first demonstration that deer disperse viable spores of epigeous, wind-dispersed fungi. Our soil bioassays suggest that suilloid spores are abundant in soils, as others have also shown (Baar *et al.*, 1999; Taylor & Bruns, 1999; Kjøller & Bruns, 2003). Here we provide evidence that spores can survive desiccation for at least 1 year, and that *Rhizopogon* survives desiccation better than *Suillus*. While our results suggest the spores can survive as a resistant spore bank in soils, the deer pellets would likely be subjected to repeated wetting and drying on the dunes, eventually disintegrating into the soil matrix. We do not know if spores are capable of withstanding such conditions, but Terwilliger and Pastor (1999) report that 8 wk of flooding did not completely eliminate the ectomycorrhizal potential of forest soils.

Suilloid fungi (*Rhizopogon* and *Suillus* spp.) were the principle EMF on pines establishing where mycelial networks were absent. This suggests that EMF are not functionally redundant and that suilloid fungi provide an excellent example of a group with specific ecological adaptations for establishment of pines in harsh or early successional habitats. Whether other species are absent on the isolated seedlings because they are not dispersing to the area or are incapable of surviving the harsh conditions requires further study.

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