Ectomycorrhizal fungi above and below ground in a small, isolated aspen stand: A simple system reveals fungal fruiting strategies and an edge effect

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Cripps, C. L. (Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717, U.S.A.). Ectomycorrhizal fungi above and below ground in a small, isolated aspen stand: A simple system reveals fungal fruiting strategies and an edge effect. Memoirs of The New York Botanical Garden 89: 249–265, 2004.—Ectomycorrhizal fungi are an important aspect of forest dynamics; however, it is not trivial to analyze mycorrhizal communities *in situ*, particularly in quantitative terms. Above-ground sporocarps and below-ground ectomycorrhizae often give contrasting pictures of mycorrhizal communities. This study examined a *whole*, simple ectomycorrhizal community with limited plant-fungal diversity (a small, isolated aspen stand) to assess quantitative measures. This contrasts with most studies where a *fragment* of a complex mycorrhizal forest system is selected. Eight species of ectomycorrhizal fungi were detected, half represented by sporocarps. *Inocybe lacera, Paxillus vernalis, Laccaria proxima*, and *Tricholoma scalpturatum* dominated in importance (frequency plus abundance), and comprised 94% of ectomycorrhizae. *Cenococcum geophilum, Populorhiza nigra, Hebeloma mesophaeum*, and *Hebeloma* sp. were detected only below ground. Fruiting strategies varied. *Inocybe lacera* and *L. proxima* fruited prolifically each year only at the edge of the stand, while their mycorrhizae existed throughout the stand. Spore production at the edge of a forest could be advantageous for these early colonizers. In contrast, *Paxillus vernalis* produced only 1 sporocarp in 4 years, yet mycorrhizae were distributed throughout the stand. Co-occurrence of mycorrhizal types in the soil showed 2–5 types in close proximity. Sporocarp locales rarely overlapped, and are mapped for each species. Edge effect and fruiting strategies could be particularly relevant in certain ectomycorrhizal systems and for selection of study sites.

KEY WORDS: aspen, fungi, ectomycorrhizae, *Populus tremuloides*, sporocarps, community ecology

Introduction

The importance of ectomycorrhizae in forest systems has been increasingly recognized over the last century. Historically studied as individual mutualisms between a host tree and a fungus, mycorrhizae are now perceived in more encompassing terms as the associations between microscopic fungi and plant roots on which many forest ecosystems are predicated. A diversity of ectomycorrhizal fungi thrive in coniferous and deciduous forests and in arctic-alpine tundra with woody shrubs, and more are being discovered in tropical ecosystems (Smith & Read, 1997). Ectomycorrhizal symbiosis occurs wherever potential host plants exist, or, from another point of view, trees are able to exist in many habitats because of partnerships with ectomycorrhizal fungi.

While particular species of mycorrhizal fungi can occur across many forest types, it is becoming evident that particular assemblages (or communities) of mycorrhizal fungi are characteristic of each forest type. Sporocarps are often used to detect diversity and infer abundance of macrofungi (including mycorrhizal species) in forest systems. Studies of mycorrhizal diversity relying on sporocarps in western forests include those for Douglas fir (Norvell & Exeter, 2004; O'Dell et al., 1999; Smith et al., 2002), pine (Gehring et al., 1998), aspen (Cripps, 2001; Cripps & Miller, 1993), and timberline forests (Kernaghan & Currah, 1998; Kernaghan & Harper,

Fungi in Forest Ecosystems: Systematics, Diversity, and Ecology, Edited by Cathy L. Cripps © 2004 THE NEW YORK BOTANICAL GARDEN

2001). It is important to understand mycorrhizal communities because of their influence on forest dynamics; however, it is not trivial to analyze these communities *in situ*. Easily accessed epigeous sporocarps are not necessarily indicative of unseen fungal processes below ground.

In a previous study, more than 50 species of ectomycorrhizal fungi were recorded in aspen forests in the northern Rocky Mountain region (Cripps, 1997a, 2001; Cripps & Miller, 1993). One-third of the fungal species occurred on three very different soil types with aspen, although frequency varied among sites which appeared related to microhabitat and/or tree age. Another third were restricted to smelter-impacted sites with acidic, sandy soils (Cripps, 2003). Like some other studies of ectomycorrhizal communities, sporocarps were used to infer fungal diversity, abundance, and distribution of mycorrhizal fungi in the soil. Differences in the composition of the fungal communities appeared to be real in our study. However, it was not clear how sporocarp parameters related to measures of the fungal organism as a whole. Were the dominant fungi above ground also the most important species in below-ground processes? What proportion of the mycorrhizal community was even represented above ground?

A study of the annual fungal standing crop in Douglas fir forests in western Oregon showed that sporocarps comprised only 0.3% of the biomass, while 50% was attributed to mycorrhizae, 33% to mycelium, and 14% to sclerotia (Fogel & Hunt, 1979). Ectomycorrhizal mycelium can contribute a third of the microbial biomass in the soil (Högberg & Högberg, 2002). The unreliability of sporocarps as a quantitative representation of the fungal organism might be attributed to their comparatively small biomass, ephemeral and seasonal nature, dependency on precipitation, and annual variation (Straatsma et al., 2001). Mycorrhizae could be a more reliable measure of the abundance and distribution of mutualistic fungi in forest systems.

Before the 1990s, mycorrhizae were recognized generically as mycelium wrapped around roots, or as vague "morphotypes," described primarily in physiological studies. Advances in methodology have made it possible to recognize individual species of ectomycorrhizal fungi on the roots of higher plants, using both morphological and molecular techniques. Agerer (1987–1999) and others have used the structural details of mantle, rhizomorphs, and cellular layers in mycorrhizae to distinguish genera and species of fungi on roots. The problem for ecologists is that it is still difficult to identify fungal mutualists in forest soils by root morphology alone, since there is no basis of comparison for many types, and thousands of possible mycobionts exist.

Molecular methods have allowed recognition of fungal species on roots through ITS-RFLP analysis and subsequent comparison to results from sporocarps or a reference library (Gardes & Bruns, 1993, 1996b; Gardes et al., 1991). Newer methods, using the ITS region and phylogenetic analysis, can place an ectomycorrhizal fungus with its nearest relatives or identify it to species (Bruns et al., 1998).

Various combinations of these techniques have been used in more recent ecological studies to examine mycorrhizal communities both above and below ground. To date, only rather complex mycorrhizal communities, comprising *a fragment* of a larger forest, have been examined (Dahlberg, 2001; Dahlberg et al., 1997; Dahlberg & Stenlid, 1995; Danielson, 1984; Gardes & Bruns, 1996a; Grogan et al., 2000; Horton & Bruns, 1998; Luoma & Eberhart, 1997; Mathiasen & Albion, 2001; Mehmann et al., 1995; Peter et al., 2001; Taylor & Bruns, 1999; Wurzburger et al., 2001; Yamada & Katsuya, 2001). In most studies, there was not a one-to-one correspondence of fungal species above and below ground, and only a portion of the fungal diversity was typically represented above the soil surface. In addition, molecular analysis has revealed that one morphotype can include several species, and conversely, several morphotypes can represent one species (Mehmann et al., 1995; Taylor & Bruns, 1999). Quantitatively, there appears to be a low correlation between abundance of sporocarps and their respective mycorrhizae. This suggests that the relationship between sporocarps and mycorrhizae is complex, and not directly proportional, at least not in quantitative terms.

The purpose of the present research was to examine a simple but *whole* mycorrhizal community in detail, to get a "real feel" for the abundance and distribution of the specialized organs of mycorrhizal fungi, i.e., the sporocarps and mycorrhizae. A small discrete system was selected in the form of an isolated aspen stand (*Populus tremuloides*) surrounded by open fields of non-ectomycorrhizal plants located in southwestern Montana. The aspen stand was purposely chosen for its small size, isolated nature, and limited diversity of ectomycorrhizal fungi (as initially indicated by sporocarps). A second mission was to identify factors such as edge effect and individual fungal life strategies, which might be important considerations in unraveling more complex mycorrhizal communities, particularly as related to plot selection and sampling procedures in a forest ecosystem.

Additionally, the system is unique in that aspen reproduces primarily by root suckering, and the study area

was colonized by aspen after airborne pollution from copper smelters acidified the soil and killed most of the previous vegetation (Cripps, 1996, 2003). Aspen clones are common near smelters in the region, and this study also allows us to examine how these areas recover from this kind of disturbance, the specific mycorrhizal fungi involved, and the environmental factors relating to successful tree establishment. Aspen is a significant forest type in the western United States and its decline of 40% or more in recent years is of high concern (Shepperd et al., 2001).

Methods

Study Site

The study area is located east of Butte, Montana, in Silverbow County (tp. 3N, rge. 7W), at an elevation of 1800 m, in the foothills of the Rocky Mountains, just west of the Continental Divide. Yearly normal precipitation is 31 cm, with nearly 40% falling in May and June. The mean annual temperature is 3.8°C. Winters are cold with record lows to –46°C, and summers are mild with rare highs above 35°C.

The soil is 70–84% sand with elevated levels of copper, iron, and other heavy metals. Outcroppings of granitic parent material jut from the sandy soil in many areas. Little or no organic layer is present on the soil surface, but organic matter is distributed with depth, probably due to the colluvial nature of the soil and adjacent rugged terrain. The soil is dystrophic, with base saturations of 45% at the surface to 11% at 20 cm. The pH ranges from 5.3 at the surface to 4.3 at 15 cm and 5.7 at 97 cm. This suggests the acidity does not come from the parent material but, rather, from previous heavy smelter emissions in the area found in a buried A horizon. Additional soil information is available in Cripps (2001).

Slopes in the area were devoid of aspen 70 years ago, as reported by the land owner, due to smelter activity. Today, aspen are colonizing the area as a pioneer species, as is common in other smelter sites in the region. A small discrete aspen stand was selected for study because of its isolation from other ectomycorrhizal systems (Fig. 1). It is surrounded by open slopes, sparsely vegetated by *Centaurea* sp., *Artemesia tridentata* Nutt., and *Chrysothamnus nauseosus* (Pall.) Britton, which are not ectomycorrhizal and indicate the xeric nature of the area. A 20 × 11 m plot, covering a majority of the canopy area, was laid out within the aspen stand. Aspen is clonal, and the stand could well represent one individual with numerous ramets springing from a single root system. Aspen ramets over 2.5 cm in diameter were plotted to provide a sense of their spatial arrangement. Larger ramets averaged 37 years of age, the oldest being 46 years.

Sporocarp Sampling

Sporocarps of ectomycorrhizal fungi were collected within the aspen stand every 10 days throughout the 1990, 1991, 1992, and 1993 field seasons, and casually for an additional 3 years, and locations recorded. The fungi were identified and reference specimens are in the Massey Herbarium, Virginia Tech (VPI) and the Montana State University Herbarium (MONT). Three categories were used to describe the abundance of sporocarps of each ectomycorrhizal species: over 30 sporocarps per year (+++), 2–10 sporocarps per year (++), and 1

FIG. 1. The study site is a small aspen stand representing a discrete ectomycorrhizal community surrounded by arbuscular-mycorrhizal plants in southwest Montana.

sporocarp per year (+). These categories were consistent for each species over the four-year study period. One-centimeter cubes of tissue were removed from representative sporocarps for ITS-RFLP analysis. Cubes were dried, placed in vials, and mailed to the Bruns Lab at the University of California at Berkeley for analysis.

SAMPLING AND IDENTIFICATION OF ECTOMYCORRHIZAE ON ROOTS

Ectomycorrhizae were sampled in the spring of 1993 in early July. Sampling was done to coincide with the high precipitation period, and before expected summer droughts. Mycorrhizal numbers have been shown to peak in spring in Montana soils (Harvey et al., 1978). Root samples were taken every 2 m along a central lengthwise transect of the plot, and at a distance to the left or right as specified by a random number table. This quadrat method was used to ensure a sample was taken from each of 10 subplots, a common technique for vegetation sampling (Cox, 1967).

Preliminary sampling revealed that the top 16 cm of soil was virtually devoid of ectomycorrhizae. This was unexpected since ectomycorrhizae are commonly restricted to the top few centimeters of soil (Fogel, 1980). Further sampling revealed ectomycorrhizae deeper in the soil, from 16 to 48 cm. Ten soil cores were taken with a 6.5 × 16 cm soil auger to a depth of 48 cm. Each core was divided into three lengths and sections were placed in separate Ziploc bags for a total of 30 samples. Samples were refrigerated at 5°C and processed within 3 weeks.

Each sample was added to water in a ridged plastic pan, normally used for panning gold. Roots and organic matter were separated from the heavier soil by the typical panning method of swirling and pouring off lighter material which contained the roots. This method worked well for sandy soil, and periodic observation showed that no roots remained in the discarded portion. Roots were gently washed on a 2 mm mesh screen and rinsed a second time over a finer-meshed screen to remove adhering fine silt and clay, and examined with a dissecting scope (Harvey et al., 1979; Vogt et al., 1981).

All ectomycorrhizae were counted in each sample (no subsampling) and sorted by types. Only those which were turgid with fresh-looking mantles were counted (Harvey et al., 1976). Dead, shriveled, hard ectomycorrhizae were not counted. Spring sampling helped keep this distinction clear, as previous years' dead mycorrhizae contrasted with the new mycorrhizae forming with renewed root growth. Mycorrhizae were removed with fine forceps, and sorted into distinct types, which were periodically checked microscopically. Mycorrhizae that appeared to consist of more than one fungus were put in the miscellaneous category.

For morphotype identification, complete descriptions were made with fresh ectomycorrhizae (Agerer, 1987–1999). Selected mycorrhizae were dehydrated, embedded in paraffin, sectioned with a microtome, and stained with safranin O and fast green. The complete method is described in Cripps and Miller (1995).

Ectomycorrhizal types were identified by morphological comparison with those previously synthesized *in vitro* using isolates of known fungi from local aspen stands and aspen seedlings (Cripps, 1997b, unpubl. data; Cripps & Miller, 1995). Additional evidence for identification of morphotypes were the mycelial connections to sporocarps in areas depauperate of other mycorrhizal types in the study area. Identification was further supported by ITS-RFLP analysis performed by T. Bruns and M. Gardes (pers. comm).

ANALYSIS OF SPECIES RICHNESS

The cumulative number of species of ectomycorrhizal fungi fruiting above ground over a four-year period was used to represent species richness above ground. It has been shown that the number of fungal species accumulated over several seasons correlates better with number of mycorrhizal types than an annual count (Jansen, 1991).

Species richness below ground was the total number of ectomycorrhizal types in 30 soil samples. Since species richness depends on the number of samples taken, the cumulative number of mycorrhizal types which accrues as more samples are taken was plotted against sample size to assess if a majority of the dominant mycorrhizal types were discovered. This is similar to the species increment curves used to determine a sufficient sample size for examining arbuscular mycorrhizal species on a specific site (Tews & Koske, 1986). For the present study, *all* possible permutations of samples were calculated, averaged, and plotted to give an estimate of the number of mycorrhizal types which are expected in a certain number of random samples (Taylor, 2002).

DENSITY OF ECTOMYCORRHIZAL ROOT TIPS IN THE SOIL

The density of mycorrhizal root tips in the aspen stand was determined by averaging the number of mycorrhizae in each sample and adjusting to a standard volume of soil (100 cc). The equation employed by Tews and Koske (1986) for AM fungi was used to assess if our

sample size was sufficient to determine the overall density of mycorrhizal types in the soil: $N = [t^2 (CV)^2]/E^2$, where N = sample size, t = 1.96 (95% is acceptable), E = specified accuracy (0.1 for a standard error within 10% of mean), and CV = coefficient of variation ($S_x/\bar{X}$). This equation has also been used by Alexander (1985) to examine the effect of different-sized soil cores for mycorrhizal determination. Data were not sufficient to determine density of *individual* types with accuracy, but trends are presented.

Abundance and Frequency of Mycorrhizal Types

The term 'abundance' was used in lieu of 'density' as suggested for clonal organisms which cannot be delineated as individuals (Pielou, 1974). The abundance of a mycorrhizal type in the soil was considered to be the average number of root tips of that type per 100 cc of soil. Tews and Koske's (1986) formula was used to assess how many samples would be needed to accurately determine species abundance of mycorrhizal fungi in a small aspen stand for this study. The utility of this method for larger, more complex mycorrhizal communities can then be inferred.

The frequency of each mycorrhizal type is defined as follows: percent frequency = [number of samples containing species *x*/total number of samples] × 100. The frequency of fungi fruiting in subplots has been used as a measure of fungal dominance (Bills et al., 1986) and is employed similarly here for mycorrhizal roots, equating a core sample with a subplot. In large-scale mycorrhizal studies, where sample sizes are large, it may be more feasible to determine frequency of a fungal species (presence/absence in a soil core) rather than abundance of ectomycorrhizal types (counting individual root tips) (Alexander, 1985).

Spatial Distribution of Ectomycorrhizal Fungi above Ground and in the Soil

Spatial distributions of a fungal species above ground (sporocarps) and in the soil (mycorrhizae) were compared by delineating both on a plot layout diagram. Examination of an entire, clearly delineated ectomycorrhizal system (a discrete aspen stand) allows us to assess the importance of plot location within larger mycorrhizal communities. For example, if edge effect, microclimate, and/or successional factors influence sporocarp distribution (fruiting strategies), the choice of plot location within a larger forest system could affect results of above- and below-ground comparisons.

Vertical Distribution of Ectomycorrhizal Fungi in the Soil

Each soil core was divided into three parts: 0–16 cm, 16–2 cm, and 32–48 cm. Friedman's test (Hollander & Wolfe, 1973) was used to determine if significant differences existed in species richness and abundance of mycorrhizal root tips per 100 cc of soil among the three depths sampled. This nonparametric test is for data with large variations among samples which are filtered out by ranking samples before comparison. Sample sizes were insufficient to compare the abundance and frequency distributions of *individual* species vertically, but a few possible trends are discussed.

Results

Sporocarp Diversity (Species Richness) above Ground

The species richness of ectomycorrhizal fungi fruiting above ground was low as expected in the small aspen stand. Only four species of ectomycorrhizal fungi produced epigeous sporocarps over four years within the plot (Table 1). *Inocybe lacera* (Fr.:Fr.) Kummer var. *lacera* and *Laccaria proxima* (Boud.) Pat. fruited annually and prolifically each year (Fig. 2, Table 1). Thirty to well over 100 sporocarps of each of the two species were estimated on the plot annually. Both species fruited primarily in spring but also fruited after rain into September. Only one fruiting body of *Paxillus vernalis* Watling was collected during the four-year study. Sporocarps of *Paxillus* are remarkably durable and are unlikely to be overlooked. *Tricholoma scalpturatum* (Fr.) Quél. did not fruit until the fourth year of the study and produced two small clusters of sporocarps. These records likely reflect maximum sporocarp production for the aspen

TABLE I
ABUNDANCE OF EPIGEOUS ECTOMYCORRHIZAL SPOROCARPS OBSERVED IN A SMALL ISOLATED ASPEN STAND IN SOUTHWESTERN MONTANA[a]

Mycorrhizal species	1990	1991	1992	1993
Inocybe lacera (Fr:Fr) Kummer v. *lacera*	***	***	***	***
Paxillus vernalis Watling	–	–	*	–
Tricholoma scalpturatum (Fr.) Quél.	–	–	–	**
Laccaria proxima (Boud.) Pat.	***	***	***	***

[a] ***, 30–100 sporocarps; **, 2–10 sporocarps; *, 1 sporocarp; –, sporocarps absent.

FIG. 2. Top left. *Laccaria proxima*. Top right. *Inocybe lacera*. Bottom left. *Paxillus vernalis*. Bottom right. *Tricholoma scalpturatum*.

stand, since above-normal precipitation in 1992 and 1993 contributed to unusually high sporocarp production in Montana and larger aspen stands near the study area supported a higher diversity of fungi than normal. No additional species of ectomycorrhizal fungi were noted fruiting in the stand in three subsequent years of casual observation.

MYCORRHIZAL DIVERSITY (SPECIES RICHNESS) BELOW GROUND

Eight distinct ectomycorrhizal morphotypes were distinguished in the soil of the aspen stand (Table 2). Four of the ectomycorrhizal types were correlated with species that fruited on the plot: Type I = *Inocybe lacera*, Type II = *Paxillus vernalis*, Type III = *Tricholoma scalpturatum*, and Type IV = *Laccaria proxima*. The ectomycorrhizal types were morphologically identical to those produced *in vitro* by isolates of these species from the study site and aspen seedlings (Cripps, 1997b; Cripps & Miller, 1995). In addition, all four ectomycorrhizal types were found associated repeatedly with corresponding sporocarps in areas depauperate of other ectomycorrhizal fungi. A preliminary ITS-RFLP analysis by T. Bruns and M. Gardes at University of California, Berkeley supported identifications made by morphological methods. RFLP analysis (with Hin F and Ala I) matched *Inocybe lacera, Paxillus vernalis*, *Tricholoma scalpturatum*, and *Laccaria proxima* mycorrhizae to sporocarps. Type V (white) turned out to be a color variant of Type II (*Paxillus vernalis)*, and data were pooled. Young mycorrhizae of *Paxillus vernalis* are white, and become mottled brown, eventually maturing a brown color. This is similar to *P. vernalis* sporocarps which are initially white, and eventually turn brown.

The four additional mycorrhizal types were not correlated with sporocarps from the plot. Type VI was a "*Cenococcum*-type" of mycorrhiza; however, the typical stellate mantle pattern was not always fully formed. Root tips were not counted individually since mycorrhizae were often small and partially formed, making quantification difficult. Type VII was designated *Populariza nigra* after Agerer's (1987–1999) terminology for unidentified ectomycorrhizae. The rare clamp connec-

TABLE II

EVIDENCE SUPPORTING THE IDENTIFICATION OF MYCORRHIZAL MORPHOTYPES IN THE SOIL OF A SMALL ASPEN STAND. SAMPLED MYCORRHIZAE WERE COMPARED WITH: 1) MYCORRHIZAE FORMED *IN VITRO* WITH ISOLATES FROM KNOWN SPOROCARPS AND ASPEN, 2) MORPHOLOGY OF MYCORRHIZAE IN PUBLISHED DESCRIPTIONS, 3) SPOROCARP RFLP PATTERNS FROM PLOT, 4) NATURALLY OCCURRING MYCORRHIZAE BENEATH SPOROCARPS ON OR NEAR THE PLOT.

Mycorrhizal morphotype = species	1) *In vitro* synthesis	2) Morphology		3) RFLP analysis	4) Natural mycorrhizae
		Genus	Species		
Type I = *Inocybe lacera*	+	+	+	+	+
Type II = *Paxillus vernalis*	+	+	+	+	+
Type III = *Tricholoma scalpturatum*	+	+	–	+	+
Type IV = *Laccaria proxima*	–	+	–	+	+
Type VI = *Cenococcum*-type	+	+	+	–	–
Type VII = *Popularhiza nigra*	–	–	–	–	–
Type VIII = *Hebeloma mesophaeum*	–	+	+	–	+
Type VIII = *Hebeloma* sp.	–	+	–	–	+

tions attest it is a basidiomycete. It is robust, smooth, deep brownish black, and somewhat similar to Type ITE.5 on birch (Ingelby et al., 1990).

Hebeloma mesophaeum (Fr.) Quél. and an unidentified species of *Hebeloma* fruited near, but not on, the plot. Type VIII matched mycorrhizae found beneath *H. mesophaeum* sporocarps off the plot and matches descriptions of *H. mesophaeum* mycorrhizae on birch (Ingleby et al., 1990), except the aspen variety is more yellow. Mycorrhizae matching type IX were observed in conjunction with sporocarps of an unidentified *Hebeloma* species that fruited just south of the plot. The morphology of Type IX is typical for *a Hebeloma,* with a thin, white mantle and long silky rhizomorphs (Agerer, 1987–1999). These two types were not subjected to RFLP analysis. Photographs of mycorrhizal types and complete descriptions are in Cripps and Miller (1995).

In addition, detailed morphological descriptions of these ectomycorrhizal fungi matched published descriptions: *Inocybe lacera* with pine (Chu-chou & Grace, 1983) and birch (Ingleby et al., 1990), *Paxillus involutus* with aspen (Godbout & Fortin, 1985) and alder (Miller et al., 1991), *Laccaria* and aspen (Godbout & Fortin, 1985), *Cenococcum geophilum* (Ingleby et al., 1990), and *Hebeloma mesophaeum* (Ingelby et al., 1990).

CUMULATIVE-SPECIES/SAMPLE CURVE

The cumulative-species/sample curve levels off, suggesting that sample size was sufficient to determine a majority of ectomycorrhizal fungi in the soil of the aspen stand (Fig. 3). This leveling is expected for ecosystems of low diversity (Pielou, 1977; Taylor, 2002; Tews & Koske, 1986).

OVERALL ABUNDANCE (DENSITY) OF MYCORRHIZAE IN THE ASPEN STAND

Overall abundance (density) of mycorrhizal root tips in the small aspen stand was 72 ± 54 tips/100 cc of soil, with a range of 9–190 tips/100 cc. Ten samples were sufficient to estimate the number of mycorrhizae per 100 cc of soil within 46% of the mean, and 216 samples would be required to estimate within the accepted confidence level of 10% with same size cores. Samples from mature aspen stands in the region (with silty soil) ranged from 570 root tips/100 cc if sampled to 48 cm, to a high of 1215/100 cc if sampled only in the top 16 cm where mycorrhizae were concentrated. Conversely, a low number of mycorrhizae were found in the aspen plot after the 1994 drought, and averaged less than 7 mycorrhizae per 100 cc of soil.

The total number of root tips in the soil of the aspen plot (20 m × 11 m × 48 cm deep) was calculated to be 76 ± 56 million, or between 19 and 133 million. This is likely an underestimate, and underscores the logistical and statistical problems inherent in mycorrhizal studies.

ABUNDANCE (DENSITY) AND FREQUENCY OF MYCORRHIZAL TYPES IN THE SOIL

The overall abundance (density) and frequency of individual mycorrhizal types is shown in Table 3, and graphically in Figure 4. One-third of the mycorrhizae were of *Inocybe lacera,* one-fourth *Laccaria proxima,* one-fourth *Paxillus vernalis,* with the remaining 20% split between the less common species (*Cenococcum* not included). In terms of frequency: *Inocybe lacera* mycorrhizae were found in 90% of the samples, *Paxillus ver-*

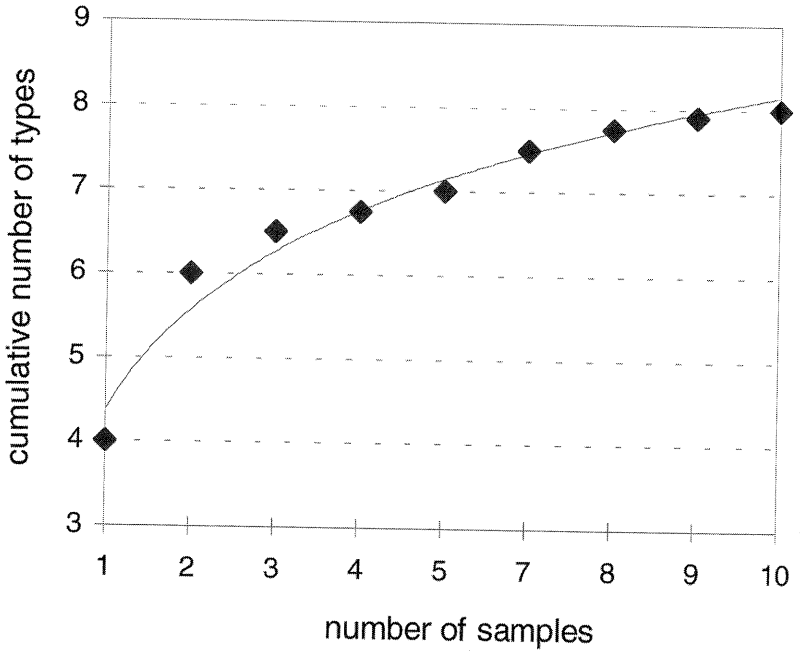


FIG. 3. Average number of morphotypes in a given number of soil samples. The curve levels off, indicating that a majority of ectomycorrhizal fungi were discovered in the soil of the aspen stand.

nalis in 80%, *Laccaria proxima* in 70%, *Cenococcum* in 60%, *Tricholoma scalpturatum* and *Populariza nigra* each in 40%, with the two *Hebeloma* species at lesser frequencies. Viewed as relative frequencies, the order of species remains the same but in different proportions. In this study, the relative abundance (column 2) and relative frequency (column 4) give similar estimates for most of the individual mycorrhizal species (Fig. 4). The importance value, the sum of abundance and frequency (Horton & Bruns, 2001), clearly shows the dominance of *Inocybe, Laccaria,* and *Paxillus*. Approximately 94% of the mycorrhizae counted were of species represented above ground for this study.

VERTICAL DISTRIBUTION OF MYCORRHIZAE IN THE SOIL

A majority of the mycorrhizal root tips were distributed from 16 to 48 cm in the soil, with a paucity of mycorrhizae in the top 16 cm (Fig. 5). An average of 26 ± 33 mycorrhizal root tips was found in the top 16 cm, 120 ± 117 at 16–32 cm, and 70 ± 66 at 32–48 cm (Fig. 5). The Friedman test found the difference to be statistically significant. In contrast, a brief examination of mycorrhizae in a mature aspen stand in silty soil in Idaho revealed a majority of the abundant mycorrhizae in the top 16 cm which declined rapidly with depth as is considered typical for many forest types.

While data were not sufficient to statistically determine depth preferences for individual mycorrhizal species, trends were suggested (Table 4). Type VII occurred only in the surface soil in dead wood, and *Inocybe* mycorrhizae were the other primary type in the top 16 cm of soil. *Inocybe, Laccaria, Tricholoma,* and *Cenococcum* mycorrhizae all reached maximum abundance at 16–32 cm. *Paxillus* mycorrhizae were primarily deeper in the soil at 32–48 cm, but *Inocybe* and *Laccaria* mycorrrhizae also reach this depth.

SPATIAL DISTRIBUTION OF SPOROCARPS AND ECTOMYCORRHIZAE

In general, each species of mycorrhizal fungus fruited in clusters restricted to one or more areas on the edge of the stand, and respective mycorrhizae were more widespread throughout the plot (Figs. 6 & 7). *Inocybe lacera* fruited in restricted areas on the northern and western edges of the plot and in large numbers, some fruiting outside the canopy zone of the aspen stand (Fig. 6A). In contrast, mycorrhizae of this species were found

TABLE III

ABUNDANCE (MYCORRHIZAE/UNIT OF SOIL), RELATIVE PERCENT OF ROOT TIPS, FREQUENCY (% OF SOIL CORES IN WHICH THE SPECIES OCCURRED), RELATIVE FREQUENCY, AND IMPORTANCE VALUES (ABUNDANCE + FREQUENCY) OF MYCORRHIZAL FUNGI IN A SMALL ASPEN STAND

Mycorrhizal type	Mycorrhizae/ 100 cc soil	Percent of total mycorrhizae	Percent frequency	Relative frequency (%)	Importance values
I: *Inocybe lacera*	24 ± 22	34	90	22	56
II: *Paxillus vernalis*	16 ± 20	23	80	20	43
III: *Tricholoma scalpturatum*	7 ± 16	10	40	10	20
IV: *Laccaria proxima*	17 ± 23	24	70	17	41
VI: *Cenococcum geophilum*	–	–	60	15	15
VII: *Popularhiza nigra*	4 ± 8	6	40	10	16
VIII: *Hebeloma mesophaeum*	1 ± 2	1.5	10	2.5	4
IX: *Hebeloma* sp.	1 ± 3	1.5	20	5	6.5

throughout the plot. Likewise, *Laccaria proxima* fruited regularly on the north side of the clone, and mycorrhizae were distributed throughout most of the plot (Fig. 6C). The lone fruiting body of *Paxillus vernalis* (in four years) was found toward the north side of the plot, while mycorrhizae occurred throughout the whole aspen stand and in over 80% of samples (Fig. 6B). *Paxillus* mycorrhizae were found as far as 11 m from the single sporocarp. *Tricholoma scalpturatum* fruited entirely within the canopy of the aspen stand, and mycorrhizae were limited to the center area of the stand (Fig. 6D).

Regarding species not represented above ground, *Cenococcum* mycorrhizae occurred over most of the plot, except the southwest corner (Fig. 7A). Type VII *Popularhiza nigra* occurred on the western half of the plot where dead wood was prevalent (Fig. 7B). Mycorrhizae

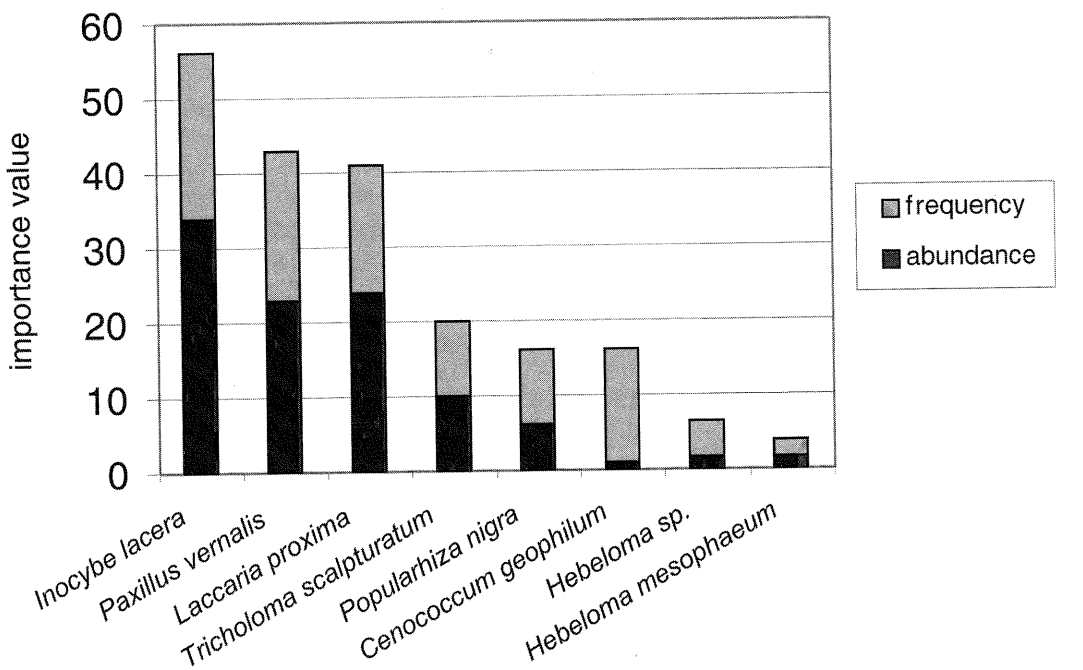


FIG. 4. Importance values of ectomycorrhizal fungi in a small aspen stand determined by combining relative abundance and relative frequency values.

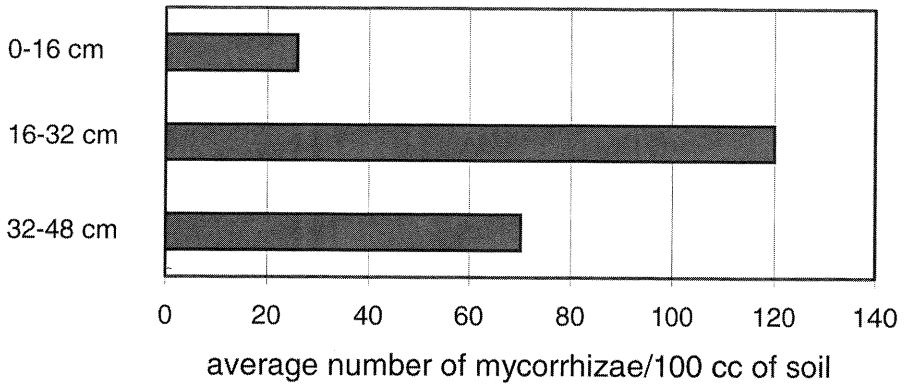


FIG. 5. Vertical distribution of ectomycorrhizae in the soil of the small aspen stand.

were mostly inside well-decayed wood near the surface. Distribution of *Hebeloma* mycorrhizas was limited, and fruiting bodies of both species occurred south of the plot in a ravine, but none were recorded within the plot (Fig. 7A,B).

Discussion

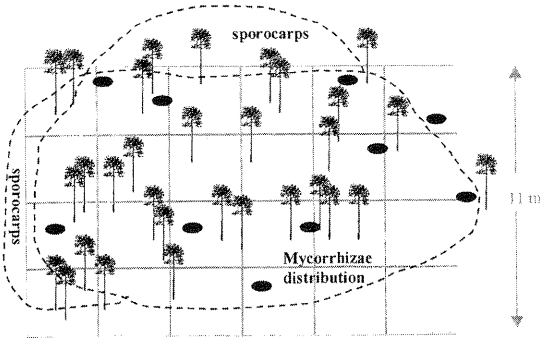
The isolated aspen stand was selected because of its limited size, so that the *whole* ectomycorrhizal community could be examined, not just a forest fragment as in most studies. The diversity (species richness), abundance, frequency, and distribution of each species of ectomycorrhizal fungus could then be addressed quantitatively, both above and below ground. The number of mycorrhizal roots in the small stand was calculated at between 0.2 and 1.3 million/m^3 and is likely an underestimate. The numbers are daunting in terms of sheer quantity, and underscore the logistical problems inherent in examining mycorrhizal communities even on a small scale. Similar high numbers have been cited by Taylor (2002) and Dahlberg et al. (1997).

While ten samples were sufficient to determine the overall density of mycorrhizal root tips (72 ± 54) per cc of soil to within 46% of the mean, a staggering 216 samples would be necessary to attain an acceptable confidence level of 10% even at this small scale. Fogel and Hunt (1979) suggested that "large errors in some measurements may have to be tolerated (for mycorrhizal studies) unless sampling or design methods are refined." Alexander (1985) found that variation in the number of mycorrhizal root tips among soil cores could be reduced by selecting the optimum core width, which was 39 mm for his study. Larger and smaller core sizes necessitated much larger sample sizes to achieve the same confidence level (431 samples for 12 mm core, 44 for 39 mm core, and 125 samples for 113 mm core). Methods for determining mycorrhizal density are not uniform and depend on the depth of the soil sampled, which can skew results and thus make comparisons difficult. The problem of ascertaining the density of mycorrhizae of *individual species* requires even larger sample sizes.

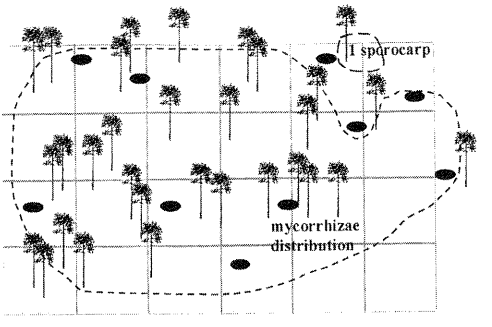
Eight species of ectomycorrhizal fungi (Table 2) were discovered in the soil of the aspen stand and four were correlated with sporocarps. Morphological and molecular methods used for identification concurred in this simple system. An additional four species were detected only below ground. Even in this small aspen stand, only half of the mycorrhizal fungi were observed above ground, and if this holds for more complex systems, underground studies are imperative to understanding the whole ectomycorrhizal community. More complex

TABLE IV
VERTICAL DISTRIBUTION OF ECTOMYCORRHIZAL FUNGI IN THE SOIL OF A SMALL ASPEN STAND. AVERAGE NUMBER OF MYCORRHIZAE PER 100 CC OF SOIL AT THREE DEPTHS

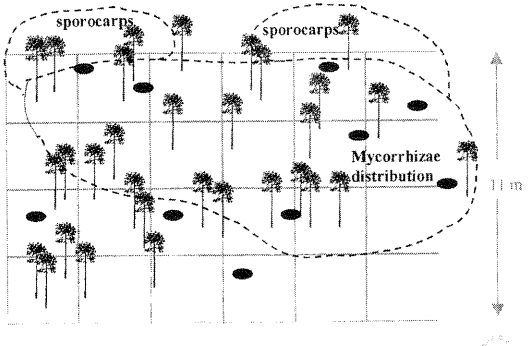
Species (morphotypes)	0–16 cm	16–32 cm	32–48 cm
II: *Inocybe lacera*	9	44	18
II: *Paxillus vernalis*	<1	18	27
III: *Tricholoma scalpturatum*	0	16	4
IV: *Laccaria proxima*	4	31	17
VII: *Populorbiza nigra*	13	<1	<1
VIII: *Hebeloma mesophaeum*	0	0	2
IX: *Hebeloma* sp.	0	2	<1



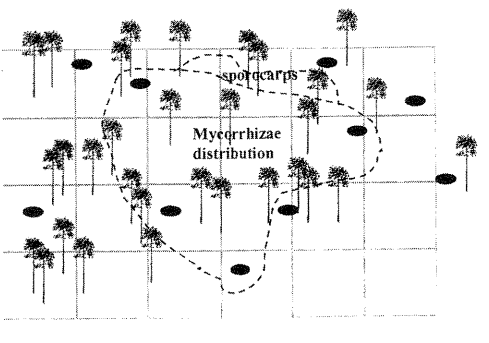
A. *Inocybe lacera* sporocarp and mycorrhizae distribution. *I. lacera* produced 100's of sporocarps annually.



B. *Paxillus vernalis* sporocarp and mycorrhizae distribution. *P.vernalis* produced 1 sporocarp over 4 years.



C. *Laccaria proxima* sporocarp and mycorrhizae distribution. *L. proxima* produced 100's of sporocarps annually.



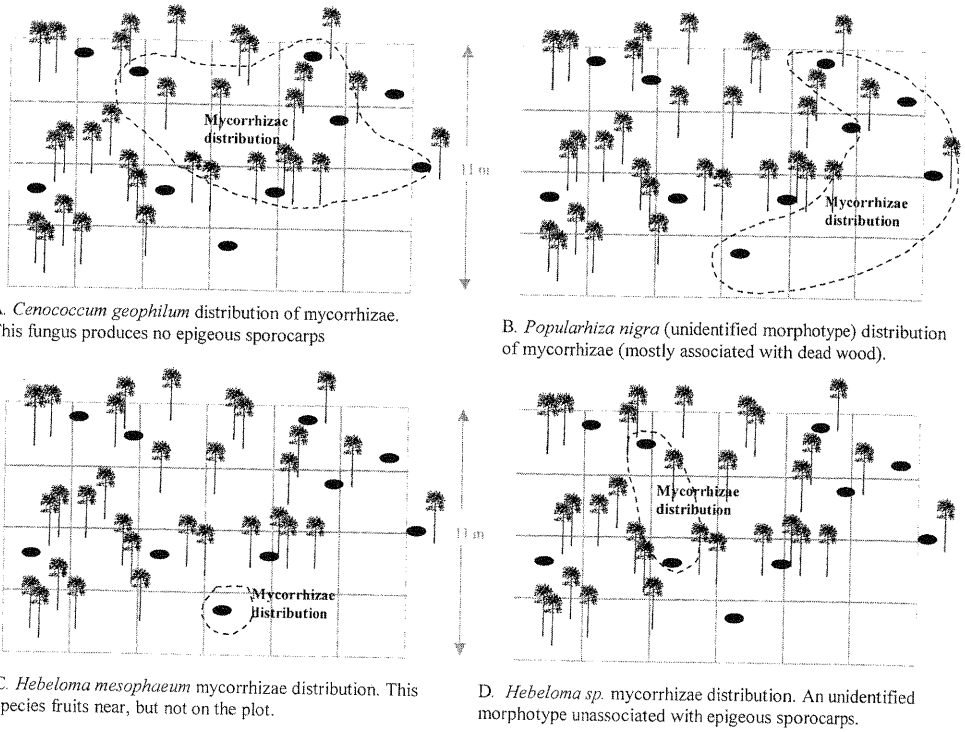
D. *Tricholoma scalpturatum* sporocarp and mycorrhizae distribution. *T. scalpturatum* produced 15 sporocarps in the 4th year of the study.

FIG. 6. Distribution of sporocarps and mycorrhizae in a small discrete aspen stand. The plot is 20 × 11 m and covers a majority of the aspen stand. Ramets with diameters >5 cm are indicated as trees, and soil cores for mycorrhizal sampling are shown as dark ovals.

systems likely contain more rare and difficult-to-detect species as discussed by Taylor (2002) and Horton and Bruns (2001).

Similarly, in *Pinus banksiana* forests in Alberta, only two-thirds (56 of 82) of the mycorrhizal types identified by root morphology were represented by sporocarps (Danielson, 1984), suggesting sporocarps represent minimal diversity. More than 20 taxa of ectomycorrhizal fungi (eight species, and others to genus or family) were distinguished on roots in *Pinus muricata* forests in California using RFLP matching of ectomycorrhizae to sporocarps and/or phylogenetic analysis of the mitochondrial LrRNA gene, while only 10 taxa were observed fruiting (Gardes & Bruns, 1996a). In *Picea abies* forests in Switzerland, 18 species of ectomycorrhizal fungi were discovered by root morphology alone, and 23 by independent RFLP analysis. Of these, seven RFLP patterns were matched to sporocarps. There was not a one-to-one correspondence for others, and molecular analysis revealed that one morphotype included several species and, conversely, that several morphotypes represented one species (Mehmann et al., 1995). Other studies found a lower diversity of morphotypes than epigeous fruiting bodies (Jansen, 1991; Menge & Grand, 1978), and Jansen and DeNie (1988) suggested that for some systems morphotypes equate better with genera than with species. Although molecular methods have improved some distinctions, they are not always possible. For example, what was thought to be one *Rhizopogon* species in a bishop pine forest turned out to be a 'species conglomerate' of six related taxa with RFLP analysis (Taylor & Bruns, 1999).

Examination of the relative abundance and frequency of various ectomycorrhizal types in the aspen stand revealed a few rather well-distributed species and a few uncommon types in the soil (Table 3, Fig. 4). The



A. *Cenococcum geophilum* distribution of mycorrhizae. This fungus produces no epigeous sporocarps

B. *Popularhiza nigra* (unidentified morphotype) distribution of mycorrhizae (mostly associated with dead wood).

C. *Hebeloma mesophaeum* mycorrhizae distribution. This species fruits near, but not on the plot.

D. *Hebeloma sp.* mycorrhizae distribution. An unidentified morphotype unassociated with epigeous sporocarps.

FIG. 7. Distribution of mycorrhizae for fungal morphotypes not correlated with epigeous sporocarps in a small discrete aspen stand. Plot is 20 × 11 m and covers a majority of the canopy zone.

species curve levels off (Fig. 3), suggesting that a majority of the major types have been discovered, as expected for systems of low diversity (Pielou, 1977; Taylor, 2002; Tews & Koske, 1986). This low diversity could reflect the young age of the aspen, its early successional status, limited size, and/or abiotic conditions which few fungi tolerate (low nutrient, low pH, high heavy metals, low moisture).

The frequency and abundance of the eight ectomycorrhizal types found in the soil are contrasted in Table 3 and Figure 4. Abundance measures (column 1) elevated the significance of major species and decreased that of minor species, and the reverse was true for frequency. One advantage of using frequency (presence or absence in a soil core) as a measure is that it is more efficient than counting and identifying all individual root tips in a core (abundance). Thus, many more samples can be processed, which might allow better analysis of patchy systems. An "importance value" was included (sum of relative frequency and relative abundance), as suggested by Horton and Bruns (2001), to give a better representation of the contribution of each fungal species in a community (Fig. 4). *Inocybe lacera, Laccaria proxima,* and *Paxillus vernalis* showed clear dominance. In the aspen stand, over 94% of the root tips counted represented four species. In a pine–Douglas fir forest over 74% of the mycorrhizae counted were of Thelephoraceae and Russulaceae (Horton & Bruns, 1998; Taylor & Bruns, 1999), suggesting underground dominance of a few fungal taxa in some systems.

Overall, there was not a one-to-one correspondence of fungi above and below ground. *Inocybe lacera* and *Laccaria proxima* did fruit prolifically and both had high importance values below ground. However, *Paxillus vernalis* produced only one fruiting body in four years and yet 25% of all mycorrhizae were of this species. *Paxillus* sporocarps are large, persistent, and unlikely to be overlooked. The importance of *Paxillus* would be highly underrated from only an above-ground perspective. *Tricholoma scalpturatum* fruiting bodies did not appear until the fourth year of the study (in two small clusters), and their mycorrhizae were restricted to the same area. There is no *a priori* reason to expect that structures designed to gain nourishment from plant roots (mycor-

rhizae) should correlate quantitatively with structures for reproduction (sporocarps), although they could do so in some systems.

Fruiting Strategies of Ectomycorrhizal Fungi

Differences in fruiting patterns of various fungal species became apparent during the study. In this system, *Laccaria proxima* and *Inocybe lacera* expended energy in both prolific annual fruiting and extensive underground mycorrhizal colonization. Both genera are known as early colonizers, and are common in stressed systems (Cripps, 2003). Other studies have shown the importance of sexual spore propagation in genus *Laccaria*. *Laccaria amethystina* is reported to produce numerous small genets and close sporocarps which are primarily genetically unique (Fiore-Donno & Martin, 2001; Gherbi et al., 1999). Conversely, de La Bastide et al. (1995) found that well-separated sporocarps of *L. bicolor* around a spruce were from the same individual. Genets were not examined in the present study, but it should be noted that the small aspen stand likely shares one large root system and could behave more as a single tree. While the canopy area within the aspen stand was devoid of fruiting bodies of *Laccaria* and *Inocybe* (Fig. 6A,C), their mycorrhizae were common throughout the stand. It is possible that development of understory, organic matter, and litter build-up eventually preclude fruiting of these species in the center of the stand. It would be interesting to know if small genets existed in the fruiting locale with larger genets in the primarily somatic area. *Inocybe lacera* is also an early colonizer of disturbed sites (Cripps, 2001; Schramm, 1966), and appears to have a fruiting strategy similar to that of *L. proxima,* but no genet information is available. We do know that each of these two species can have two RFLP types in a limited area (Horton, 2002).

In contrast, only a single fruiting body of *Paxillus* was observed in four years, and a second four years later in the same location. Years passed between spore production events. Mycorrhizal and rhizomorphic development was extensive throughout the stand (Fig. 6B), and contributed one-fourth of all mycorrhizae. *Paxillus* mycorrhizae were, at maximum, farther than 11 m from the sporocarp. Again we have no genet information, but some boletoid fungi such as *Xerocomus* are known to have large persistent genets (Fiore-Donno & Martin, 2001), and *Paxillus* is related to boletes.

Tricholoma scalpturatum was restricted to a small area of the aspen stand where it produced mycorrhizae in dense clusters and a few local sporocarps. It has been shown to produce small genets under some conditions. For example, more than 60 genets of *T. scalpturatum* were associated with 63 fruiting bodies in a black poplar stand, suggesting the population was structured mostly by sexual reproduction. This was in contrast to *T. populinum* which produced large genets in the same area (Gryta et al., 2001). A few *Hebeloma* mycorrhizae were noted in the aspen stand, but no fruiting bodies were found on site (Fig. 7C,D). Another species, *H. cylindrosporum*, can also produce small nonpersistent genets (Guidot et al., 2001). *Cenococcum geophilum* was common in low numbers throughout the stand as is usual for this species. *Populorhiza* mycorrhizae occurred in dead wood in the half of the plot where downed wood was most common. Other factors that might be considered in population structure are longevity of spores in the soil, durability of sporocarps, and breeding systems of various species.

Mycorrhizal Fungi and Edge Effect

Laccaria, *Inocybe*, and *Paxillus* species all fruited on the edge of the stand, primarily the northern edge (Fig. 6). At first, this was thought to be a result of shade, cooler soils, and/or more moisture retention on the north side of the stand. Schramm (1966) reported a shading effect and observed mycorrhizal fungi fruiting on the north side of trees on coal spoils where high temperatures often inhibited seedling establishment. To test for an edge effect versus shading (or other microhabitat/microclimate) factors, additional aspen clones in the area were examined for the location of sporocarps of *Inocybe lacera.* Results indicated that *Inocybe lacera* fruited on northern, southern, and western edges of other aspen stands in the area, but primarily outside the canopy zone, indicating an edge effect (Cripps, unpubl. data). Ruderal/early colonizing species are known to associate with younger outer roots of a tree (Last et al., 1987). In this case, the aspen stand could act as a single tree, with stress-tolerant species fruiting at the perimeter where conditions are less favorable for other species (less organic matter, drier soils, etc.). As primarily wind-dispersed pioneers in mycorrhizal succession, the predominance of sporocarps at the edge of the stand would confer an advantage. Allen (1991) noted that wind turbulence is limited on forest floors, which limits turbulence-induced dispersal of ectomycorrhizal fungi. Turbulence is higher at the edge of stands. Spores of *Thelephora*, an early colonizer, were found repeatedly on the edge of forests on Mt. St. Helens in Oregon (Allen, 1987). In addition, *Inocybe lacera* and *Laccaria* spp. often fruit at the time of seed dispersal for aspen, but are

broad host fungi, capable of associating with a variety of young tree species. Aspen clones reach considerable size, often with thick understories, which could influence fruiting patterns. In adjacent, larger aspen stands, competitive K-selected mycorrhizal fungi (*Lactarius*, *Russula*, *Cortinarius*) typically fruited in the interior where there is increased understory, leaf litter, organic matter, and moisture (Cripps & Miller, 1993). In these larger stands, mycorrhizae were concentrated more in the few centimeters of the surface soil, instead of being distributed to 48 cm as in the smaller stand (with sandy soil), revealing a different pattern of mycorrhizal fungi under aspen.

Whether this edge pattern is unique to aspen as a clonal organism or is found in other forest types remains to be seen. However, an abundance of non-host-specific fungi has also been noted on the edge (interface) of forests and alpine habitats (Kernaghan & Harper, 2001). The importance of forest edges, with both positive and negative aspects, is well known and studied by ecologists (Matlack, 1994), but the relationship of forest edge and mycorrhizal fungi has not been well delineated. The numerous abiotic (light, organic matter, moisture, shading, litter, oxygen) and biotic (understory plants, tree root age) gradients which occur at the forest edge could be of high importance in the dynamics of some mycorrhizal fungi and forest trees as it is the boundary of the system itself. It is well known to mushroom collectors that "edges" of meadows and forests can be prime collecting areas for sporocarps. Edge should be taken into consideration in plot selection for mycorrhizal studies, and edges also occur on a small scale in forest gaps and meadows.

Co-occurrence of Mycorrhizal Fungi above and below Ground, and Vertical Distribution in the Soil

If distributions of ectomycorrhizae and sporocarps (Figs. 6, 7) are overlain, a co-occurrence of mycorrhizal types is observed across the plot. Root cores themselves contained one to five species of fungi in close physical proximity. There appeared to be some resource partitioning, particularly as pertains to depth (Table 4), but data was minimal. *Inocybe lacera* and *Populorhiza nigra* were found in the top 16 cm of soil, and, with one exception, did not occur in the same soil cores. At 16–32 cm, where most mycorrhizae occurred, 2–5 types were found in each soil core, usually with one type dominating. *Inocybe*, *Laccaria*, *Tricholoma*, and *Cenococcum* mycorrhizae reached their peak at this depth, and *Paxillus* was also common. Below 32 cm to depths of 48 cm, *Paxillus* dominated, although *Inocybe* and *Laccaria* were still frequent, and cores again contained 2–5 types. The values for vertical distributions reflect only trends, and are not statistically meaningful due to small sample size. In contrast, fruiting areas were more restricted than that of mycorrhizae (Fig. 6), and co-occurrence of sporocarps was rare.

Mycorrhizal samplings are only "snapshots in time," and systems are dynamic, changing both seasonally and long term. New mycorrhizae are continually formed as roots proliferate, and other mycorrhizae die, and new species can come to dominate. Drought plays a crucial role in mycorrhizal dynamics in semi-arid areas, and a midsummer sampling showed a severe paucity of functioning mycorrhizae (Cripps, unpubl. data). Sampling over time would add further information; however, once the first set of soil cores is taken, the system is no longer undisturbed. For example, in the aspen stand, *Laccaria* fruited in core holes in the stand interior after termination of the experiment.

As for regeneration and recovery from airborne pollution, we now know that aspen (*Populus tremuloides*) is an important species, naturally colonizing areas around smelter sites in the northern Rocky Mountains. Some of the ectomycorrhizal fungi in this study are important in aspen establishment along with others such as *Thelephora terrestris* (Cripps, 1996, 2003). Most are considered ruderal/stress-tolerant/early colonizers, which typically have a broad host range. While some improve aspen growth *in vitro*, it is not yet known if mycorrhizal fungi can be used to enhance aspen establishment on mining impacted areas (Cripps, 2001). Some of the ecological findings from this study can suggest which mycorrhizal fungi might be of benefit, considering the more precise ecological role of each.

In conclusion, quantification of macrofungi beyond species richness is still problematic. However, the value of working towards quantification of ectomycorrhizal fungi lies not only in delineating basic ecological parameters but also in helping us understand how ectomycorrhizal forests function, and further serves as an aid in early detection of responses to natural and anthropogenic disturbances in forest systems. Aspen has the largest distribution of any tree in North America, and there is growing concern about its decline in the western United States (Shepperd et al., 2001). The role of mycorrhizae has not been considered in this regard.

Acknowledgments

I would like to thank Orson K. Miller Jr. for his part in this research. I also take this opportunity to thank

him for his prodigious accomplishments which have spurred the field of mycology forward. As a teacher, his encouragement of students helped spawn many mycologists, his involvement with amateur groups gave them the benefit of professional expertise, his books gave North America its own field guides, his numerous papers gave us new knowledge; as a mentor, he made difficult goals seem attainable. I especially thank Orson for personal encouragement, and for making the academic world fun! I also thank my husband for many hours of companionship in aspen forests everywhere.

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