A COMPARISON OF ECTOMYCORRHIZAL HYPOGEOUS FUNGI IN SPRUCE-FIR AND NORTHERN HARDWOOD FORESTS ON ROAN MOUNTAIN (NC/TN)

A Thesis

by

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Submitted to the Graduate School

Appalachian State University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2001

Major Department: Biology

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ABSTRACT

A COMPARISON OF ECTOMYCORRHIZAL HYPOGEOUS FUNGI IN SPRUCE-FIR AND NORTHERN HARDWOOD FORESTS ON ROAN MOUNTAIN (NC/TN)

(December 2001)

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Hypogeous sporocarps were sampled during two years in spruce-fir and northern hardwood forests on Roan Mountain TN/NC. Ectomycorrhizal root tips were sampled in each forest during fall 1999 and spring 2001. Molecular techniques were used to compare sporocarp and ectomycorrhizal DNA. *Elaphomyces muricatus* and *E. granulatus* were present in spruce-fir forest transects; only *E. muricatus* was found in northern hardwood forests. There was no significant effect of forest type (northern hardwood or spruce-fir) or year sampled (2000 or 2001) on sporocarp biomass or sporocarp number. Fraser fir (*Abies fraseri*) and *E. muricatus* had a moderately positive association (association value = 0.47). Ectomycorrhizal morphotype evenness was similar to other studies, with one or two morphotypes dominant and others less abundant. A multivariate analysis of variance (MANOVA) on morphotypes present in more than one soil core showed significant effect of forest type (Wilk's Lambda = 0.0019). Different morphotypes were affected by forest type and time sampled differently. *Cenococcum geophilum* was the most common ectomycorrhiza;

present in every core sampled, and in most cores the dominant ECM morphotype. In this study, 14 of 26 ECM morphotypes were found in both forest samples, 4 were unique in northern hardwood samples, and 8 were unique in spruce-fir samples. *Elaphomyces muricatus, E. granulatus, Alpova* sp. and *Scleroderma* sp. were not present as ECM morphotypes in either forest type.

ACKNOWLEDGEMENTS

This thesis would never have been completed without the assistance of many individuals. The initial interest in the Carolina northern flying squirrel came from a request from Ron Hughes to analyze some squirrel scat. Conversations with Ron and Dr. Peter Weigl at Wake Forest University started my "gears" turning. Through Dr. Weigl, S. Coleman McCleneghan was able to contact Dr. Susan Loeb who turned us on to the possibility of funding for this project. I would like to thank my committee members, for their continued support and advice throughout my study. Dr. Mary Connell provided me with lots of reassurance and suggestions in the molecular lab. Dr. Robert Creed assisted with experimental design and statistical analysis, and provided me with excellent theoretical questions to keep my mind whirling. Their input as my research progressed and during the writing phase was immeasurably valuable.

Funding for this research was provided by a research grant from USDA Forest Service, the Graduate School of Appalachian State University, the Graduate Student Association Senate of Appalachian State University, and the Athena Award sponsored by Joyce Lawrence. The James Greene Fellowship provided assistance during the first two years of my research.

The staff and faculty of the Biology department were absolutely appreciated during this study. Betsy Harris graciously answered any question I

had, Val Somers handled all of my supply orders and travel requests without a whimper, and Vicki Isaacs was always there to happily assist me. The faculty members of ASU's Biology Department truly have a special place in my heart, as they all in some way contributed to my development as a young scientist. Dr. Gary Walker helped me especially with many administrative questions.

Randall Fowler, Michael Hambourger, and Carrie Gass assisted me in the rather tedious, and at times unrewarding, task of truffle hunting, with very few complaints! Joanne Holden helped immensely with technical advice in the molecular lab, helping me through a particularly rough few months of contamination problems. Michael Hambourger also conducted the sclerotia quantification in this study, again without complaints. Dr. Tom Horton, at State University of New York – Environmental Science and Forestry, helped immensely by answering several e-mails on molecular techniques.

I would also like to acknowledge the support of my families throughout my years at Appalachian, as they never faltered in their support, and did not even balk when I suggested that what I truly wanted to do with my life was sit in the woods and dig in the dirt. They possessed confidence in me when I was certain I might fail. There is no way to quantify the amount of love they poured my way. My thanks are also extended to my ever-present companion, the beagle mix Isabelle.

Finally, I would like to acknowledge my major professor, Dr. S. Coleman McCleneghan. She played so many essential roles for me over the past 3 years: friend, confidante, counselor, supervisor, expert mycologist, and advisor. She

somehow always knew exactly when I needed a kind word, or a firm order to get to work, depending on my mood at the time. She continued to support and guide me when I was most frustrated, and then stood back and let me take credit when I succeeded. It has been an honor and privilege to work with her.

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INTRODUCTION

Hypogeous fungi form sporocarps (fruiting bodies) underground and are presumed ectomycorrhizal symbionts of trees in temperate forests. Some members of the *Basidiomycota* and *Ascomycota* form hypogeous sporocarps. Ectomycorrhizas (ECM) are associations between tree roots and fungi where the fungi grow between the cortical cells of the root, without penetrating the actual cells. Typically, the fungus partner forms a thickened mantle around the root tip, and alters both the morphology and color of the tree root (Smith and Read 1997). Ectomycorrhizas increase the growth and fitness of many species of trees, especially conifers. Some hypogeous species, such as *Rhizopogon*, frequently exhibit species-specific associations with particular plant hosts (Molina and Trappe 1994). Other hypogeous species, such as *Elaphomyces granulatus*, are found in a variety of forest types suggesting that it is a generalist with respect to plant hosts (Zhang and Mintner 1989). Most studies of hypogeous fungi have been conducted in the western parts of the United States (e.g., Luoma et al. 1991; North et al. 1997; States and Gaud 1997; Waters et al. 1997). Fewer studies have been conducted in the Southern Appalachian Mountains to characterize hypogeous fungal communities (Miller 1986; Loeb et al. 2000).

The interactions between fungi and small mammals have been studied in

various forests in the United States and Australia for several decades (e.g., Fogel and Trappe 1978; Kotter and Farentinos 1984; Cork and Kenagy 1989; Hall 1991; Claridge and May 1994; Johnson 1994; Claridge and Lindenmayer 1998). Some hypogeous fungi, such as *E. granulatus* and *Mesophellia pachythrix,* may require passage through a mammal digestive system before germination starts (Cork and Kenagy 1989; Claridge et al. 1992). Further, some small mammals, such as northern flying squirrels and California red-backed voles consume a lot of hypogeous fungi, suggesting they are important in the dispersal of these fungi (Ure and Maser 1982; Maser et al. 1985; Hall 1991; Rosentreter et al. 1997).

In the past decade, it has become apparent that sporocarp presence and abundance do not correlate with occurrence as an ectomycorrhizal symbiont for all ECM fungal species (Gardes and Bruns 1996; Karen and Nylund 1997; Dahlberg et al. 1997). Some fungi, for example *Suillus pungens*, which produce lots of sporocarps, are not commonly found as ectomycorrhizal symbionts (Gardes and Bruns 1996). Other species however, such as *Amanita francheti*, produce lots of sporocarps and are equally present as ectomycorrhizal symbionts. Some species, for example *Russula amoenolans* and a boletoid type, are common as ECM symbionts but fruiting bodies are rare (Gardes and Bruns 1996). Some species of ECM do not produce fruiting bodies, or rarely fruit. The lack of a relationship between sporocarp abundance and frequency of ECM associations means that direct observation and identification of ECM from roots is required. Relying solely on sporocarp surveys is insufficient to describe

ECM associations (Gardes and Bruns 1996).

Ford et al. (1985) demonstrated that symbiotic fungal species vary in terms of their impacts on their tree hosts. Species found to form ECM with loblolly pine (e.g., *Pisolithus tinctorus*, *Thelophora terrestris*, *Rhizopogon roseolus,* and *Scleroderma aurantium*) were not equally effective in promoting shoot growth in loblolly pine. Seedlings forming ECM with *S. aurantium* had significantly greater shoot weights. Laboratory studies such as the one conducted by Ford et al. (1985) demonstrate the need for understanding which fungi are forming ECM with which forest trees, which could lead to improved forest management.

Many studies indicate that ECM communities can be very diverse, even in forests with only one or two tree species (Gardes and Bruns 1996; Dahlberg et al. 1997; Gehring et al. 1998; Horton and Bruns 1998; Massicotte et al. 1999). It is important for conservation and ecological knowledge to know which fungi are present in various forests, due to the varied benefits different ECM provide their tree hosts and the forest as a whole (Massicotte et al. 1999). The importance of hypogeous fungi as ECM symbionts has been assumed in mycophagous mammal studies, but direct observations from the root tips of trees have not been documented for all species of hypogeous fungi. The lack of correlation between sporocarp production and ECM formation found by Gardes and Bruns (1996) indicates the need to examine both sporocarp production and root tips with ectomycorrhizas in order to characterize an ectomycorrhizal community accurately. This can then increase the understanding of the hypogeous fungi as

ectomycorrhizal symbionts, the role of the mammal in spore dispersal, and overall forest health.

In the Pacific Northwest a number of researchers have examined ECM community structure. Horton and Bruns (1998) found several ECM species colonizing both Douglas fir and bishop pine. The only hypogeous species they found was *Rhizopogon parksii*, which was specific to Douglas fir. Because of the difficulty of identifying ECM based on morphology alone, this study incorporated molecular methods to compare DNA from sporocarps with ECM DNA. Fungal specific primers constructed by Gardes and Bruns (1993) allow for the amplification of fungal DNA from a mix of plant and fungal DNA extract. These primers amplify the internal transcribed spacer (ITS) regions of ribosomal DNA. Internal transcribed spacer regions are non-coding, and thus able to accumulate mutations without being detrimental to the organism. For this reason, species level differences can be observed and allow for identification of the fungal hyphae, following restriction fragment length polymorphism (RFLP) analysis (Gardes and Bruns 1993).

The Southern Appalachian Mountains offer an interesting opportunity to study the interactions between hypogeous ectomycorrhizal fungi, trees, and small rodents. At higher elevations (above 1500 m) the forests of the Southern Appalachians resemble those of more northern latitudes, being dominated by red spruce and Fraser fir (White and Cogbill 1992). These unique forests are a highly threatened ecosystem, facing the threats of the balsam woolly adelgid, logging, road building, pollution, and temperature increase due to global warming

(Noss and Peters 1995). Below the spruce-fir forest, species more common in the northeastern United States and Canada are found (American beech, yellow birch, mountain maple). Roan Mountain, on the border of Tennessee and North Carolina, has a very well studied population of Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*) (CNFS), an endangered subspecies of northern flying squirrel (Weigl et al. 1999). Although this species is common in Canada and western United States, the CNFS subspecies is threatened by loss of the spruce-fir habitat (Weigl et al. 1999).

Understanding the ecology of the CNFS has been hampered due to its nocturnal foraging habits and small population size. General habitat requirements for the CNFS appear to be stands dominated by spruce, yellow birch, American beech, Fraser fir, and maples, often on northern facing slopes (Weigl et al. 1999). Carolina northern flying squirrels have been tracked with radio telemetry and found to forage in spruce-fir dominated stands, while nesting more often in mature hardwood trees (Weigl et al. 1999). Feces collected from CNFS that live in the ecotone between spruce-fir and northern hardwood forests on Roan Mountain contained 6 genera of hypogeous fungi: *Geopora, Elaphomyces, Picoa, Rhizopogon, Melanogaster*, and *Pachyphloeus* (Weigl et al. 1999). The only hypogeous species found on Roan Mountain by Loeb et al. (2000) was *E. granulatus*. The source of the hypogeous fungi found in the CNFS feces is still in question. Abundance of red spruce was the best predictor of *E. granulatus* sporocarps (Loeb et al. 2000). This suggests that there might be more hypogeous sporocarps in the spruce-fir forests than in the lower

northern hardwood dominated stands.

The species composition of ECM on Roan Mountain is also still in question. Bills et al. (1986) compared epigeous sporocarps of ECM in red spruce and northern hardwood forests in West Virginia, and found that of 54 species, only eight species were commonly found in both forest types. Few other studies have compared ECM of red spruce-Fraser fir forests with forests composed of northern hardwood species. Meier (1989) found the most common ECM of red spruce were *Cenococcum geophilum*, which is a mitosporic fungus, and an unidentified tannish brown ECM morphotype.

The objectives of this study were two-fold: first, to compare the hypogeous fungal species in spruce-fir and northern hardwood forests in order to further understand the habitat of the CNFS; second, to assess the ECM communities of the two forests and determine which ectomycorrhizas form hypogeous sporocarps. Molecular techniques were employed to help achieve the second objective. From these objectives, the following hypotheses were formed:

1. Hypogeous fungal diversity:

   H_0 = The same diversity of hypogeous fungal species occur in both spruce-fir and northern hardwood forests.

   H_a = Hypogeous fungal species are more prevalent in spruce-fir than northern hardwood forests.

2. ECM diversity

   H_0 = The ectomycorrhizal communities exhibit the same diversity in

spruce-fir and northern hardwood forests.

H_a = The ectomycorrhizal communities are more diverse (species rich) in spruce-fir than northern hardwood forests.

The directionality of the alternative hypotheses for both objectives stems from previous research which has demonstrated that many hypogeous species appear specific to members of the Pinaceae (which includes *Picea* and *Abies*) and members of the Pinaceae have been found to have very diverse ECM assemblages (Horton and Bruns 2001). Further, the local studies by Loeb et al. (2000) and Weigl et al. (1999) both suggest more hypogeous fungal associations in the spruce-fir forest.

MATERIALS AND METHODS

Study Site

Roan Mountain is in the Southern Appalachian Mountains located on the border of North Carolina and Tennessee (36° 12' N, 82° 04' W), with forests in the Cherokee National Forest and in the Pisgah National Forest. The peak, at 1915 m, has many plant species that are more commonly found in Canada. These plants have disjunct populations at the higher elevations of the Southern Appalachians (Brown 1941). Numerous shrub balds and Fraser fir (*Abies fraseri*)-red spruce (*Picea rubens*) forests dominate the plant community above 1700 m. Northern hardwood species, such as American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), and yellow birch (*Betula lutea*), dominate at lower elevations. Soils on Roan Mountain are a gray-brown podsol (Brown 1941), with a parent material of migmatitic biotite-hornblende gneisses (NC Department of Natural Resources and Community Development 1985). Cooler temperatures, increased precipitation, and higher wind speed than the surrounding lower elevations characterize the climate of Roan Mountain (Brown 1941).

Human activities have influenced the forest composition on Roan Mountain. By 1937, every tree larger than six inches in diameter had been removed by loggers (Brown 1941). The U.S. Forest Service now manages the

area, which is used mostly for recreation and conservation purposes. The Appalachian Trail traverses the mountain, and a Forest Service road allows visitors to drive to the summit.

The location of my transects was chosen based on maps describing Carolina northern flying squirrel ranges (Weigl et al. 1999), distance from the Appalachian Trail, and distance from other trails to minimize human disturbance. One hundred meter transects in northern hardwood were established at 1550 m, two in Tennessee, and one in North Carolina. Spruce-fir transects were located at 1700 m, again with two in Tennessee and one in North Carolina. Five 1 m^2 plots in each transect were established using random numbers to generate one plot within each twenty meter segment of the transect.

Sporocarp sampling

In order to prevent consumption of sporocarps by mammals, plots were covered with aluminum window screening (North and Trappe 1994). Plots were covered for approximately three to four months. Sampling occurred during the months of April through July 2000 and March through May 2001. Sampling was attempted during fall of 2000, but was not completed due to snow and frozen soils. Plots were excavated to a depth of 15 cm. A sieve (#4: 5.15 mm mesh size; Newark Wire Cloth Company, NJ) was used in the field to help sort through the soil that tended to clump, especially in the hardwood forests. Sporocarps were placed in wax paper and labeled with date and plot location.

Specimens were photographed and identified using Castellano et al. (1989) for genera and Zhang and Mintner (1989) for descriptions of *Elaphomyces*. Small amounts of material were preserved in a 2X CTAB buffer [100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 2% cetyltrimethylammonium bromide (CTAB)] at -20 C. Sporocarps were then dried at 140 C for at least 48 hours. Dried sporocarps were weighed, and the total biomass per plot recorded. Specimens were deposited into the Appalachian State University Herbarium (BOON).

Vegetation surveys

The tree and shrub species within 1.5 m of each plot were recorded. Circumference at breast height was sampled for conversion to diameter at breast height. These data were used to calculate an association index (see statistical analysis section below).

Ectomycorrhiza sampling

Soil cores were taken from the perimeter of plots established for sporocarp sampling. Each core was 8 cm in diameter. Cores were placed in a resealable plastic bag and returned to the lab. Five cores each were taken from spruce-fir and northern hardwood transects in November 1999, and five more were taken from each forest type in February of 2001.

Each core was rinsed through a series of soil sieves (#4, #12: 1.52 mm mesh size; and #30: 0.5 mm mesh size; Newark Wire Cloth Company, NJ), and

the contents of the #4 sieve were sorted for mycorrhizal tips. These tips were stored in sterile distilled water for up to two weeks prior to counting. During counting, morphological observations were made, using characteristics described by Agerer (1987-1993) (Appendix 1). Ectomycorrhizas were quantified by recording the number of root tips per morphotype per core. Each branching portion of an ECM was considered to be one root tip. Descriptions were made using check sheet A from Agerer (1987-1993). Photographs were made with a stereoscope (Olympus SZX12, Olympus Optical Co. LTD, Japan). In cases of thin or non-apparent mantles, sections were made to determine presence of a Hartig net at 1000X magnification using a compound microscope. Samples of each morphotype were stored at -20 C in a 2X CTAB solution.

Sclerotia sampling

During the February 2001 ECM collection, each soil core was subsampled for *Cenococcum* sclerotia, using methods developed by Trappe (1969). Soil was collected from the #30 soil sieve, the finest mesh, and placed in a resealable plastic bag. Four subsamples of 15 ml were randomly taken from the sample, and visually inspected for sclerotia. Numbers of sclerotia were recorded for all soil cores from the 2001 sampling.

DNA extraction

Molecular methods followed Gardes and Bruns (1993), with some modification (Horton, personal communication). DNA was extracted from one

ECM root tip or a small amount of sporocarp tissue using a 2X CTAB buffer with 2% β-mercaptoethanol. Three freeze-thaw cycles were performed, using liquid nitrogen and a 65 C water bath. Samples were then ground in 1.5 ml Eppendorf tubes using micropestle grinders. Solutions were incubated for 1 hour at 65 C. Proteins were extracted using chloroform, and DNA was precipitated using isopropanol. DNA was subsequently washed with -20 C 70% ethanol, and dried in a speed vacuum. Twenty to 30 µl of TE buffer solution were added to DNA pellets, and then the samples were vortexed and centrifuged.

Fungal DNA amplification

The primers ITS1-f and ITS4 (Gardes and Bruns 1993) were used to amplify the fungal DNA. These primers were selected because they amplify ascomycete and basidiomycete DNA. Primers were synthesized by Marshall University DNA Core Facility (Huntington, WV). The ITS region of rDNA was amplified in a Perkin Elmer 9600 Gen Amp PCR system (PE Applied Biosystems, Forest City, CA). Amplification reactions of 25 µl were set up with 1µl of DNA isolate, 1 µmole of each primer, 1 "Ready to go" PCR bead (Amersham Pharmacia Biotech, Inc. Piscataway, NJ) and 23 µl of sterile distilled water. Two negative controls were prepared with each set of samples. One control was prepared prior to any samples, and one was prepared following the samples. Filtered micropipettor tips were used to prevent DNA aerosols. Amplification followed a three-temperature cycle from Gardes and Bruns (1993). For some ECM morphotypes, no fungal DNA was obtained the first run, and

these were repeated. If no fungal DNA was obtained from the second run, the morphotypes were considered non-mycorrhizal. Notes on morphology of these types correlated (generally thin, or unapparent mantles or Hartig nets).

RFLP analysis

Amplification products were digested using the restriction enzymes *Hinf* I and *Alu* I (Promega Corporation, Madison WI). Eight µl of DNA, 8 µl of 2X "B" buffer from the manufacturer and 0.5 µl of restriction enzyme were mixed and incubated for one hour in a 37 C water bath. Loading dye was added to the products, and DNA was electrophoresed using a 1%Agarose/2%NuSieve (Biowhittaker) gel. Electrophoresis was performed for 150 minutes at 100 volts in TBE [89mM Tris, 89 mM of boric acid, 2 mM EDTA (pH 8.0)] buffer. Two lanes of *Phi*X174/*Hind*III size markers were used. Gels were then stained in a 2 mg/ml ethidium bromide solution, and viewed on a UV 400-M UV Transluminator using the Alph Innotech Digital Imaging and Analysis System. Restriction fragment length polymorphisms (RFLP's) were sized using ProRFLP Molecular Weight software (DNA Pro Scan, Inc., Nashville, TN). Sporocarp RFLP's were then compared with ectomycorrhizas RFLP's to determine identification of ectomycorrhizas. I compared RFLP patterns from voucher specimens of *E. muricatus* and *E. granulatus* (from Loeb et al. 2000) to account for any geographic variation in ITS regions (Farmer and Sylvia 1998). Voucher specimens of *Alpova* and *Scleroderma* species (from Loeb et al. 2000) were also compared with ECM RFLP's.

Data analysis

Associations between sporocarp and occurrence of tree species in plots were determined using the following association formula based on presence/absence data.

$$Association = \sqrt{\frac{(ad - bc)}{[(a+b)(c+d)(a+c)(b+d)]}}$$

Where a= number of plots with both species present, b=number of plots with species one present, c= number of plots with species two present, and d=number of plots with neither species present (Krebs 1999). A one way analysis of variance (ANOVA) was used to analyze of sporocarp biomass and sporocarp number with respect to plot location and sample year using SAS Software, version 8 (SAS Institute, Cary NC).

Rank abundance was used to compare species richness and evenness for ECM samples. Ectomycorrhizal root tips were counted for each morphotype in each soil core. Root tip abundance data were transformed using *log (x+1)* in order to reduce the effect of extreme abundances of some morphotypes (Krebs 1999). Univariate and multivariate analyses were performed using SAS Software, version 8 (SAS Institute, Cary NC). Effect of forest or season on total ectomycorrhizal abundance was analyzed using a one-way ANOVA. A multivariate analysis of variance (MANOVA) was performed with morphotypes that appeared in more than one core to determine any effects of forest and seasonal factors on ECM root tip abundance. Sclerotia data were analyzed using a one-way ANOVA with SAS Software (SAS Institute, Cary NC)

RESULTS

Sporocarp diversity

The sporocarps of two species of hypogeous fungi were collected in red spruce/Fraser fir (SF) plots, *E. granulatus* and *E. muricatus*. The only species found in northern hardwood (NH) plots was *E. muricatus*. In the first sample period, April through June of 2000, three plots in SF contained sporocarps, and two plots in NH contained sporocarps (Table 1).

Table 1. Number of plots with *Elaphomyces muricatus* sporocarps in spruce-fir and northern hardwood forests. Numbers in parentheses indicate numbers of sporocarps found total for each forest and sampling season.

Sampling period	Spruce-fir	Northern Hardwood
Plots with sporocarps		
2000	3 (14)	2 (7)
2001	3 (6)	3 (12)
Plots without sporocarps		
2000	12	13
2001	7	7

** One spruce-fir plot contained one Elaphomyces granulatus sporocarp during October 2000.*

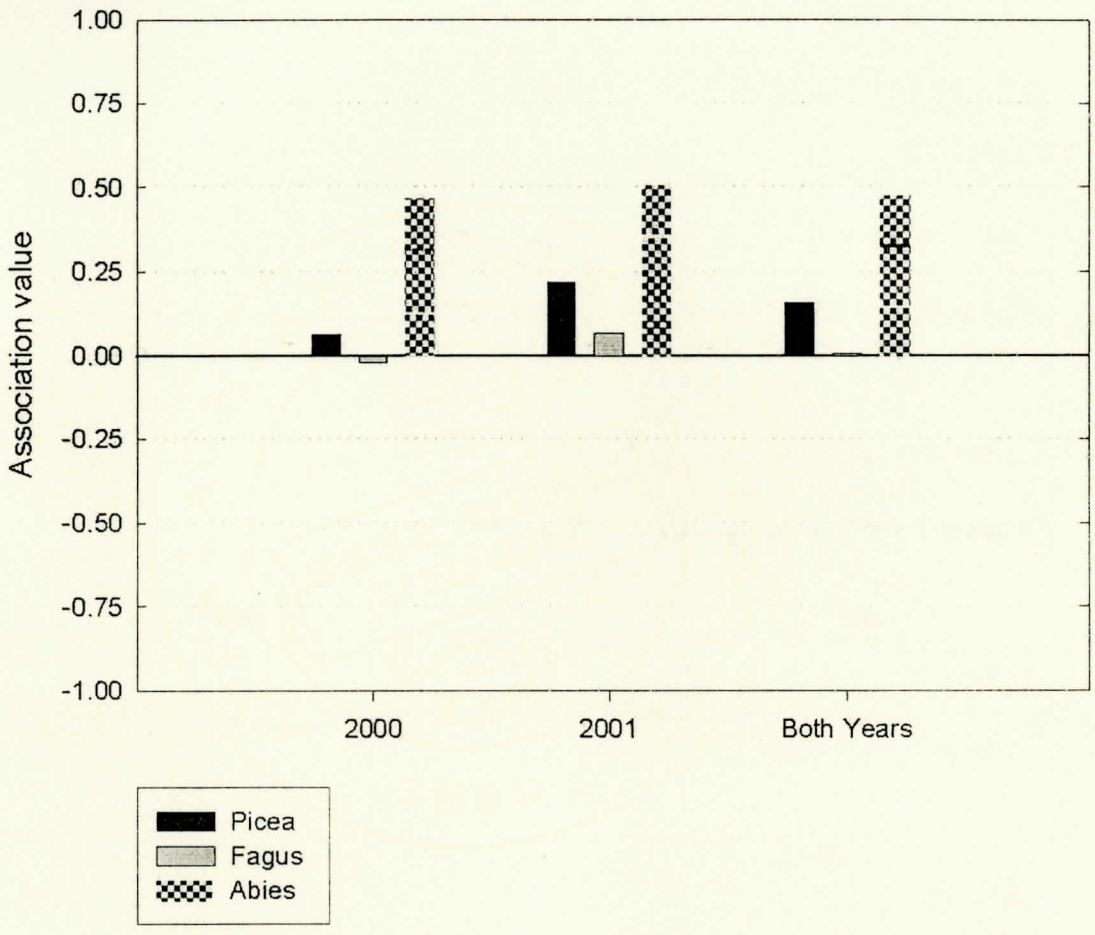
All five plots with sporocarps in the first sampling period contained *E. muricatus*. One sporocarp of *E. granulatus* was collected in SF during the attempted October sampling of 2000. During the second sampling, March through May of 2001, a total of six plots contained sporocarps. Three SF plots

and three NH plots contained *E. muricatus* in April 2001. There was no significant effect of sampling time (2000 vs. 2001) with forest type (SF vs. NH) nested on sporocarp biomass (df=3, F= 0.52, P=0.67). There was no significant effect of time with forest type nested on numbers of sporocarps (df=3, F=0.31, P=0.82).

An association statistic was used to determine associations between tree species and *E. muricatus* based on presence / absence data of each species (Fig. 1). All northern hardwood plots had at least one beech tree within one meter of the perimeter, and three had small (<6cm diameter) Fraser fir trees within one meter of the perimeter. All spruce-fir plots had Fraser fir within one meter of the perimeter, 6 plots had red spruce within one meter of the perimeter, and 3 had a beech tree within one meter of the perimeter.

A value of 1.0 indicates a strong positive association, whereas a value of –1.0 indicates a strong negative association. Values between –0.1 and 0.1 represent a neutral association. *Abies fraseri* and *E. muricatus* had the strongest association (2000= 0.47, 2001=0.50, combined=0.47) (Fig. 1). *Picea rubens* and *E. muricatus* show a neutral to slight positive association (2000= 0.063, 2001=0.22, combined= 0.16). *Fagus grandifolia* did not exhibit any association with *E. muricatus*, either positive or negative (2000= -0.02, 2001=0.07, combined=0.01

Figure 1. Association values of *Picea rubens*, *Abies fraseri*, and *Fagus grandifolia* with *Elaphomyces muricatus* sporocarps. Values are given in text. +1.0=obligate association, 0=no associations; -1.0=obligate exclusion.


1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
Association value
2000
2001
Both Years
Picea
Fagus
Abies

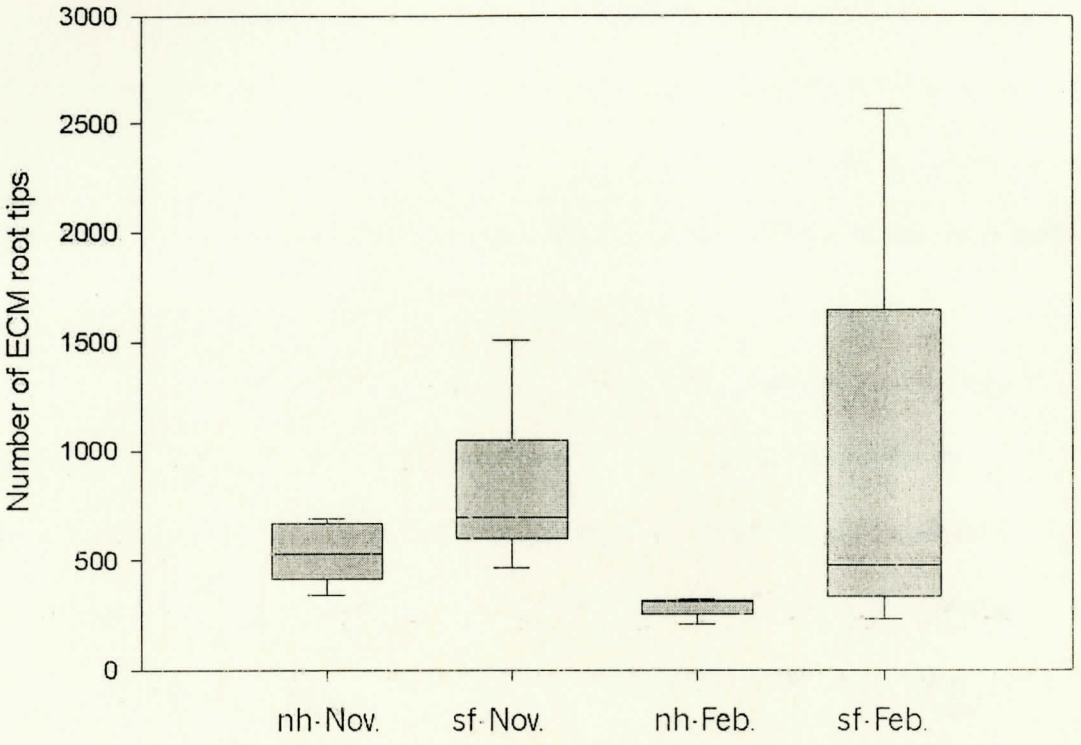
Ectomycorrhizal analysis

Ectomycorrhizas were found in all soil cores in both forests. The numbers of ectomycorrhizal root tips varied greatly between soil cores regardless of forest type (Figure 2). The range in numbers of root tips with ECM found in northern hardwood soil cores was from 211-691 root tips. The mean number of root tips with ECM found in northern hardwood soil cores was 410.5 (S.E. = 52.0). The range of root tips with ECM found in spruce-fir soil cores was 233-2566. The mean number of root tips with ECM in spruce-fir was 920.9 (S.E.=224.3). An abundance of morphotype 18 in one soil core greatly increased the range in the spruce-fir samples. With this sample omitted, a decrease in the number of ectomycorrhizal root tips from fall to spring was observed. More ectomycorrhizal root tips were found in soil cores from spruce-fir forests than northern hardwood forests.

Morphotype descriptions are given in Appendix I, using terminology from Agerer (1987-1993). Number identifications refer to morphotypes that were separated visually and with RFLP analysis. A MANOVA analyzing ECM abundance based on forest or season found a significant amount of variance explained by forest type (Wilk's Lambda df=8, F=7.9, P=0.0019). The difference between seasons was not statistically significant (Wilk's Lambda df=8, F=1.74, P=0.2038), however there was a trend of fewer ECM in spring than in fall.

Comparison of species diversity in each forest was analyzed by observing rank abundance (to compare evenness). All sampled communities had similar evenness, with one or two morphotypes dominating the soil cores, and many

Figure 2. Abundance of total ectomycorrhizal root tips in soil cores collected from northern hardwood and spruce-fir forests in November 1999 and February 2001. Boxplots are of numbers of root tips per soil core (n=5 for each forest/time combination). Shaded boxes represent the middle 50% of the distribution (inter-quartile range), with the horizontal line inside the boxes representing the mean. The outer horizontal lines represent the range of values. nh= northern hardwood forest, sf=spruce-fir forest.


Number of ECM root tips
3000
2500
2000
1500
1000
500
0
nh·Nov.
sf·Nov.
nh·Feb.
sf·Feb.

morphotypes appearing rarely (Fig. 3-6). During both sampling times, more morphotypes were apparent in spruce-fir soils, i.e., species richness was higher in spruce-fir. Fifteen morphotypes were found in the northern hardwood samples collected in November 1999, 12 morphotypes were found in northern hardwood samples collected in February 2001. Seventeen morphotypes were found in spruce-fir samples collected in November 1999, and 16 morphotypes were found in spruce-fir samples collected in February 2001. Most ECM were found in both forests, with 13 morphotypes in common in November 1999, and 12 morphotypes in common in February 2001. Northern hardwood forests had 2 unique morphotypes and spruce-fir forests had 4 unique morphotypes in November 1999. Northern hardwood forests had 1 unique morphotype and spruce-fir forests had 3 unique morphotypes in February 2001. *Cenococcum* and morphotype 18 were dominant taxa in spruce-fir soil cores, whereas *Cenococcum* and morphotype 3 appear as dominants in northern hardwood soil cores. Numbers of morphotypes were higher in spruce-fir and November 1999 samples. In this study, 14 of 26 ECM morphotypes were found in both forest samples, 4 were unique in northern hardwood samples, and 8 were unique in spruce-fir samples.

Seasonal and spatial patterns of the various morphotypes were very different. Some morphotypes, such as *Cenococcum*, displayed significant differences in abundance between sampling times, but not between forest types (Season df=1, F=6.89, P = 0.0178; Forest df=1, F=0.00, P = 0.9739) (Fig. 7). Other morphotypes, such as morphotype 18, exhibited differences between

Figure 3. Rank abundance of ECM morphotypes from northern hardwood samples collected in November 1999. Morphotype number is the assigned designator for ECM separated morphologically and by comparing RFLP patterns of the internal transcribed spacer region. Morphotype 1 is *Cenococcum geophilum*. The remaining morphotypes are unidentified.

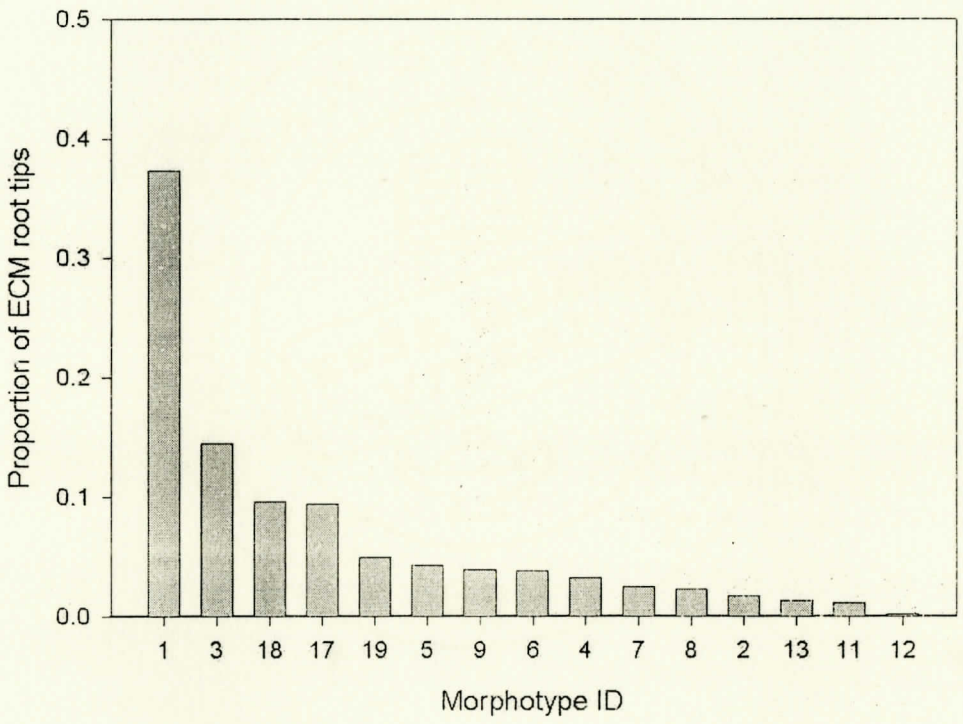

0.5
0.4
0.3
0.2
0.1
0.0
Proportion of ECM root tips
1
3
18
17
19
5
9
6
4
7
8
2
13
11
12
Morphotype ID

Figure 4. Rank abundance of ECM morphotypes from northern hardwood samples collected in February 2001. Morphotype number is the assigned designator for ECM separated morphologically and by comparing RFLP patterns of the internal transcribed spacer region. Morphotype 1 is *Cenococcum geophilum*. The remaining morphotypes are unidentified.

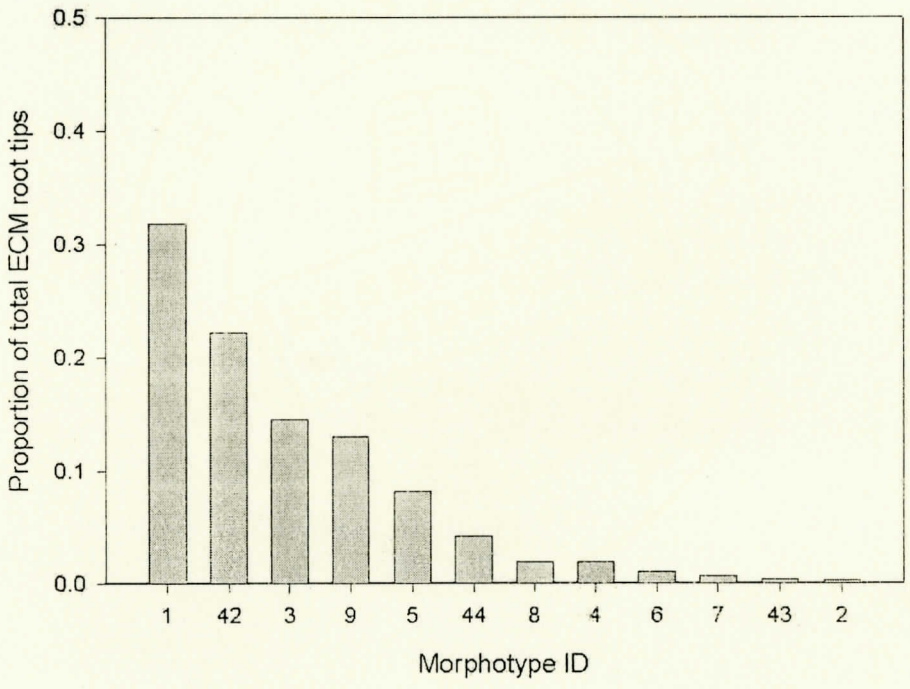

0.5
0.4
0.3
0.2
0.1
0.0
Proportion of total ECM root tips
1
42
3
9
5
44
8
4
6
7
43
2
Morphotype ID

Figure 5. Rank abundance of ECM morphotypes from spruce-fir forest samples collected in November 1999. Morphotype number is the assigned designator for ECM separated morphologically and by comparing RFLP patterns of the internal transcribed spacer region. Morphotype 1 is *Cenococcum geophilum*. The remaining morphotypes are unidentified.

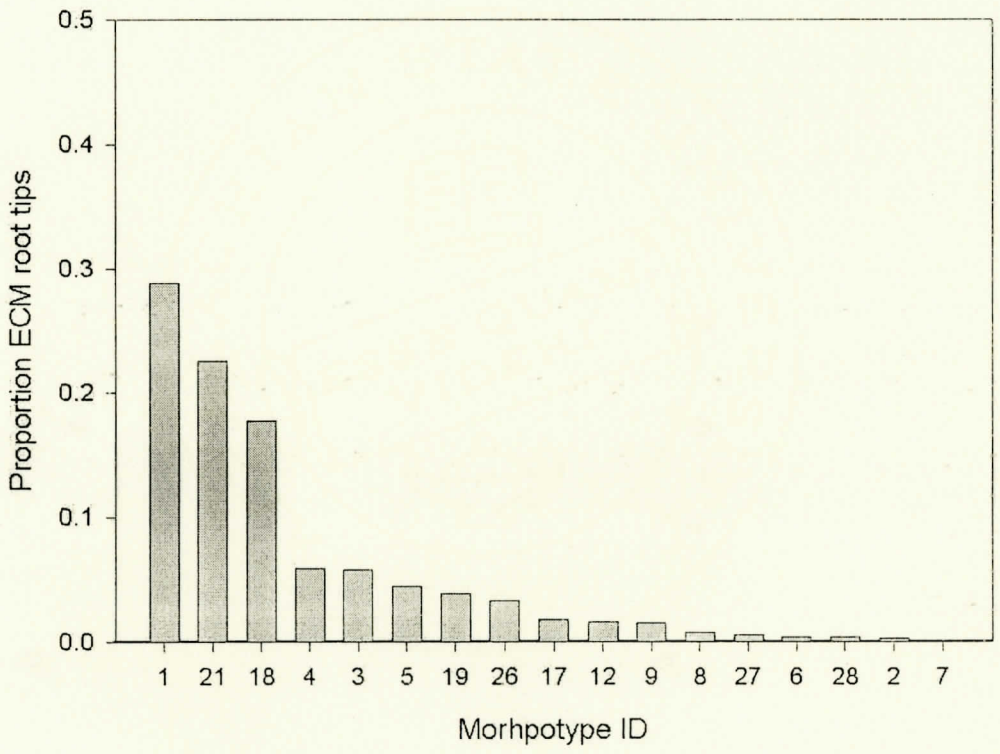

0.5
0.4
0.3
0.2
0.1
0.0
Proportion ECM root tips
1
21
18
4
3
5
19
26
17
12
9
8
27
6
28
2
7
Morhpotype ID

Figure 6. Rank abundance of ECM morphotypes from spruce-fir forest samples collected in February 2001. Morphotype number is the assigned designator for ECM separated morphologically and by comparing RFLP patterns of the internal transcribed spacer region. Morphotype 1 is *Cenococcum geophilum*. The remaining morphotypes are unidentified.

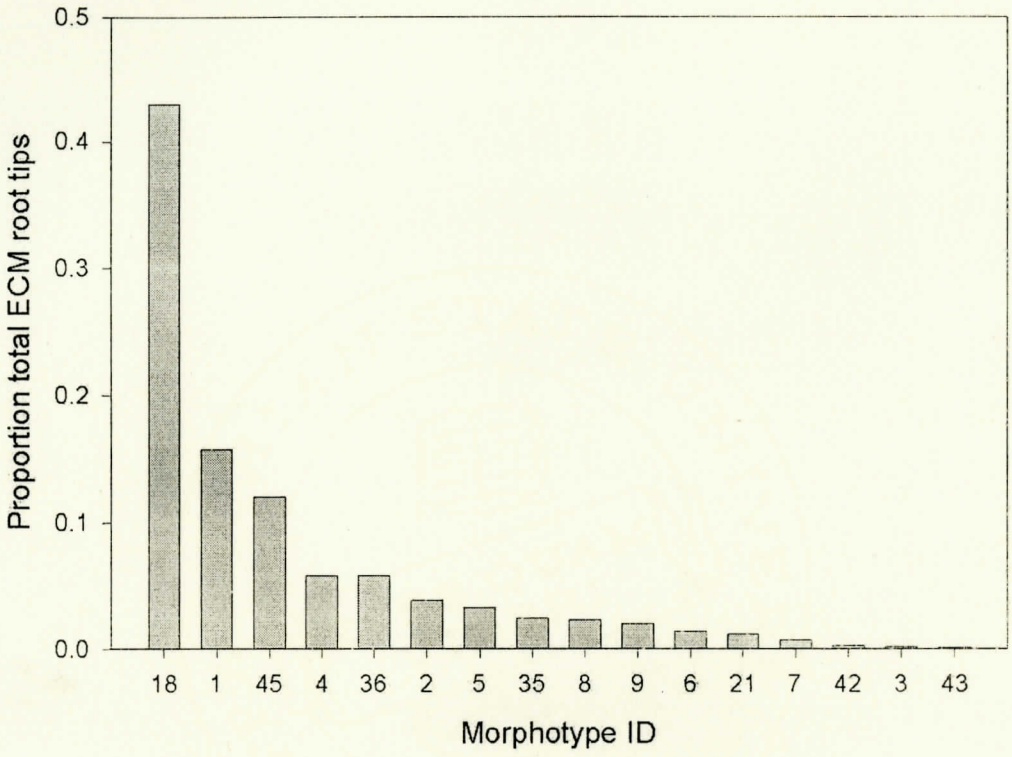
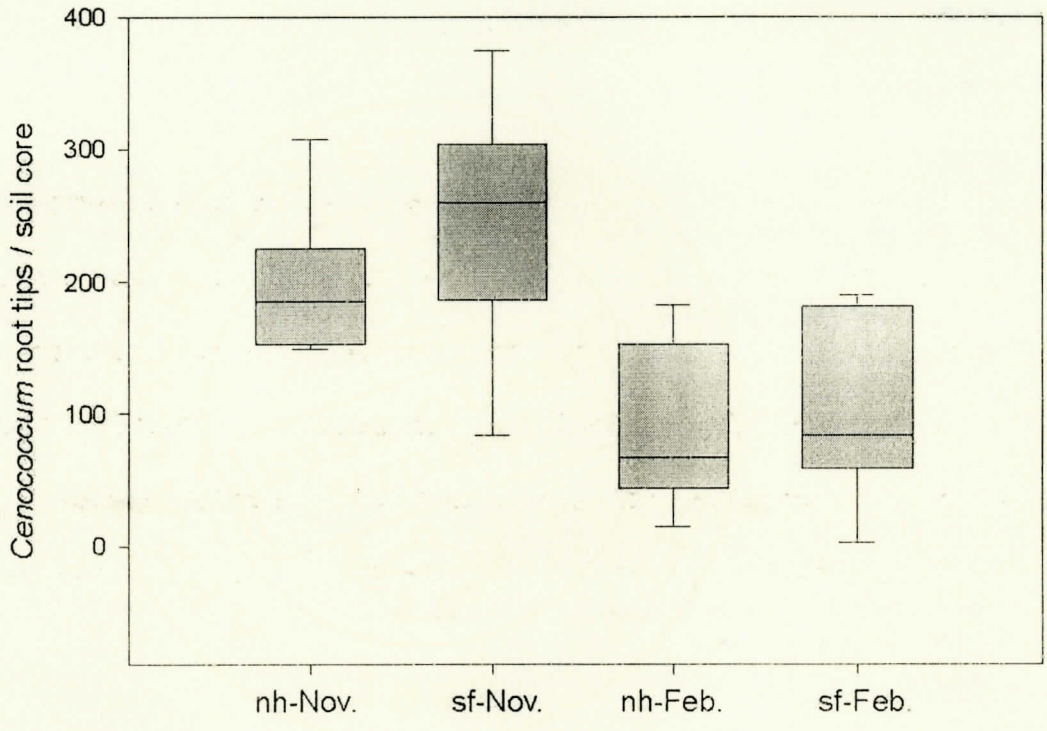

0.5
0.4
0.3
0.2
0.1
0.0
Proportion total ECM root tips
18
1
45
4
36
2
5
35
8
9
6
21
7
42
3
43
Morphotype ID

Figure 7. *Cenococcum* ECM abundance in soil cores collected from northern hardwood and spruce-fir forests in November 1999 and February 2001. Boxplots are of numbers of root tips per soil core (n=5 for each forest/time combination). Shaded boxes represent the middle 50% of the distribution (inter-quartile range), with the horizontal line inside the boxes representing the mean. The outer horizontal lines represent the range of values. nh= northern hardwood forest, sf=spruce-fir forest.


400
300
200
100
0
Cenococcum root tips / soil core
nh-Nov.
sf-Nov.
nh-Feb.
sf-Feb.

forest types (df=1, F=35.97, P= <0.0001), rather than season (df=1, F=1.01, P=0.3288) (Fig. 8 and 9).

Morphotype 21(M21) was only found in spruce - fir forests. There was a significant difference between forest type (df=1, F=12.64, P = 0.0024) but not season, although the effect of season was nearly significant (df=1, F=3.68, P = 0.0720). Most M21 root tips were collected in November 1999 (Fig. 10). Morphotype 3 was found in both forests in November 1999 and only the northern hardwood forest in February 2001. Forest and season effects were marginally significant (Forest df=1, F=3.78, P = 0.0684; Season df=1, F=4.33, P = 0.0529) (Fig. 11). Morphotypes 4, 5, 6, and 9 showed no statistically significant effect of either forest or season. Data for morphotype 5 are displayed in figure 12.

Abundance of sclerotia

All sclerotia found in soil samples were *Cenococcum. Cenococcum* sclerotia were more abundant in samples from spruce-fir forests than northern hardwood forests (df=38, F= 7.10, P=0.01) (Fig. 13). Mean sclerotia abundance in northern hardwood and spruce-fir forests was 19 per 15 mL sample and 29 per 15 mL sample respectively.

RFLP analysis of hypogeous species in the ECM community

In order to account for possible variability of the internal transcribed spacer region of *Elaphomyces*, I compared the RFLP patterns of fruiting bodies I collected on Roan Mountain across plots, and voucher specimens from the

Figure 8. Abundance of morphotype 18 in soil cores collected from northern hardwood and spruce-fir forests in November 1999 and February 2001. Boxplots are of numbers of root tips per soil core (n=5 for each forest/time combination). Shaded boxes represent the middle 50% of the distribution (inter-quartile range), with the horizontal line inside the boxes representing the mean. The outer horizontal lines represent the range of values. nh= northern hardwood forest, sf=spruce-fir forest.

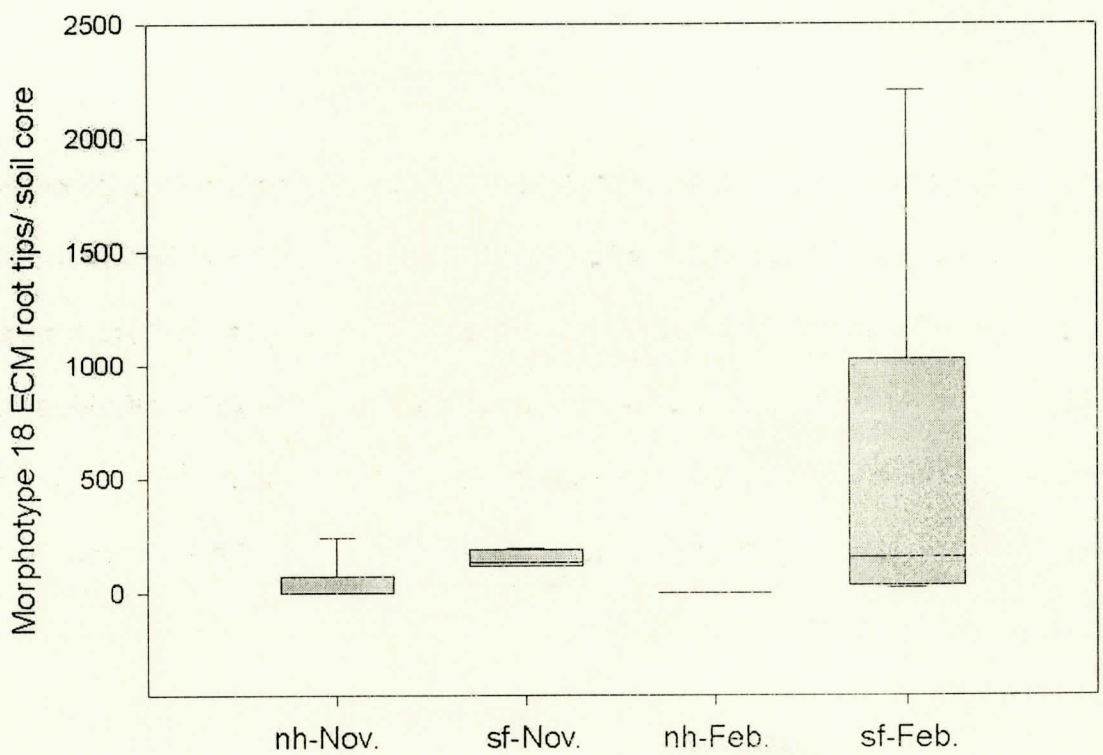

Morphotype 18 ECM root tips/ soil core
2500
2000
1500
1000
500
0
nh-Nov.
sf-Nov.
nh-Feb.
sf-Feb.

Figure 9. Morphotype 18 ECM root tips abundance in soil cores collected from November 1999 and February 2001, with largest sample from February 2001, spruce-fir omitted. Boxplots are of numbers of root tips per soil core (n=5 for each forest/time combination). Shaded boxes represent the middle 50% of the distribution (inter-quartile range), with the horizontal line inside the boxes representing the mean. The outer horizontal lines represent the range of values. nh= northern hardwood forest, sf=spruce-fir forest.

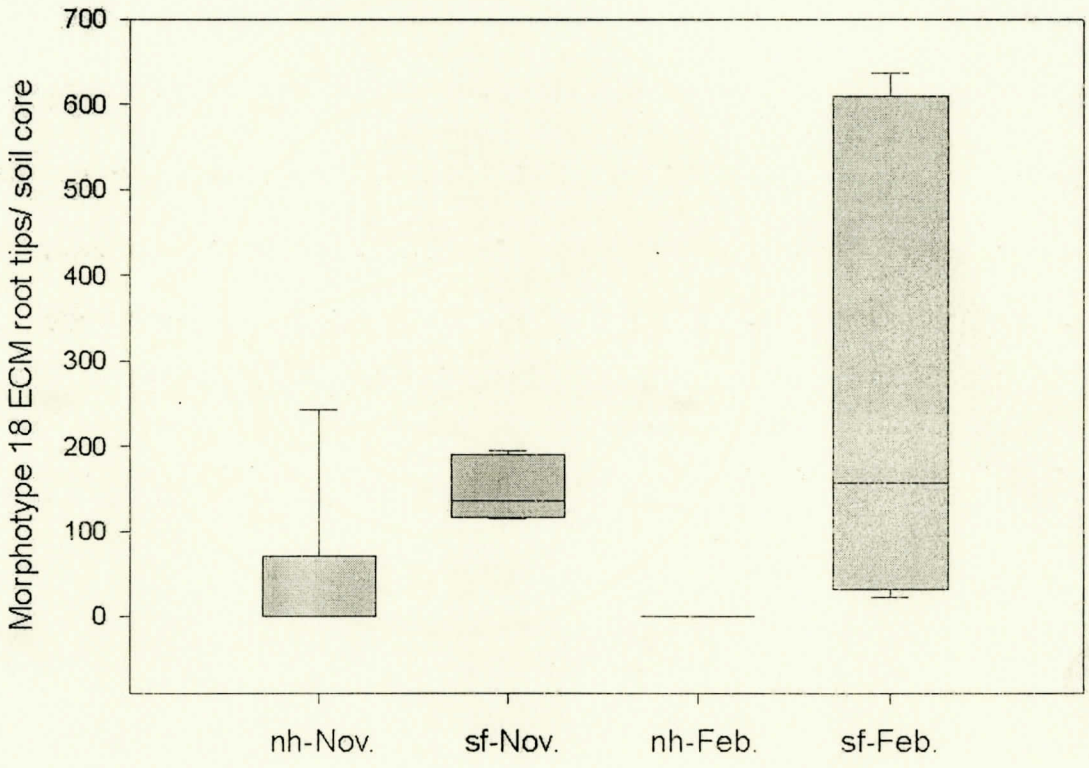

Morphotype 18 ECM root tips/ soil core
700
600
500
400
300
200
100
0
nh-Nov.
sf-Nov.
nh-Feb.
sf-Feb.

Figure 10. Morphotype 21 ECM root tip abundance in soil cores collected from northern hardwood and spruce-fir forests in November 1999 and February 2001. Boxplots are of numbers of root tips per soil core (n=5 for each forest/time combination). Shaded boxes represent the middle 50% of the distribution (inter-quartile range), with the horizontal line inside the boxes representing the mean. The outer horizontal lines represent the range of values. nh= northern hardwood forest, sf=spruce-fir forest.

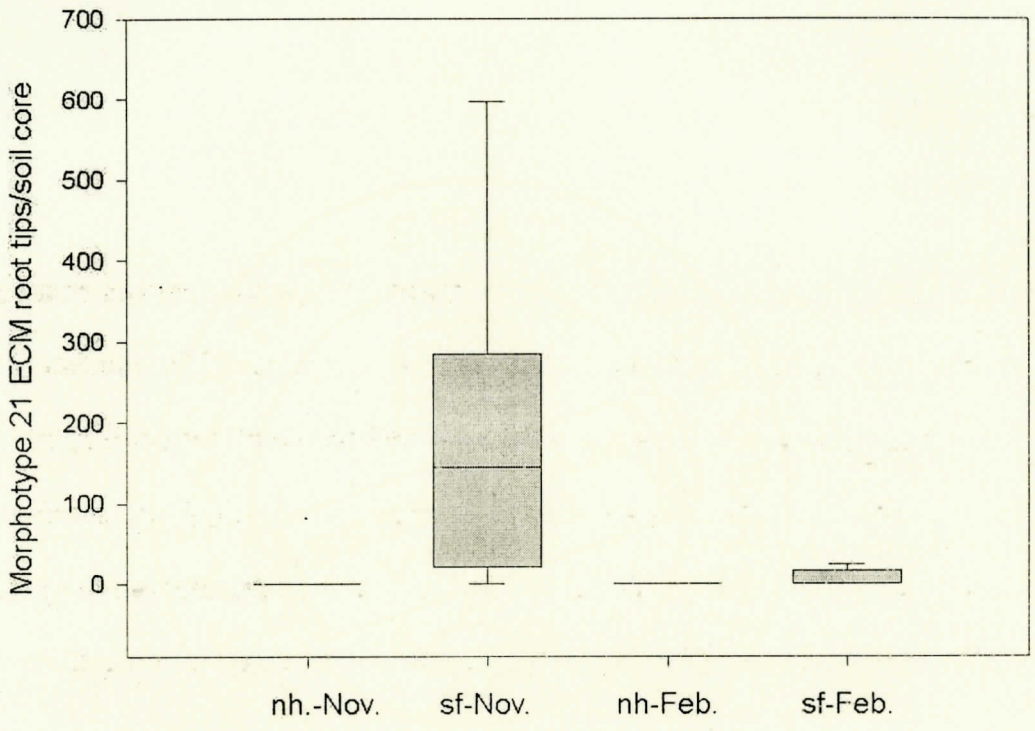

Morphotype 21 ECM root tips/soil core
700
600
500
400
300
200
100
0
nh.-Nov.
sf-Nov.
nh-Feb.
sf-Feb.

Figure 11. Morphotype 3 ECM root tips abundance in soil cores collected from northern hardwood and spruce-fir forests in November 1999 and February 2001. Boxplots are of numbers of root tips per soil core (n=5 for each forest/time combination). Shaded boxes represent the middle 50% of the distribution (inter-quartile range), with the horizontal line inside the boxes representing the mean. The outer horizontal lines represent the range of values. nh= northern hardwood forest, sf=spruce-fir forest.

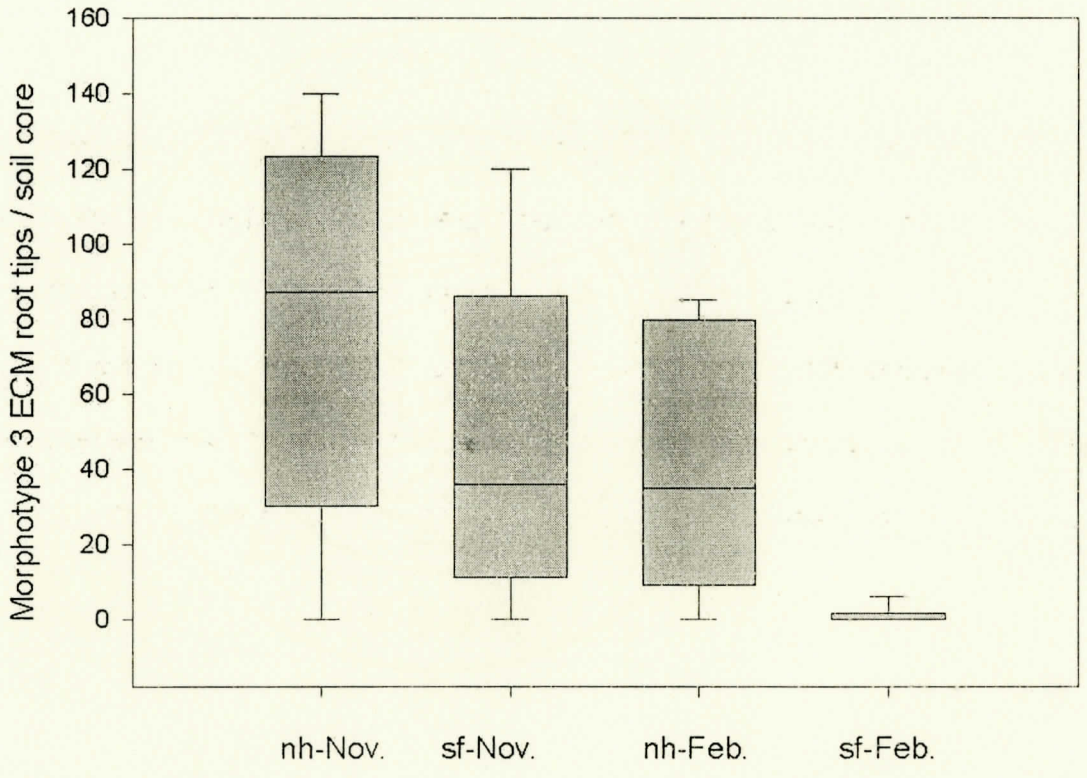

Morphotype 3 ECM root tips / soil core
160
140
120
100
80
60
40
20
0
nh-Nov.
sf-Nov.
nh-Feb.
sf-Feb.

Figure 12. Morphotype 5 ECM root tip abundance in soil cores collected from northern hardwood and spruce-fir forests in November 1999 and February 2001. Boxplots are of numbers of root tips per soil core (n=5 for each forest/time combination). Shaded boxes represent the middle 50% of the distribution (inter-quartile range), with the horizontal line inside the boxes representing the mean. The outer horizontal lines represent the range of values. nh= northern hardwood forest, sf=spruce-fir forest.

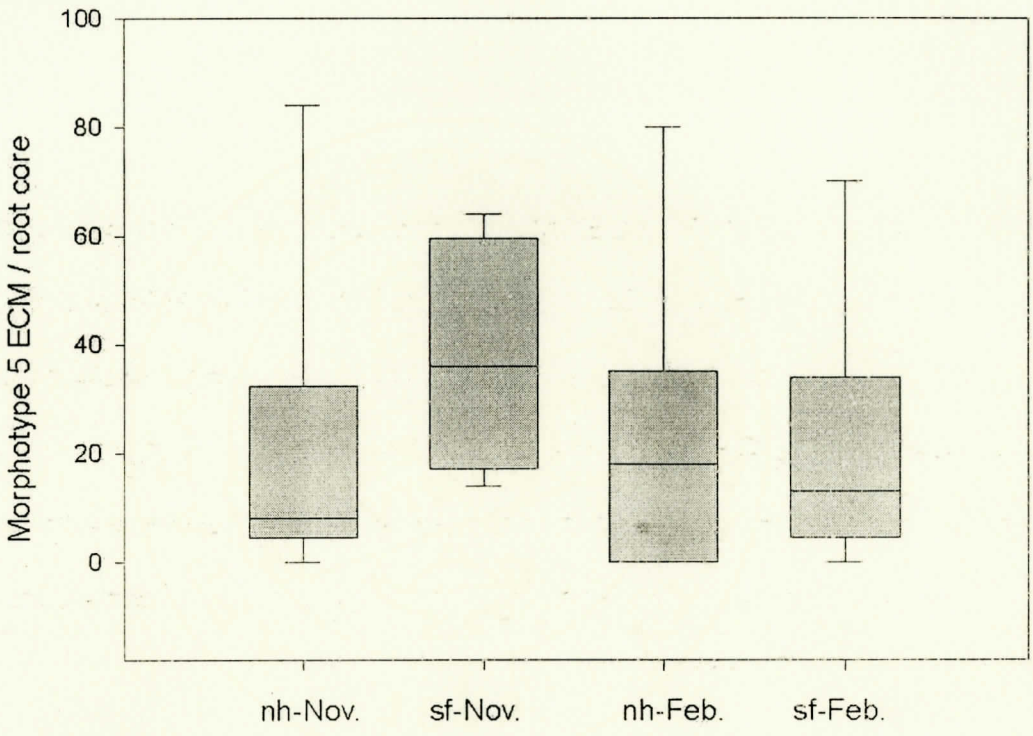

Morphotype 5 ECM / root core
100
80
60
40
20
0
nh-Nov.
sf-Nov.
nh-Feb.
sf-Feb.

Figure 13. Abundance of *Cenococcum* sclerotia in soil cores collected spruce-fir and northern hardwood forests. All samples are from February 2001. Boxplots are of numbers of sclerotia per 15 cm^3 root tips per soil core (n=20 for each forest). Shaded boxes represent the middle 50% of the distribution (inter-quartile range), with the horizontal line inside the boxes representing the mean. The outer horizontal lines represent the adjacent values. The black dots represent outliers. nh= northern hardwood forest, sf=spruce-fir forest.

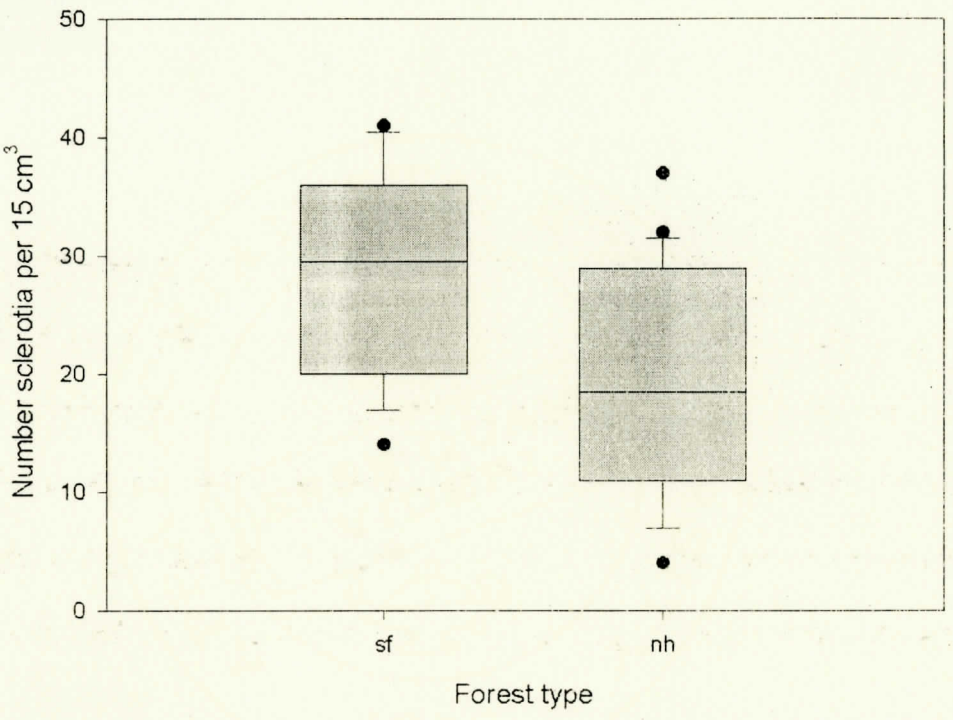

50
40
30
20
10
0
Number sclerotia per 15 cm3
sf
nh
Forest type

Figure 14. Gel of *Elaphomyces muricatus* sporocarps collected on Roan Mountain: Lanes 1 and 11: *Phi*X174/*Hin*dIII size marker, lanes, 2, 5, 8, 12, 15= cut with *Hinf* I; Lanes 3, 6, 9, 13, 16; cut with *Alu* I; lanes 4,7,10,14,17 uncut amplification products.

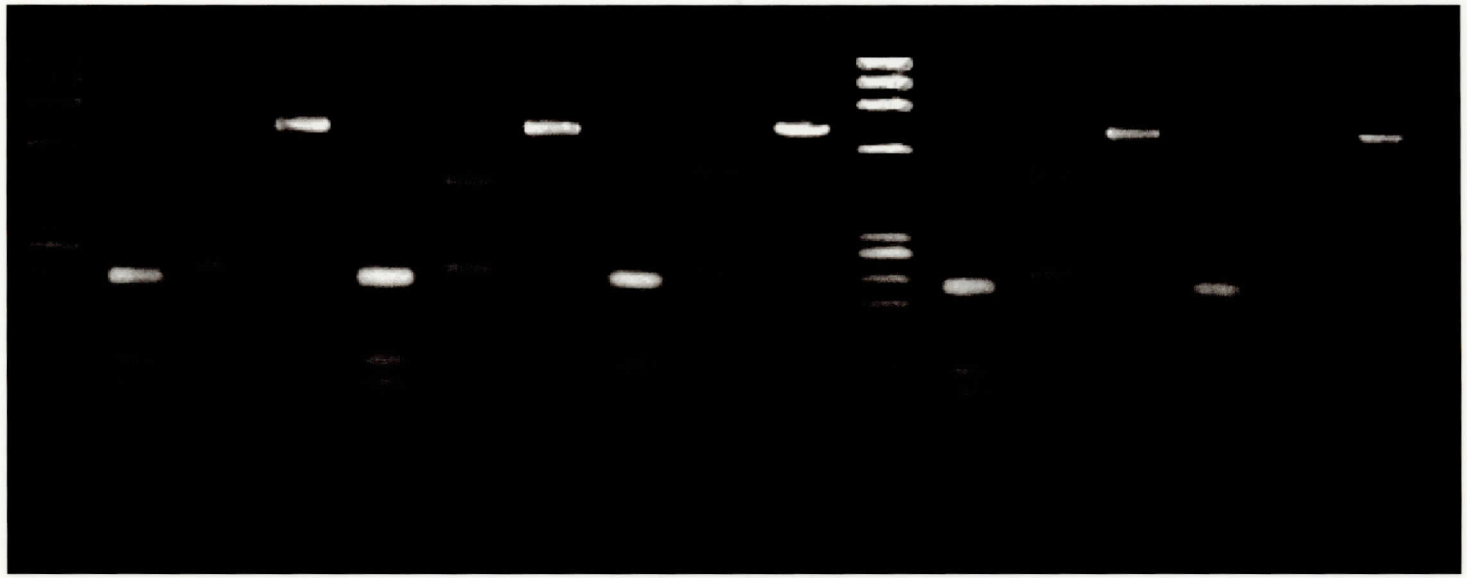

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Figure 15. Close up of gel with *Alpova* sp. and *Scleroderma* sp.. Lane 1: *Phi*X174/*Hin*dIII size marker. Lane 2, uncut *Elaphomyces* species. Lanes 3,4,5 *Alpova* sp. Lane 3, *Hin*fI; lane 4 *Alu*I; lane 5 uncut amplification product. Lane 6,7,8 *Scleroderma* sp. Lane 6 *Hin*fI, lane 7 *Alu*I, lane 8 uncut amplification product.

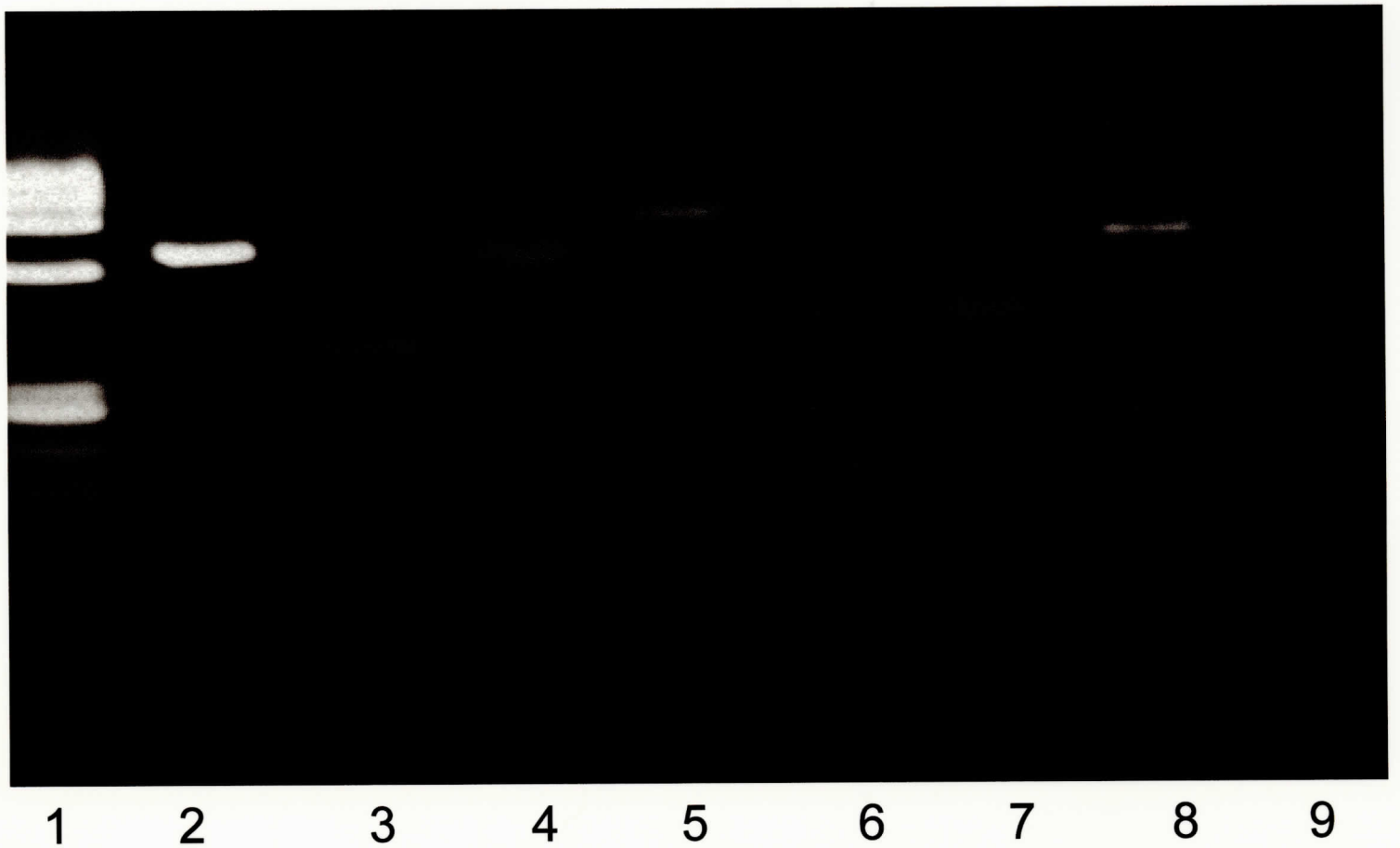
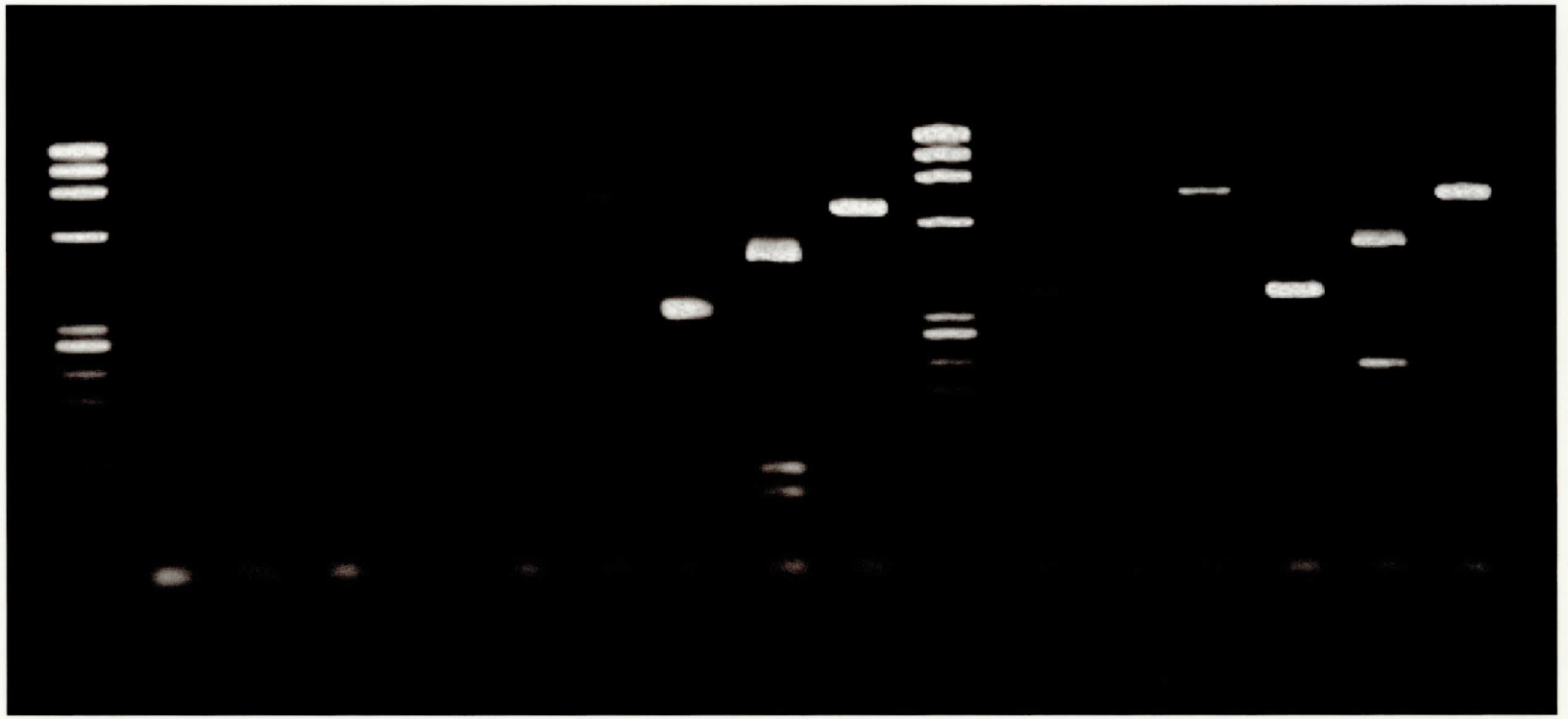

1
2
3
4
5
6
7
8
9

Figure 16. Gel of morphotypes (2nd gel): Lanes 1 and 11: *Phi*X174/*Hin*dIII size marker, lanes, 2,5,8,12,15= cut with *Hin*fI; Lanes 3,6,9,13,16; cut with *Alu* I; lanes 4,7,10,14,17 uncut amplification products. Samples are from northern hardwood forest, morphotype 7 (lanes 2-4); morphotype 8(lanes 5-7); morphotype 9 (lanes 8-9), morphotype 10(lanes 12-14: probably contamination from 11); morphotype 11 (lanes 15-17).


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Loeb et al. (2000) study. All of the RFLP patterns were the same (Fig. 14, Appendix II). *Alpova* and *Scleroderma* RFLP's were different from either *Elaphomyces* species (Fig. 15).

RFLP comparisons confirmed the visual separation of many morphotypes (Fig. 16, Appendix II), and resulted in the combining of a few others, such as Morphotypes 19 and 20 (Appendix II). Faint RFLP bands were not included in the comparisons. The lack of species richness in sporocarp collections limited my ability to identify the species involved in ECM associations. However, I was able to determine that neither *E. muricatus* nor *E. granulatus* were present among ECM root tips. Restriction fragment length polymorphisms of *Alpova* and *Scleroderma* (sporocarps from Loeb et al. 2000) did not match any RFLP's from ECM root tips.

DISCUSSION

Sporocarp diversity

The null hypothesis for the first objective, that hypogeous fungal diversity is the same in northern hardwood and spruce-fir forests, was not rejected in this study. The presence of sporocarps representing only one genus of hypogeous fungus was unexpected. Previous studies by Weigl et al. (1999) on Roan Mountain found CNFS consumed at least 6 genera of hypogeous fungus, based on fecal analysis. Those genera that were found in CNFS fecal samples but not in plots I sampled might produce sporocarps during late summer or fall. Loeb et al. (2000) only found *E. granulatus* on Roan Mountain but not *E. muricatus*. My study was different from that conducted by Loeb et al. (2000) in that I collected sporocarps in two years, from March-June (2000 and 2001). Loeb et al. (2000) collected one year during mid-summer. This suggests that there may be seasonal differences in which species are dominant.

Sporocarp and tree species association

Luoma et al. (1991) found more *E. granulatus* and *E. muricatus* in spring collections in Douglas fir forests. Luoma et al. (1991) found that *E. granulatus* was a much more abundant sporocarp than *E. muricatus*. The one *E. granulatus* sporocarp that I found was in October 2000, near red spruce trees. The other

sporocarps I found were *E. muricatus,* near red spruce, beech, and Fraser fir trees. The association values indicate a moderately positive association between *E. muricatus* and *Abies fraseri.* This contrasts with the results of Loeb et al. (2000). They found that the presence of *E. granulatus* was best explained by red spruce (*Picea rubens*). These differences in findings may represent a species-specific association between *E. granulatus / Picea rubens* and *E. muricatus / Abies fraseri.* All *E. muricatus* sporocarps were found on the TN, north facing side of the mountain. This correlates with the general description of habitat given by Weigl et al. (1999) for CNFS. The one *E. granulatus* sporocarp was found on the NC, south facing side.

Although a moderate association was found between *E. muricatus* and *A. fraseri* in this study, *E. muricatus* and *E. granulatus* are common sporocarps and are found across a wide range of forest types in the Northern Hemisphere (Zhang and Mintner 1989, Trappe 1979). They are each associated with several genera of trees. Studies have found both *Elaphomyces* species fruiting in association with conifers such as Douglas fir (Luoma et al. 1991), red spruce (Loeb et al. 2000), and fir species (Zhang and Mintner 1989), as well as with hardwood species, such as *Quercus* (Trappe and Guzman 1971). My results suggest that *Elaphomyces* species are generalists with respect to host tree. The moderate association found between *E. muricatus* and *Abies fraseri* may indicate a preference for Fraser fir as a host, but not an obligate association between these two species. Continued sampling of root tips may allow for the vegetative

portion of *Elaphomyces* species to be found at the root level, which would clarify with which host tree(s) *Elaphomyces* species form ECM

Miller (1986) documented several *Rhizopogon* species occurring in the southeastern United States. These *Rhizopogon* species have been found in association with spruce and fir trees in the west, and others have found these species with conifers such as *Tsuga canadensis*, a common tree in the Southern Appalachians (Miller 1986). Weigl et al. (1999) found spores from the genus *Rhizopogon* were found in CNFS feces. I did not find *Rhizopogon* in either forest type I sampled, however their presence cannot be ruled out. The most likely explanation for the paucity of hypogeous fungal species recovered in my study was the small area sampled and the duration of this study. Typical studies documenting sporocarp occurrence have involved sampling from 100 m^2 to 8064 m^2 (e.g., Loeb et al. 2000; States and Gaud 1997; Luoma et al. 1991; Waters et al. 1997). I sampled approximately 100 m^2, which is at the lower end of this range. Fungal sporocarps are not produced every year by all species. Estimates suggest that as many as eight years of sampling is necessary to document all species which are in an area (States and Gaud 1997). I sampled during two years.

The presence of *E. muricatus and E. granulatus* in both northern hardwood and spruce-fir forests indicates that CNFS may forage in both of these forest types. However, it is also possible that the fruiting bodies that are in the spruce-fir forest obtain different chemical components than those in the northern hardwood forests, due to the difference in tree hosts. Carbohydrates from tree

hosts are transferred through mycorrhizas, possibly to fruiting bodies. Differences in chemical composition may alter the nutritional value or palatability of the sporocarps. North et al. (1997) found *Elaphomyces* was not as preferred as other species such as *Rhizopogon subcaerulescens* or *Truncocolumella citrina*. Cork and Kenagy (1989) found *Elaphomyces* could not sustain the golden-mantled ground squirrel (*Spermophilus saturatus*) due to the low amount of digestible nitrogen.

I expected to find sporocarps of *Geopora* in this study, based on the very high frequency of spores (>90%) found in CNFS scat by Weigl et al. (1999). However, no *Geopora* sporocarps were found. It is possible that these sporocarps were not fruiting during the years I sampled, or they were not fruiting in the plots I sampled. However, no reports of *Geopora* in the southeastern United States have been found, and some suspect that spores of *Geopora* have been confused with the eggs of a parasite that look like *Geopora* spores (Mitchell Donna personal communication).

Ectomycorrhizal analysis

The uneven distribution of species in each forest type is similar to ectomycorrhizal communities found in other studies. Typically, one to two species dominate, and the remaining morphotypes are much less abundant (Gehring et al. 1998; Horton et al. 1999; Horton and Bruns 2001). The increase in morphotype number in November 1999 is to be expected, due to overall warmer temperatures. The similarity of the two forest types in terms of species

presence, however, was not expected. Beech (*Fagus grandifolia*) was the dominant tree in hardwood forests and red spruce and Fraser fir dominated the spruce-fir forests. Bills et al. (1986) found little similarity in epigeous sporocarps of ECM fungi produced in a red spruce versus a northern hardwood forest. Of 54 species collected, only 8 were in common to both forests. Sampling at the root level in my study revealed a greater similarity between the two forests in terms of species composition.

Previous studies describing ECM communities have often found *Cenococcum* as a frequent component, but not as a dominant species. In this study, *Cenococcum* was the most dominant species in northern hardwood forests and spruce-fir forests in fall. One extreme sample of morphotype 18 moved *Cenococcum* into second most dominant for spruce-fir samples in spring. Meier (1989) found *C. geophilum* in all samples of red spruce roots from the Southern Appalachians, however it was not the most abundant ECM in any sample. Horton et al. (1999) found *C. geophilum* in the majority of their cores (10 of 12). However, it never dominated in terms of biomass.

Byrd et al. (2000) found *Cenococcum* as a dominant ECM symbiont in clear-cut lodgepole pine forests that had undergone regrowth for eight years. *Cenococcum* was also present in undisturbed lodgepole pine sites, although not as a dominant species (Byrd et al. 2000). *Cenococcum* has been found in both the northern and southern hemispheres, and in a variety of ecosystems, ranging from subtropical forests to arctic forests. It is often a pioneer species that colonizes after a variety of disturbances, including clear-cutting and volcanic

eruptions (LoBuglio 1999). *Cenococcum* has long been described as a generalist fungus; it has been found with most trees that can form ectomycorrhizas (LoBuglio 1999).

Cenococcum geophilum is a mitosporic species, which has not been found to reproduce sexually. The species forms sclerotia, hard spheres of hyphae that can over winter in an area, but are generally not dispersal agents (LoBuglio 1999). Although mycophagous mammals may disperse the sclerotia inadvertently, they are generally not thought to be a food for the mammals (LoBuglio 1999). The larger amount of sclerotia found in the spruce-fir forest in my study is probably due to environmental variables, such as temperature and moisture; the colder temperature of the spruce-fir forest may stimulate production of these over wintering structures.

Researchers have found similar ECM morphotypes in association with red spruce and Fraser fir trees as I found in this study. Gibson (1979) found the same ECM associated with Fraser fir that I found associated with spruce fir and northern hardwood forests. Morphotype 18 from my study is similar to Gibson's "Brown Type Two," which lacked a distinctive mantle, and was found in plantations of Fraser fir in Gibson's study (1979). Morphotype 6, which I found in small amounts in cores from both the northern hardwood and spruce fir forests, was similar to Gibson's "White Type 2." Gibson also found *Cenococcum* present on Fraser fir roots, which he described as common in all treatments. Morphotypes 4 and 5 may be the same as Gibson's "Pale Brown Type," although the lack of color photographs hinders this comparison.

Meier (1989) found other morphotypes on red spruce similar to those found in this study. *Cenococcum* was present as a common ECM, however one described as "tannish brown" was the most common ECM of all samples (Meier 1989). The lack of photographs greatly impedes comparisons, however it could be similar to morphotypes 4, 5, or 21.

Hypogeous fungi as ECM symbionts.

The common hypogeous fungi *Elaphomyces granulatus*, *E. muricatus*, *Alpova* sp. and *Scleroderma* sp. were not present as dominant ECM symbionts in soil cores from either spruce-fir forests or northern hardwood forests, based on RFLP comparisons. There are several possible reasons for this result. Ectomycorrhizas may be patchily distributed in both forests and thus were underrepresented in my samples. Ectomycorrhizas of *Elaphomyces muricatus* and *E. granulatus* may be present in one or both of these forests, but were missed in this study. Morphotype 11, which was not matched with any sporocarp RFLP's, does have a similar morphology to some *Rhizopogon* species. Sequencing of morphotype 11's DNA would allow for a more reliable identification.

The dominance of *Cenococcum*, morphotype 18, morphotype 3, and others in both forest types may prevent *E. muricatus* and *E. granulatus* from gaining access to abundant numbers of root tips. *Elaphomyces* appeared to be a common fruiter and a rare ECM symbiont on Roan Mountain, as *Suillus pungens* is in California *Pinus muricata* forests (Gardes and Bruns 1996). The

rarity of these fungi as ECM symbionts may be due to competition with other fungi, or perhaps they are so efficient at obtaining carbohydrates from their tree hosts they only need a few connections (Bruns 1995). The carbohydrate resources needed for formation of ECM and hypogeous sporocarps need to be further explored.

Alpova sp. and *Scleroderma* sp. were not found in this study, either as sporocarps or ECM symbionts. These two species are present in other high elevation forests of the Southern Appalachians (Loeb et al. 2000), which suggests they may be present in the forests sampled in this study. Again, the area sampled and duration of this study may have prevented their detection.

Ectomycorrhizal community research

The complexity of a community of symbiotic organisms such as ectomycorrhizas is tremendous. The genetics of the host tree affects the formation of ectomycorrhizas in one fashion, and the fungal symbiont also has genetic variability in its ability to form connections with the host tree, to compete with other ECM fungi, to obtain photosynthates from the tree host, and to convey phosphorous, nitrogen, and water to the tree host (Smith and Read 1997). The variety of spatial colonization methods by the trees and fungi involved in ectomycorrhizal symbioses may explain the different distributions found for the various ECM morphotypes in this study. Spore dispersal mechanisms of different fungal symbionts vary from wind dispersed to animal dispersed. Some ECM fungi, such as *Cenococcum,* do not produce spores and appear to rely on

vegetative growth for dispersal. Wind dispersed spruce and fir seeds distribute differently than animal dispersed beech seeds. All of these factors may affect the associations found in ectomycorrhizal communities.

These intricate relationships between fungi and trees are further complicated when one considers the actual nature of the rhizosphere: a tight mat of root tips from a variety of trees, dozens of fungal ECM symbionts, as well as fungivorous arthropods, bacteria, and other soil fungi in a large heterogeneous mixture with decaying litter. The immense number of interactions that must be occurring in even the smallest sample of soil is daunting. Understanding these interactions at an ecosystem level will require many focused studies. This and other similar studies are the beginning of an understanding of the complex rhizosphere in spruce-fir and northern hardwood forests of the Southern Appalachian Mountains.

The community of fungi present as mycorrhizal symbionts must be sampled both in terms of fruiting and vegetative structures. This provides understanding of each fungus throughout its life cycle. Some species might be more efficient receiving photosynthates in the symbiosis, producing abundant sporocarps with few mycorrhizal connections. Others may not be as efficient at the uptake of carbohydrates, producing few sporocarps or requiring many ECM connections. Other fungal species may provide essential nutrients for specific tree hosts resulting in increased forest health. For many years, the difficulty of identifying ECM in their vegetative state has limited data collection to total numbers of ECM, rather than determining the roles of various fungal species in

an ECM community. Community ecologists have long known that various animals and plants play different roles in communities. The molecular methods refined by others and used in this study allowed more delineation of the variety of fungal species present as ECM (Horton and Bruns 2001). Continued research of ectomycorrhizal fungi will allow individual species' roles to be described, rather than assuming the same niche for all fungi which form mutualistic associations with trees.

The tripartite interaction. OR *What about the squirrel?*

The tripartite interaction between rodents, trees, and fungi may not be as tightly coupled in this ecosystem as it is in Pacific Northwest forests. The lack of matching ECM for hypogeous species found in this study suggests that CNFS are not playing a large role in spore dispersal of ECM for either forest. The lack of understanding of *Elaphomyces* as a symbiont for the tree hosts involved limits this statement however, because it may in fact be that *Elaphomyces* contributes something to the tree health that other ECM are not able to provide. The unidentified ECM morphotypes may match other genera of hypogeous fungi that were found in CNFS scat; future work to sequence the DNA of the morphotypes will allow more meaningful identifications.

The genera of fungi found in CNFS scat are all presumed to be mycorrhizal fungi. Mycorrhizal fungi are assumed to require connections with plant hosts to obtain carbohydrates. Therefore, it can be deduced that even though they were not found in this study, these fungi are fruiting in one or both

forests and are connected to some of the trees. However, this study indicates that these hypogeous fungi are not dominant in terms of abundance. The dominance of an ectomycorrhizal species should not be confused with its importance in the ecosystem (Bruns 1995). The roles of individual species of fungi in promoting forest health are not well understood.

Mycophagous mammals also consume epigeous species (above-ground fruiting), of which many are presumed to be ectomycorrhizal (Fogel and Trappe 1978). However, these are usually less abundant throughout the year, as above ground temperatures and moisture levels are subject to more extreme variation than underground. Most epigeous species rely on wind for spore dispersal and do not need to be extracted from the soil by a mammal. The effect of mycophagous mammals on epigeous species of fungi needs to be explored further.

Future Research

The anthropogenic influence on Roan Mountain has been very high over the past century. The early 1930's saw the removal of all large trees from the mountaintop, and the continued impact of visitors since the creation of the recreation area is hard to ignore. In order to better understand this fragile ecosystem, it is very important to understand the community dynamics of the organisms that inhabit the high elevation forests. Hypogeous fungi may not be dominant members of the vegetative ECM community, but the effect *Elaphomyces* has on a tree has not been examined. They may be more efficient

than other fungi at providing some vital nutrient for the host. The role of *Cenococcum geophilum* in these forests also needs to be determined. Greenhouse studies comparing the effectiveness of *C. geophilum* versus other native and non-native ECM would be very enlightening. Do spruce-fir and northern hardwood forests of less disturbed peaks of the Southern Appalachians also have *C. geophilum* as a dominant ECM? Mills (1995) found that hypogeous sporocarp abundance declined near forest edge. Peaks of the Southern Appalachian Mountains with undisturbed forests may exhibit greater hypogeous sporocarp diversity, due to increased continuous forest patches.

The temporal and spatial extent of this study was minimal. In order to understand the dynamic nature of communities, of both the fungal component and the trees, it is important to continue sampling, increasing the area and time sampled. The fascinating biogeographical patterns of organisms in the northern, central, and Southern Appalachians (White and Cogbill 1992) will be further understood with continued research of the fungal communities across these mountain ranges. Conservation of spruce-fir and other high elevation forests of the Southern Appalachians will be aided by additional knowledge of ectomycorrhizal fungi.

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APPENDIX I

Descriptions of Ectomycorrhizal Morphotypes

Morphotype ID	Similar Species	Color	Mantle	Rhizomorph	Ramification
1	*Cenococcum geophilum*	Black	Star-like hyphal arrangement	covered with emanating hyphae	Simple to monopodial pinnate
2	?	Brown with white mantle	Silvery mantle	common, white, smooth margin	monopodial pinnate to pyramidal
3	?	red brown	mantle surface not distinct	no rhizomorphs, reticulate pattern of *Cenococcum* hyphae surrounding	monopodial pinnate
4	*"Fagirhiza cystidiophora"?, Tuber puberulum?*	yellow brown, ochre	shiny, densely short-spiny	none	simple
5	*Lactarius sp.?, "F. granulosa"?*	yellow brown	silvery, grainy, short spiny	none	monopodial pinnate to pyramidal
6	*Cortinarius sp.?*	white	densely cottony/woolly	Frequent, white	dichotomous to monopodial pinnate
7	?	bright yellow	shiny, densely woolly	hyphal fans	monopodial pinnate
8	?	white (cream)	short spiny	none	monopodial pinnate

Morphotype ID	Similar Species	Color	Mantle	Rhizomorphs	Ramification
9	?	white	shiny	scarcely, smooth	monopodial pinnate pyramidal
11	*Rhizopogon sp. ?*	green yellow	smooth	none	coralloid
12	?	white yellow	very densely woolly	emanating hyphae dense over tip	simple
13	?	yellow, w/green tips	grainy	none	monopodial pinnate
14	?	light brown yellow	stringy	none	monopodial pinnate
17	?	black blue	shiny smooth	none	monopodial pinnate
18	?	light red brown (pink) white lilac	smooth, not distinct	none	monopodial pinnate
19	?	white green	smooth	none	pinnate
20	?	ochre	smooth	none	pinnate
21	*"Piceirhiza conspicua"*		smooth	none	irregularly pinnate

Morphotype ID	Similar Species	Color	Mantle	Rhizomorph	Ramification
22	*"Piceirhiza conspicua"*	ochre, with brown tips	smooth	none	irregularly pinnate
23	?	ochre	loosely stringy	none, black hyphae surrounding	irregularly pinnate
24	?	flesh colored	loosely cottony, loosely long-spiny	none	monopodial pinnate
25	?	black green	cottony	none	monopodial pinnate
26	?	green silver	shiny smooth	none	dichotomous, monopodial pinnate, pyramidal
27	?	blue silver	shiny woolly	infrequent, blue	irregularly pinnate
28	?	ochre white	densely cottony	frequent, white	dichotomous, monopodial pinnate
29	?	orange	shiny, grainy	none	monopodial pinnate
35	?	red brown	smooth	none	irregularly pinnate

Morphotype ID	Similar Species	Color	Mantle	Rhizomorphs	Ramification
36	?	dark brown	smooth	none	irregularly pinnate
37	?	olive	smooth	none	simple
40	?	yellow	loosely cottony	infrequent	irregular pinnate
41	*Piceirhiza nigra*	black	shiny	none	monopodial pinnate to pyramidal
42	?	red	thick smooth	none	tortuous
43	?	yellow	smooth	none	tortuous monopodial
44	?	yellow	smooth	none	monopodial
45	?	white	thin cottony	frequent white	tortuous

APPENDIX II

Restriction fragment length polymorphisms (RFLP's) for ectomycorrhizal morphotypes and hypogeous sporocarps. Fragment sizes are given in approximate base pair numbers.

Morphotype	*Hinf* I fragments	*Alu* I fragments	Uncut PCR product
1 (Cenococcum)	631, 398, 356, 295, 245, 226, 199, 129		1298, 846, 692
2	403, 366, 302, 265, 190, 150		771, 692, 622
3	No DNA amplified	No DNA amplified	No DNA amplified
4	381, 348	621	757
5	381, 348, 279	538, 246	735
6			
7	231, 214, 195, 180, 132	290, 259	717
8	394	560, 273	817, 672
9	361, 343	581, 520, 198, 167	777
10	No DNA amplified	No DNA amplified	No DNA amplified
11	458, 383, 225	530, 440, 267	771
12	408, 315		712
13	376, 284, 250, 214, 179, 130, 82		761
14	410, 350, 284, 272, 250, 233		677, 615
15	Same as Morph. 1 *(Cenococcum)*		
16	Same as Morph. 1 *(Cenococcum)*		
17	475, 399, 341, 302, 284, 264, 228		1106, 810, 752
18	415, 377, 331, 280, 218, 193, 162		791
19	702, 518, 499, 375, 328, 311, 283, 252		872, 766
20	Same as Morph. 19		
21	382, 341, 306, 235, 212, 143	499	782, 616
22	Same as Morph. 21		
23	Same as Morph. 21		
24	Same as Morph. 21		
25	Same as Morph. 21		
26	781, 674, 421, 361, 279, 234, 207	436,380	1012,892,674

Morphotype	***Hinf* I fragments**	***Alu* I fragments**	**Uncut PCR product**
27	306, 276, 257, 231, 216, 132	513, 315, 244, 185	637, 554
28	374, 292, 277, 181, 141	394, 231	649, 554
35	320, 274, 244	499	815,645
36	No DNA amplified		
37	383, 281, 264, 214	624	815, 645
42	352, 279	453, 279	645
43	387, 320, 276, 244	645	667
44	407, 273, 220	585, 214, 180	843
Sporocarps			
E. muricatus	262, 189, 151, 79	473, 273	700
E. muricatus	262, 189, 155, 77	455, 272	700
E. muricatus	258, 185, 148, 75	464, 269	700
E. muricatus	259, 185, 150, 77	477, 273	700
E. muricatus	263, 189, 153, 81	486, 272	727
E. muricatus (from Loeb et al. 2000)	264, 189, 150, 76	470, 270	715
E. granulatus	389, 316, 283, 259, 199		717
Alpova sp. (from Loeb et al. 2000)	385, 284	677, 276	970
Scleroderma sp. (from Loeb et al. 2000)	278, 253	485, 289	823

VITA

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