DIVERSITY OF ECTOMYCORRHIZAL FUNGI IN MINNESOTA'S ANCIENT AND YOUNGER STANDS OF RED PINE AND NORTHERN HARDWOOD-CONIFER FORESTS

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ABSTRACT

Diversity of ectomycorrhizal fungi in Minnesota's ancient and younger stands of red pine and northern hardwood-conifer forests

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Remnant ancient forests were investigated to examine the magnitude of the ectomycorrhizal fungal communities, integral components of forest ecosystems. Quantitative data were obtained for these fungi in red pine and northern hardwood-conifer forests in northern Minnesota, using dispersed, circular, 4-m^2 sampling areas along permanent transects in half hectare plots. Basal areas of woody plants were measured for the plots, and soil organic matter and pH were determined. Diversity, species frequencies, and fruitbody densities were examined in ancient and younger stands. The red pine forest, with a major conifer component, harbored a larger community of ectomycorrhizal fungi with two to three times more species, and abundance an order of magnitude greater than that observed for northern hardwood-conifer forest. Differences were found in species composition between the two age classes. Some species characterized old-growth and others, mature red pine forest. Russulaceae were major components in, and included the most abundant taxon of each forest: *Russula silvicola* for red pine forest, and *Lactarius thejogalus* for northern hardwood-conifer forest. *Laccaria laccata*, the second most frequent species, had higher densities in old-growth stands of both forest types. *Cortinarius* was a dominant genus in the red pine forest with an unknown number of species. By comparison all but a few northern hardwood-conifer forest species were infrequent or rare, and a smaller percentage of species were shared among plots. Even though frequency and diversity were similar or less than in younger forest, old-growth stands of both forest types had greater total fruitbody density: 1.1 times greater for red pine and a significant 1.8 times greater for northern hardwood-conifer forest. Greater productivity in these two old-growth forests may be an important factor in ecosystem function. The hypothesis that fungal diversity declines with advanced forest age is not supported by the findings of this study where species diversity and abundance are maintained in these old-growth stands.

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I. INTRODUCTION

ECTOMYCORRHIZAL FUNGI AND THEIR DECLINE IN FOREST COMMUNITIES

Fungi form mycorrhizal associations with about 85% of the world's vascular plants. Two of the seven types of mycorrhizae are well studied. Endomycorrhizae or arbuscular mycorrhizae are formed with a wide range of plants by a limited number of fungal species (ca. 150) in the Glomales, Phylum Zygomycota. These species generally display little specificity to host plants. Ectomycorrhizal fungi are much more diverse (>5400 spp.) members of Basidiomycota and some Ascomycota. They are primarily associates of woody plants with broad to narrow specificity (Allen et al. 1995). These fungi are vital to plants by providing access to phosphorus (by endomycorrhizae) and nitrogen (by ectomycorrhizae) in soils (Read 1991). Mycorrhizal fungi as a whole serve as keystone resource species by facilitating the absorption of mineral nutrients, and as keystone mutualists essential to the success of the plant associates (Hawksworth and Ritchie 1993). The mycelial systems of ectomycorrhizal fungi also have a significant input of nitrogen and carbon into soil communities (Read 1991).

Catastrophic declines in ectomycorrhizal fungi have been reported in Europe, and Red Data Lists of threatened fungi have been compiled (e.g. Arnolds 1989, Rassi and Väisänen 1987, Ing 1996). Two major factors are involved in these declines of population numbers and geographic ranges: loss of seminatural, ancient forest and changes resulting from atmospheric pollutants (Ing 1996). Some of the ectomycorrhizal fungi that are extirpated (locally extinct) or threatened in The Netherlands (Arnolds, 1988, 1989) are currently found in Minnesota; these include *Amanita verna*, *Astraeus hygrometricus*, *Boletinus cavipes*, *Cortinarius traganus*, *Hydnellum aurantiacum*, *Hygrophorus pudorinus*, *H. russula*, *Lactarius piperatus*, *L. volemus*, *Phellodon niger*, *Rozites caperata*, *Strobilomyces floccopus*, *Suillus aeruginascens*, *S. placidus*, *Tricholoma sejunctum*, and *T. virgatum*. Loss of these fungi in parts of Europe appears to be an advance indicator of forest decline. This species loss is attributed in part to increased availability of nitrogen in soils, which is an indirect effect of air pollution (Arnolds 1991, Arnolds and Jansen 1992). Other stresses on

ectomycorrhizal symbioses are heavy metal pollution and, potentially, excess CO_2 (Colpaert and Van Tichelen 1996). Climatic change may lead to loss of vegetation types and associated fungi (Ing 1996).

The effects of air pollution on North American fungi is not yet known. Many North American endemic species could be at risk. Half of the Minnesota species of *Lactarius* (Leacock, unpublished) appear to be endemic to North America. An air pollution gradient extends across the Great Lakes States with decreasing deposition from Ohio to Minnesota of H^+, SO_4^{2-}, and NO_3^-; this deposition has directly affected soil solution chemistry and has increased the leaching of nutrient cations (Ca^{2+}, Mg^{2+}) (MacDonald et al. 1992). However, MacDonald et al. (1991) found no evidence that soil nitrogen levels had increased as a result of deposition. Thus, a widespread decline of ectomycorrhizal fungi in Minnesota due to pollution is not an immediate concern.

The ectomycorrhizal fungi that are currently at risk in Minnesota are uncommon and rare species with specific habitat requirements. These include *Suillus* (*Fuscoboletinus*) *weaverae*, *Laccaria trullisata*, and *Lactarius fuliginellus*, currently on Minnesota's list of endangered and special concern species (Minn. Dept. of Nat. Res. 1996). It is not known which ectomycorrhizal species are dependent on old-growth forests in Minnesota. Data obtained now, under conditions of little disturbance, can serve as a baseline against which future comparisons are made and may help us understand which species are threatened by habitat loss.

The changes in composition of ectomycorrhizal associations as trees mature and forests become old-growth are poorly known. Studies of early ectomycorrhizal succession on newly planted trees (e.g. Last et al. 1984) demonstrate that early-stage taxa are replaced by late-stage species within 12 years. Some reports on changes of fungal diversity with stand age indicate a decline after canopy closure (Dighton et al. 1986, Dighton and Mason 1985). However, it is unclear how results from these plantation studies, with tree ages of 1 to 20+ years, relate to natural and old-growth or ancient forests where there is a mixture of tree species and age classes. Newton (1992) questions the division of ectomycorrhizal fungi into "early-stage" and "late-stage" taxa based on their apparent place in "ectomycorrhizal succession" and instead argues for a functional

classification based on the ability to spread and colonize root systems by mycelial strands and other sources of inoculum. Read (1991) argues that an important factor involved is the enzymatic ability of some ectomycorrhizae to mobilize nutrients from organic matter.

Diversity of fungi may or may not be greater in older stands. Scots pine stands in Finland, originating from both natural and artificial regeneration, had more ectomycorrhizal species in stands 20 30 years-old (41+ spp.) than 30 50 years (37+), >70 years (29+ spp.), or 5 15 years (23+ spp.) (Hintikka 1988). Although overall fruitbody production was highest for 20 30 year-old stands, certain species (e.g. *Russula*) may have been more abundant in older "over-aged" stands. In Idaho, a study with western white pine found an increase in numbers of putative ectomycorrhizal species with increasing stand age (Miller 1983): 5 species in a 15 year-old stand, 34 37 species in ca. 35 year-old plantations, and 78 species in a mature stand 175-215 years old; these counts may include hypogeous species. Doudrick et al. (1990) reported that several ectomycorrhizal species were found only in the oldest stands of black spruce in northern Minnesota. It is apparent, at least for some forest types, that older stands can contribute a notable portion of the total diversity for these forests. Conservation of old-growth forests must take into account the role these forests have in harboring fungal diversity, including rare species, and providing sources of inoculum for maturing forests.

FUNGAL COMMUNITY STUDIES IN NORTHERN FORESTS AND SAMPLING METHODS

Many qualitative and quantitative studies of fungal community diversity and structure have been done for a range of forest types from natural, often mixed, stands to monoculture plantations. These studies have been more numerous, and in some cases more extensive, in Europe than in North America. Cooke (1948, 1953) has summarized earlier studies, very few of which were quantitative. Table 1 lists some of the more recent quantitative studies of fungal communities in Europe and North America that used a design of replicate plots and measurements of fruitbody production to determine diversity.

The number of ectomycorrhizal species reported by quantitative studies varies widely. Several factors seem to be involved in the magnitude of diversity found

by these studies. One is the nature of the habitat or forest, including diversity of plant species, especially the number of ectomycorrhizal host trees and shrubs present, whether stand is natural or planted, age of stand, geographic location (latitude and altitude) which relates to biogeography, soils, and climate. Another is the design of the study, the size of plot or area sampled, and the number of replicates. A third is the number of visits to a plot during a year and the number of years. Many of these factors differ between studies, making exact comparisons difficult.

TABLE 1. No. of ectomycorrhizal species in forest types, recorded by various plot methods.

Forest type	No. of spp.	Size of plot, m^2	No. of plots	Years per plot	Location	Reference
EUROPE						
Boreal coniferous and mixed forests and peatlands	125[a]	100	596	1	Finland	Salo 1993
Heath forests with pine, spruce, birch	69	200	36	3	N. Finland	Ohenoja 1978
Oligotrophic spruce forest at three sites	132 99 68	225 225 225	10 10 10	3 3 3	Norway S. Norway Germany	Gulden et al. 1992
Oligotrophic Scots pine forest	54	750	25	3	S. Finland	Hintikka 1988
Scots pine plantation	25	200	10	1	England	Hora 1959
Scots pine plantation	12	4	12	5	Scotland	Richardson 1970
Bog, birch, spruce	60	1	130	3	Denmark	Lange 1948
Acid oak woods	102	1000	29	4	Netherlands	Jansen 1984
Beech forest	24	5	295	5	S. Sweden	Tyler 1985
Deciduous forests	70	500	228	4	S. Sweden	Tyler 1989

TABLE 1 (*continued*)

Forest type	No. of spp.	Size of plot, m^2	No. of plots	Years per plot	Location	Reference
NORTH AMERICA						
Red spruce	27	256	6	3	West	Bills et al.
Northern hardwood	35	256	6	3	Virginia	1986
Maple-yellow Birch	37	400	5	2		
Fir-paper birch	30	400	5	2	Quebec	Villeneuve
Black spruce-moss	26	400	5	2		et al. 1989
Black spruce-Cladina	28	400	5	2		
N. hardwood and hardwood-conifer mixed forests[b]	ca. 180[c]	400	11	2	Quebec	Nantel and Neumann 1992
40- to 65-year-old	24[d]	1	50	3	W. Oregon	Fogel 1976
Douglas fir		100	4			
Alder, birch forest	18	1000	4	1	Alaska	Brunner et al. 1992
Jack pine forest	56	plotless	–	2	NE Alberta	Danielson 1984
Aspen forest	43	plotless	(3)[e]	3	Montana, Idaho	Cripps and Miller 1993
Black spruce	46	plotless	(22)[e]	2	Minnesota	Doudrick et al. 1990

[a] No. of ectomycorrhizal spp. by plant associate: *Pinus sylvestris* 51; *Picea abies* 38; *Betula pubescens* 29; *Betula pendula* 6; *Populus tremula* 1.
[b] Eight ectomycorrhizal plant spp.
[c] Half of these species unidentified.
[d] Hypogeous ectomycorrhizal species.
[e] Number of stands visited.

The kind and diversity of trees present have a direct impact on the community of ectomycorrhizal fungi because of the mutualist association of the two. Tyler (1985) in a very extensive study (29.5 ha covered) found 24 ectomycorrhizal species for beech forest (some ash present), whereas 102 species were recorded for oak woods using one tenth the sampling area (Jansen 1984). Salo (1993) listed the ectomycorrhizal fungi associated with five woody plants (see Table 1) ranging from 51 for pine to 1 for aspen; these 125 ectomycorrhizal species occurred in 5.96

ha of boreal forests and peatlands of central Finland. In Quebec, Nantel and Neumann (1992) found 240 ectomycorrhizal taxa with eight host tree species (of the same genera, plus *Abies*) in 0.44 ha of northern hardwood and hardwood-conifer forests.

Edaphic factors also impact diversity. Tyler (1985) divided his 295 plots into five groups forming a gradient based on soil organic matter content and metal ion saturation percentage. Across this edaphic gradient mean number of ectomycorrhizal species increased from 1 to 6 and mean number of fruitbodies increased from <2 to >20 per plot, numbers being the highest for plots with increased soil acidity and organic matter content.

Greater diversity is frequently documented when sampling area is increased. For Scots pine plantation, Richardson (1970), using a total area of 48 m^2 (twelve 4 m^2 plots) over five years, found 12 species including new species added in the fifth year, while Hora (1959) recorded 25 ectomycorrhizal species in one year sampling 2,000 m^2. In three years, more than 54 ectomycorrhizal species were found by Hintikka (1988) in 18,750 m^2 of Scots pine forests of different ages (most stands contained small birch or spruce saplings).

Lange (1948), who did pioneering work on fungal community analysis, stated that areas to be studied must be divided into small representative plots so that measurements such as frequency can be correctly quantified. A quadrat size of 1 m^2 was recommended, using at least 10 for each specific plant community type. Arnolds (1981) estimated that a plot size of at least 1000 m^2 is preferable as a minimal area for sampling fungal diversity but that some plant communities may not have homogeneous areas this large; he states that fungal community ecologists agree that this minimal area is larger than that needed for sampling plant composition. How this sampling area is laid out can influence the data obtained. Various studies have used square or broadly rectangular plots divided into contiguous quadrats. Hintikka (1988) used a strip plot of 3 × 250 m. Arnolds (1981) relates how a study by Winterhoff in 1975 found that using a continuous area of 1000 m^2 gave 50% of the species but that 54% of the species were found in less than a seventh of that area by having 135 quadrats of 1 m^2 scattered throughout the stand. This implies that a more complete estimate of fungal diversity can be obtained by spreading sampling units of a given size over a larger

extent.

Several years are required to determine the fungal diversity of a community accurately because mushroom fruiting varies both seasonally and from year to year and is strongly influenced by weather. Lange (1948) recommended that studies comprise several years of observations with many visits made each year. He thought that a single, well chosen observation may yield 50% of the species for a community and six to eight observations may give 85 to 97% of a total year's result. Estimated yield for three years of sampling was 75 to 92% of the species for grassland sites (Arnolds 1981) and 98% of the total number of hypogeous species for Douglas fir sites (Fogel 1976). Quantitative analysis over multiple years also allows for greater understanding of the relative abundance of species and which species may be characteristic of a particular habitat. Observations over three successive years, if weather conditions are not unfavorable, may give representative results, for grassland sites (Arnolds 1981) as well as forests, although a period of more than three or four years would be preferable for complete characterization (Gulden et al. 1992, Richardson 1970).

The present state of knowledge of fleshy fungi in North America is comparable with that of the vascular plants 100 years ago; range maps are available for only a few species (e.g. Redhead 1989). Basic quantitative data on species composition, diversity, and abundance of ectomycorrhizal fungi have been absent for most naturally occurring forests. With little data on distribution it is difficult to determine whether similar species loss is occurring here as in Europe (Jaenike 1991). Table 1 includes studies that have been recently published. Those of Bills et al. (1986) and Villeneuve et al. (1989) in eastern North America, mostly involving second-growth stands, compared species diversity among different forest types (see Table 1). Plot sizes used were 256 and 400 m^2 with a total area of 1,536 and 2,000 m^2, respectively, for each forest type. Nantel and Neumann (1992) used 400 m^2 plots in 11 stands to examine a vegetation gradient and the influence of abiotic factors. Ectomycorrhizal fungi are especially poorly studied in ancient forests in North America. Villeneuve et al. (1989) is the only study for eastern North America that examined old-growth (sugar maple - yellow birch), but a comparison to younger stands was not made.

Several current studies in western North America have been documenting

differences in the ectomycorrhizal fungal communities of ancient and younger forests. Ammirati et al. (1993, 1994) examined old-growth conifer forests in western Washington state for macrofungal species composition by forest type. Walker et al. (1994) compared species richness between 70 to 120 and 200 to 250 year-old spruce-hemlock forests in Washington using narrow 10 × 250 m transect plots (abundance data were not collected); 40 50 ectomycorrhizal species were found per site-age class. O'Dell and Ammirati (1994) examined eight stands of old-growth, 250 to 300 year-old, hemlock-Douglas fir forest applying dispersed sampling units. Each sample consisted of an area of 400 m^2 divided into 100 circular, 4 m^2 plots located 5 m apart along two 250 m transects. In two years 43 samples yielded over 200 ectomycorrhizal taxa (0 42 species per sample), which appears to be greater species diversity per unit area than any similar study in North America (O'Dell and Ammirati 1994). Also using transects of 4 m^2 plots, Castellano and Trappe (personal communication, 1995) uncovered variations in abundance of subterranean fungi for young, rotation age, and old-growth Douglas fir forests in Oregon; frequency was lower in the old-growth plots.

Mycorrhizas can be identified by careful observation of morphology (Agerer 1991) as in Jansen and Nie (1988). Recently developed techniques allow identification by molecular methods of DNA amplification, restriction fragment length polymorphisms, and direct sequence comparison (Gardes and Bruns 1996). Species-specific and isolate-specific DNA probes can also be used (Gardes et al. 1991). Egger (1995) reviews current molecular methods for mycorrhizal community analysis and suggests possible future directions, such as resolving the debate whether succession of early- to late-stage ectomycorrhizae is a valid model.

Diversity and abundance of ectomycorrhizal fruitbodies does not directly correlate with the composition of mycorrhizae present on tree roots. The relation of aboveground fruiting to belowground mycorrhizas has been examined for different forests (e.g., Jansen and Nie 1988, Gardes and Bruns 1996). The number of species fruiting is not a complete representation of the diversity of mycorrhiza types. In both studies, some mycorrhiza taxa on roots were poorly or not represented by fruitbodies. Gardes and Bruns (1996) found that some taxa were common fruiters but were rarely found on roots. A review of methods for

quantification of ectomycorrhizae on roots is given by Grand and Harvey (1982).

A large portion of Minnesota's macrofungal diversity is unknown; the ectomycorrhizal fungi are a significant component. Recent work is well documented but limited to relatively few locations (Weaver and Shaffer 1969, 1972; Weaver and McLaughlin 1980). Contributions are best made by focusing effort on a particular genus (Leacock 1993), a tree host (Doudrick et al. 1990), or specific communities as in the current study.

THESIS RESEARCH OVERVIEW

This is the first study in eastern North America that compares the ectomycorrhizal fungi composition of old-growth forests to that of related younger forests to assess the influence of stand age on fungal diversity. The quantitative data based on abundance of fungal fruitbodies will assist in understanding what portion of fungal diversity is common to a forest type and which species may be dependent on old-growth. This data can be used as a baseline for comparison with potential future studies at these sites to assess forest health and environmental effects. Data on these fungal communities will add to the understanding of why old-growth forests are necessary for the conservation of fungal diversity including rare species.

The goal of this research is to quantitatively characterize the ectomycorrhizal fungi communities found in examples of Minnesota's red pine forest and northern hardwood-conifer forest, and make a comparison of fungi composition between old-growth stands and younger second-growth stands of the same forest type. The sampling techniques were designed to provide measurements of species diversity, species abundance (frequency and fruitbody density), and phenology of fruiting. Voucher specimens of all species were preserved. Data also include woody plant composition and nature of soils, to examine correlations between ectomycorrhizal fungi, host tree diversity, and edaphic factors. The sampling design is a modification of that used by O'Dell and Ammirati (1993): 4 m^2 sampling circles placed along five 100 m transects. Each 0.5 ha stand has a sampling area of 400 m^2.

Results of three field seasons at the mature and old-growth red pine forest

stands are presented in Chapter II. Diversity and abundance of ectomycorrhizal fungi are examined for differences between the two age classes and to determine whether there is decreased richness or abundance for old-growth stands. Differences in species composition between the two age classes are examined to identify species that may be restricted to or characteristic of old-growth or mature red pine forest.

Chapter III covers the similar analysis of three years of sampling for old-growth northern hardwood-conifer forest and successional northern hardwood forest. Diversity and abundance are also compared between age classes. Comparisons will be made with data of Chapter II to look for similarities and differences in fungal species composition. Diversity of both forest types will be compared to that reported for other forests in North America and Europe. Chapter IV presents a brief summary discussion of the ectomycorrhizal fungi communities in these two forest types. The observed diversity is compared to the loss of species in northern Europe, and the contribution of old-growth forests to fungal diversity is discussed.

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II. Diversity and Comparison of Ectomycorrhizal Fungi in Ancient and Younger Stands of Red Pine Forest

Introduction

Basic quantitative data on the composition of fungal communities are absent for most forests in North America (Jaenike 1991), particularly ancient, or old-growth, forests. Ectomycorrhizal fungi, important constituents of these forests, are vital resource species and mutualists that are essential for the success of their plant associates (Hawksworth and Ritchie 1993). Recent investigations in Eastern North America include Bills et al. (1986), Nantel and Neumann (1992), and Villeneuve et al. (1989), only one of which dealt with ancient forests. Villeneuve et al. (1989) examined old-growth (sugar maple - yellow birch) in addition to 70- to 90-year-old coniferous forests (spruce, fir, paper birch). Quantitative studies in western North America have been made in ancient forests of fir (Ammirati et al. 1994), hemlock-Douglas fir (O'Dell and Ammirati 1994), and spruce-hemlock (Walker et al. 1994).

Changes in composition of ectomycorrhizal associations as mature forests become old-growth forests are not well understood. Some reports indicate a decline in fungal diversity for older coniferous stands (e.g., Dighton and Mason 1985, Dighton et al. 1986). Scots pine stands in Finland had more ectomycorrhizal species in 20- to 30-year-old stands than either older or younger stands (Hintikka 1988), although certain species (e.g., *Russula*) may have been more abundant in "over-aged" stands. For western white pine in Idaho, Miller (1983) found twice as many ectomycorrhizal species in a 175- to 215-year-old stand as in 35-year-old plantations. Similarly, Visser (1995) found greater diversity in older 65- and 122-year-old jack pine stands as compared to 6- and 41-year old stands. It is unclear how results from plantation studies or single tree species stands relate to natural and ancient coniferous forests particularly where there is a mixture of tree species and age classes. Diversity of fungi may or may not be greater in these forests.

Although aboveground fruiting of ectomycorrhizal species has not been found to always correlate with abundance of belowground mycorrhizas (e.g., Jansen and Nie 1988, Gardes and Bruns 1996), data on fruitbody abundance are valuable. Sampling mycorrhizae by soil coring and the time required for processing root tips limits the area that can be studied. Morphological and molecular methods required for sorting and relating mycorrhizae to the species that are fruiting can also be a limiting factor where diversity is high and the fungi flora poorly known. Both above and belowground methods have disadvantages, and neither method is guaranteed to capture the total diversity of species present in an area particularly those species occurring at low frequency. Quantitative studies of fruitbodies provide an estimate of fungal diversity and abundance, data on seasonal patterns of fruiting, and information on the ecology of species based on their distribution in and among forest types.

This study was undertaken to document the ectomycorrhizal fungi component of red pine forest and to provide baseline data for comparisons between mature and old-growth stands. Data for assessing the influence of stand age on ectomycorrhizal fungal communities includes species composition, diversity (number of species), and abundance (frequency and density). Six half-hectare plots containing permanent transects were visited during the fruiting seasons of 1993 1995 for observations of species presence and fruiting body counts. The sampling design, modeled after that of O'Dell and Ammirati (1994), used dispersed sampling circles, rather than contiguous quadrats, for increased representation of diversity and reduced autocorrelation among sampling units (due to individuals fruiting in adjacent sampling units).

MATERIALS AND METHODS (see also Appendix I)

Location and description of the study site

Permanent plots were located in adjacent mature (100 year-old) and old-growth (230 year-old) red pine stands east of Pine Lake in Scenic State Park, Itasca County, 55 km north of Grand Rapids, Minnesota. Three plots were studied in two stands of both ages. Mature plot 1 (47°44 01"N, 93°33 46"W) was 460 m south-southeast of mature plots 2 and 3 (47°44 17"N, 93°33 59"W) which were

placed end to end. Old-growth plots 1 and 2 (47°44′08″N, 93°33′43″W) were 35 m from each other and 170 m north of mature plot 1. Old-growth plot 3 (47°44′11″N, 93°33′42″W) was 50 m north of plot 2 and 200 m from mature plot 2. These six half-hectare (50 × 100 m) plots were established in 1993 and 1994. Each plot contained five parallel 100-m permanent transects spaced 10 m apart. Each transect consisted of 20 sampling circles spaced 5 m apart, each circle having an area of 4 m^2. The sampling circles for one plot totaled 400 m^2.

The level to mildly sloping stands, at an elevation of 420 425 m, are on well drained, sandy-loam soils formed on glacial deposits. Soil samples were collected to a depth of 5 cm, litter excluded, at the periphery of five randomly selected sampling circles for each plot. For each sample the pH was measured ($CaCl_2$ method, McKeauge 1978), and the Research Analytical Laboratory, University of Minnesota, determined the percent organic matter content by loss on ignition. The soil pH ranged from 3.7 to 4.8 in the mature plots and 4.0 to 4.7 in the old-growth plots. The mean organic matter content for the mature plots, 3.5 % ± 1.5 % (mean ± 1 SD, n = 15), was higher than that for the old-growth, 2.1 % ± 1.0 % (n = 15).

The mature and old-growth forest stands contained nine conifer and hardwood tree species dominated by even-aged red pine; shrubs were a minor component. Diameter at breast height of all woody stems greater than or equal to 2 cm were measured and the basal areas computed for each plot (Table 1); all trees were mapped. Thirty to fifty vascular plant species were present in each plot. The mature stands originated from natural regeneration after harvesting. There was scattered evidence of ground fires, particularly in mature plot 1, which was the most open of the plots and had noticeably more bracken fern cover. There was some selective cutting of windfalls by the Civilian Conservation Corps in the past, particularly in old-growth plot 1. Surrounding stands had similar composition or were dominated by mature aspen or paper birch.

TABLE 1. Basal areas (square meters per hectare) of woody plants for red pine forest plots.[a]

	Mature[b]			Old-growth[c]		
Species[d] Plot[e]:	3	1	2	2	3	1
Pinus resinosa Ait.	35.78	37.52	29.49	27.40	26.32	17.59
Populus grandidentata Michx.	0.34	4.13	0.49	0.06	– –	0.32
Picea glauca (Moench) Voss	0.32	0.03	1.46	0.04	0.10	0.04
Pinus banksiana Lamb.	0.29	– –	0.48	– –	– –	– –
Populus tremuloides Michx.	0.16	– –	0.38	– –	– –	– –
Acer rubrum L.	– –	0.05	0.02	– –	0.02	0.01
Amelanchier huronensis Wieg.	– –	<0.01	– –	<0.01	– –	– –
Corylus cornuta Marsh.	– –	– –	– –	– –	<0.01	– –
Betula papyrifera Marsh.	2.50	0.77	3.32	1.79	1.39	3.22
Abies balsamea (L.) Mill.	3.13	1.18	2.46	8.39	7.76	3.97
Pinus strobus L.	1.13	3.36	3.48	14.28	18.19	16.97
Total basal area/ha	43.66	47.05	41.60	51.96	53.78	42.12

[a] Actual basal area multiplied by 2 to give basal area per hectare. Species and plots are ordered by the corresponding eigenvectors of principal component 2 (see methods).

[b] Even-aged 100 year-old pine dominant.

[c] Even-aged 230 year-old pine dominant.

[d] Vascular plant nomenclature follows that of Ownbey and Morley (1991).

[e] 50 × 100 m (0.5 ha).

Sampling procedures

Two of the old-growth plots were partially sampled the initial year (1993) on four visits (August 25 October 5). During four visits, July through September, in both 1994 and 1995, the transects of all plots were visited on a rotating basis every 3 wk. Three of the five transects were sampled each visit. The center transect was used every visit and the outside pair or inside pair of transects on alternate visits. Epigeous fruitbodies of genera containing potentially ectomycorrhizal fungi were counted in each sampling circle. Several infrequent unidentified taxa with insufficient material or fruitbodies in poor condition were not included in the analysis.

The presence of additional species occurring within the plots (but outside circles) was also recorded. In addition, counts were made of *Hypomyces lactifluuorum* (Schw.:Fr.) Tul. (an ascomycete) which parasitizes certain species of *Russula* and *Lactarius*. Data on *Cordyceps* were collected to obtain a minimum estimate of the frequency of the hypogeous ascomycete *Elaphomyces*. During

field sampling representative mushroom specimens were collected for written and photographic documentation and identification. Voucher specimens are deposited in the Herbarium of the University of Minnesota (MIN). Identification of certain taxa was made by consultation with J. Ammirati, University of Washington, Seattle (*Cortinarius*), G. Mueller, The Field Museum, Chicago (*Laccaria*), T. Baroni, State University College at Cortland, New York (Entolomataceae), and C. Ovrebo, University of Central Oklahoma, Edmond (*Tricholoma*).

Genera present in red pine forest plots, for which some data were collected, but excluded from the analysis because of questionable or doubtful mycorrhizal status include *Camarophyllus*, *Clavariadelphus*, *Clavulina*, *Clavulinopsis*, *Hygrocybe*, *Lyophyllum*, Entolomataceae other than *Entoloma s.s.*, and the species *Ramaria concolor* (Arnolds 1989, 1991, Gulden et al. 1992, Singer 1986, Villeneuve et al. 1989).

Data Analysis

Quantitative data are based on the 100 sampling circles, total area of 400 m^2, for each plot. For each species the presence and fruitbody numbers observed in sampling circles was used to determine frequency (percent of circles occupied) and density (no. of fruitbodies/unit area). These data also provided distributions of species within plots, fruiting phenology, and yearly variation. Total species diversity within each half hectare plot (5,000 m^2) also included those fungi found outside the sampling circles. Sets of species-area curves display the relationship between diversity and amount of area sampled. Dominance - diversity curves portray evenness among species' frequencies (Bills et al. 1986) and in this thesis (Fig. 2) use scales standardized to 100 percent (Villeneuve et al. 1989) where percent frequency = (freq. - minimum freq.)/(maximum freq. - min. freq.) x 100 and species sequence = (rank - 1)/(no. of spp. - 1) x 100.

The summary and comparative data analyses, in addition to the above, include coefficients of similarity to compare fungal species composition between the two red pine forest ages and with other studies in North America (Villeneuve et al. 1991); results are presented in Chapter III. I used principal component analysis on the correlation matrix of species frequencies to order the species times plot matrices for ectomycorrhizal fungi, using a SAS statistical program (SAS Institute,

Inc., Cary, NC, 1996). This ordination reveals relationships between species, plots, and forest age classes. This method was also used to order the plots and plant species in Table 1, using basal area.

RESULTS

Diversity of species

More than 146 species of ectomycorrhizal fungi in 27 genera were documented for the six plots, totaling three hectares, of red pine forest (Tables 2, 3). *Cortinarius* (including *Dermocybe*) was the most abundant and diverse group encountered; 20 taxa of *Cortinarius* were separable and are included in Table 3. An additional 20 35 undetermined species of *Cortinarius* have been combined as one taxon for purposes of analysis. These *Cortinarius*, primarily in subgenus *Telamonia*, could not be reliably separated in the field because of the overlapping spectrum of characters, variability due to fruitbody age, and sheer numbers: over 1700 fruitbodies were observed in three years. *Russula* (25 spp.) and *Lactarius* (18 spp.) were very abundant. *Laccaria* (2 spp.) was also abundant. *Tricholoma* (15 spp.) and *Inocybe* (14 spp.) were diverse but except for a few species were lower in abundance. The only ectomycorrhizal ascomycete encountered was *Elaphomyces*, which was located by fruitbodies of its parasites, *Cordyceps capitata* (Holmsk.:Fr.) Link and *C. ophioglossoides* (Ehrh.:Fr.) Link.

The mature red pine forest plots exhibited greater diversity (125 species) than the old-growth plots (109 species) (Table 2). Thirty-seven species found in the mature plots were not recorded for old-growth compared to 21 species observed only in old-growth plots. Species predominantly found in one age class are listed as potential indicator taxa if common to all three plots (Table 4). The two age classes showed a 60% overlap in species composition (88 spp. shared of a total of 146 for 1993 1995). Thirty-six species (25%) were found in all six plots (Table 3). When only the data from circles is considered, the overlap was 50% (62 out of 125 spp.) with 20 species in common among the six plots. The proportion of species shared among all three mature plots, 43%, was somewhat less than that among the old-growth plots, 49%. In addition, the difference in range of the number of species per plot was noticeably greater among the mature plots (59 73

spp. in circles) than among the old-growth plots (55 57 species) (Table 2). Ordination of the plots on frequencies of 122 taxa (Table 3) separated the two age classes along principal component 2, accounting for 11% of the variation in data. Principal component 1 (78%) ordered species in terms of their frequency because this was a non-centered analysis.

TABLE 2. Number of ectomycorrhizal basidiomycetes recorded from six plots of red pine forest in northern Minnesota in 1993 – 1995.

Area:	No. of taxa[a] in circles, 400 m^2/plot						No. of taxa[a] in plots of 5000 m^2					
Age:	Mature			Old-growth			Mature			Old-growth		
Plot:	1	2	3	1	2	3	1	2	3	1	2	3
1993[b]				24	46					28	62	
1994	53	62	45	45	48	50	70	86	85	60	68	73
1995	36	45	38	37	33	34	46	70	56	49	54	47
1994-1995	61	73	59	55	57	55	76	100	94	72	82	78
1993-1995				61	64					77	90	

	Mature	Old-growth	Shared[c]	Mature	Old-growth	Shared[d]
Total 1993		52			69	
Total 1994	93	71	52	120	93	78
Total 1995	68	58	38	86	79	59
1994-1995	105	80	62	125	104	86
1993-1995		85			109	

	Mature and old-growth	Mature and old-growth
Total 1994	112	135
Total 1995	88	106
1994-1995	123	143
1993-1995	125	146

[a] Numbers are underestimates because most unidentified *Cortinarius* are collectively counted as one taxon.

[b] Only two plots of old-growth were sampled in 1993.

[c] Number of species found in circles of both age classes.

[d] Number of species found in plots of both age classes.

Plots of the two age classes displayed essentially the same range in the number of species per circle for 1994 1995 (Table 5) and their total distribution in numbers is very similar. Both averaged more than three species per 4 m^2 circle. All plots had only one to three of the 100 circles unoccupied for the two years (Table 5). Species-area curves (Fig. 1) for mature and old-growth are similar for the circles of one plot (400 m^2) but with the addition of the second and third plots total diversity increases more for mature red pine forest. The dominance-diversity curves (Fig. 2) demonstrate that 60 or 70% of the species in both age classes had very low frequencies.

Abundance of fruitbodies, frequency and density

A wide range in abundance was displayed by species in both age classes. Frequencies for each species ranged from 0.3% (present in one of 300 circles for three plots) up to 40% for *Russula silvicola* in mature plots and 34% for *Laccaria laccata* in old-growth plots (Table 3). The total frequency of all species for 1994 1995 was 98% and equals the percent of occupied circles (Table 5).

The production of fruitbodies was very high in all plots (Table 6). Total density was greatest in 1994 with an estimated 14,000 fruitbodies per hectare (fb./ha.) in mature and 16,100 fb./ha. in old-growth stands. Density for individual visits ranged from 150 fb./ha. for August 17 18, 1995 to 11,700 fb./ha. for September 24 27, 1995. The species with the greatest estimated total densities for 1994 1995 were *Laccaria laccata*, 2960 fb./ha. in old-growth, and *Russula silvicola*, 2940 fb./ha. in mature stands. The hypogeous *Elaphomyces* was potentially very abundant; it ranked sixth in total frequency, which was a minimal estimate based on the presence of *Cordyceps* (Table 6). Minimum density ranged from 150 to over 300 fb./ha./yr., a level comparable to that of 200 fb./ha/yr. found for Douglas fir (Fogel 1976). Fruitbody densities of potential indicator species for mature and old-growth red pine forest are shown in Table 4.

TABLE 3. Ordination of species and plots by frequency[a] of ectomycorrhizal species in Minnesota mature and old-growth red pine forest for 1994-1995.

Age class:	Mature			Old-growth		
Species Plot:	1	3	2	3	1	2
Russula silvicola Shaffer	42	45	34	35	27	16
Gomphus flocossus (Schw.) Sing.	17	31	14	6	5	4
Suillus granulatus (L.:Fr.) Kuntze	11	-	4	3	1	+[b]
Entoloma aff. *politum* (Pers.:Fr.) Donk	11	1	1	1	3	-
Cortinarius subcroceofolius Ammirati & A. H. Smith	7	3	3	1	-	1
Cortinarius cf. *anomalus* (Fr.:Fr.) Fr.	4	8	10	3	1	3
Russula fragilis Fr.	5	-	9	1	3	-
Cortinarius semisanguineus (Fr.) Gill.	6	7	10	6	3	5
Russula laurocerasi Melzer	4	+	2	+	+	-
Suillus luteus (L.:Fr.) S. F. Gray	4	1	+	-	-	-
Russula adusta Fr.	3	1	2	1	1	+
Lactarius torminosus (Schaeff.:Fr.) Persoon	-	4	6	1	1	+
Inocybe sp. RP 05	2	1	2	-	-	-
Cortinarius cf. *laniger* Fr.	2	7	2	1	2	2
Inocybe sp. RP 09	3	+	-	-	-	-
Ramaria sp. RP 01	4	-	-	1	-	1
Tricholoma sp. RP 09 (Sect. Genuina)	2	1	1	-	-	-
Russula sp. RP 41	3	+	2	-	+	1
Cortinarius sp. HC 03	-	8	+	1	1	1
Lactarius glyciosmus (Fr.:Fr.) Fr.	-	3	3	-	-	-
Russula viridella Peck	2	+	+	-	+	+
Hebeloma sp. RP 05	-	3	1	-	-	-
Cortinarius armillatus (Fr.:Fr.) Fr.	1	6	5	2	4	1
Russula sp. RP 05	1	+	3	+	-	+
Suillus intermedius (A. H. Smith & Thiers) A. H. Smith & Thiers	4	3	4	2	3	3
Russula sp. RP 11	1	1	1	1	+	+
Tricholoma flavovirens (Pers.:Fr.) Lundell	2	2	3	3	+	2
Tricholoma intermedium Peck	1	1	+	+	-	-
Lactarius uvidus (Fr.:Fr.) Fr.	1	+	1	-	+	-
Inocybe sp. RP 06	1	-	1	-	-	-
Leccinum vulpinum Watling	1	+	1	+	-	+
Russula sp. RP 34	1	2	+	+	-	1
Amanita vaginata (Fr.) Vitt.	+	1	2	+	+	-
Cortinarius trivialis Lange	+	1	2	-	-	-
Clitopilus prunulus (Scop.:Fr.) Quél.	-	2	-	-	-	-
Boletus edulis Bull.:Fr. ssp. *aurantioruber* Dick & Snell	1	+	+	+	-	+
Cortinarius mucosus (Bull.:Fr.) Kickx	1	+	+	-	-	-
Tricholoma olivaceobrunneum Ovrebo	1	+	-	-	-	-
Xerocomus spadiceus (Fr.) Quél.	1	-	+	-	-	-
Cortinarius sp. RP 26	1	-	-	-	-	-
Inocybe sp. RP 10	1	-	-	-	-	-
Inocybe sp. RP 12	1	-	-	-	-	-
Ramaria sp. RP 07	1	-	-	-	+	-
Russula sp. RP 31	+	1	2	1	-	-
Russula sp. RP 27	-	-	3	-	-	-
Cortinarius cf. *claricolor* (Fr.) Fr. group	-	+	2	-	+	+
Hygrophorus sordidus Peck	+	+	2	-	-	-
Hydnellum cf. *zonatum* (Batsch:Fr.) Karst.	-	+	2	-	-	-
Hebeloma sp. RP 04	1	+	-	2	-	-
Cortinarius traganus (Fr.:Fr.) Fr.	+	1	+	+	+	+

TABLE 3. (*continued*)

Species	Age class: Mature		Old-growth			
	Plot: 1	3	2	3	1	2
Amanita fulva (Schaeff. ex) Persoon	+	1	-	-	-	-
Cortinarius sp. RP 43	-	1	-	-	-	-
Inocybe sp. RP 08	-	1	-	-	-	-
Russula albonigra (Kromb.) Fr.	-	1	-	-	-	-
Lactarius rufus (Fr.:Fr.) Fr. var. *rufus*	-	1	+	+	-	-
Cortinarius pholideus (Fr.:Fr.) Fr.	-	2	2	2	-	1
Albatrellus ovinus (Schaeff.:Fr.) Murr.	-	+	4	-	-	1
Hydnum umbilicatum Peck	-	1	-	1	-	-
Phellodon melaleucus (Sw. apud Fr.:Fr.) Karst.	-	+	1	-	-	-
Hydnellum caeruleum (Hornemann ex Pers.) Karst.	-	+	1	-	+	-
Cortinarius sp. RP 46	-	-	1	-	-	-
Cortinarius sp. RP 49	-	-	1	-	-	-
Hebeloma sp. RP 02	-	-	1	-	-	-
Russula sp. RP 53	-	-	1	-	-	-
Sarcodon sp. RP 01	-	-	1	-	-	-
Tricholoma sp. RP 07 (Sect. Genuina)	-	-	1	-	-	-
Tricholoma sp. RP 12	-	-	1	-	-	-
Ramaria sp. RP 06	1	-	1	1	-	1
Lactarius aquizonatus Kytövuori	-	1	3	-	2	+
Inocybe geophylla (Sow.:Fr.) Kumm. var. *lilacina* (Peck) Gill.	-	-	1	1	-	-
Hydnellum aurantiacum (Batsch:Fr.) Karst.	-	1	1	+	-	1
Leccinum scabrum (Bull.:Fr.) S.F. Gray	-	1	+	-	1	+
Lactarius cf. *mucidus* Burlingham	-	1	+	+	1	+
Hygrophorus cf. *purpurascens* Alb. & Schw.:Fr.	+	-	+	1	-	-
Inocybe sp. RP 07	-	-	+	1	-	-
Cantharellus cinereus Pers.:Fr.	1	-	-	+	2	+
Phellodon niger (Fr.:Fr.) Karst.	-	1	2	+	1	1
Lactarius affinis Peck var. *viridilactis* (Kauffman) Hesler & A. H. Smith	+	3	9	4	2	3
Hygrophorus fuligineus Frost apud Peck	-	-	-	2	+	+
Hydnellum cf. *pineticola* K. Harrison	-	+	1	-	-	1
Lactarius indigo (Schw.) Fr. var. *indigo*	-	+	1	+	+	1
Russula sp. RP 08	1	+	1	+	3	+
Cortinarius sp. RP 55	-	-	-	-	1	-
Suillus placidus (Bonorden) Singer	-	-	-	-	1	-
Russula galochroa Fr.	+	+	+	1	1	+
Lactarius camphoratus (Bull.:Fr.) Fr.	-	-	-	1	1	-
Russula brevipes Peck	-	+	+	+	-	1
Lactarius chelidonium Peck	-	+	-	-	+	1
Inocybe sp. RP 11	-	-	-	-	-	1
Lactarius paradoxus Beardslee & Burlingham	-	-	-	-	-	1
Chroogomphus rutilis (Schaeff.:Fr.) O. K. Miller	2	1	3	2	1	4
Cortinarius sp. RP 12 (Sect. Leprocybe)	-	+	1	-	1	1
Russula sp. RP 30	+	+	+	2	+	1
Hydnum repandum (L.:Fr.) S. F. Gray	1	2	+	1	5	-
Thelephora sp. RP 01	-	-	-	-	1	1
Hebeloma sp. RP 01	4	4	8	6	10	3
Coltricia perennis (Fr.) Murr.	1	+	1	+	2	2
Russula claroflava Grove	1	1	+	2	1	3
Cortinarius cf. *strobilaceus* Moser	-	-	-	1	1	2
Inocybe lanuginosa (Bull.:Fr.) Kumm.	-	-	-	-	-	3

TABLE 3. (*concluded*)

Species	Age class:	Mature			Old-growth		
	Plot:	1	3	2	3	1	2
Amanita porphyria (Alb. & Schw.:Fr.) Secr.		1	-	+	2	1	3
Cantharellus cibarius Fr.		2	11	7	+	8	9
Chroogomphus flavipes (Peck) O. K. Miller		-	-	-	-	2	2
Rozites caperatus (Pers.:Fr.) Karst.		+	+	-	-	1	3
Inocybe sp. RP 02		2	1	9	3	3	8
Lactarius deceptivus Peck		-	3	-	5	1	5
Lactarius vietus (Fr.:Fr.) Fr.		1	21	22	9	11	17
Lactarius thejogalus (Bull.:Fr.) S. F. Gray		4	+	1	2	7	5
Russula puellaris Fr.		1	6	10	4	8	8
Russula sp. RP 16		1	1	2	4	4	6
Tricholoma saponaceum (Fr.:Fr.) Kumm. s.l.		-	+	2	9	3	4
Hygrophorus piceae Kuhn.		-	-	-	3	4	6
Laccaria bicolor (Maire) Orton		3	9	8	14	11	10
Hygrophorus pudorinus (Fr.) Fr.		8	9	15	13	8	21
Lactarius vinaceorufescens A. H. Smith		12	15	3	18	16	18
Russula sp. RP 19		+	3	2	7	5	8
Suillus spraguei (Berk. & Curt. in Berk.) Kuntze		3	1	3	6	8	10
Entoloma cf. *sericatum* (Britz.) Sacc.		2	11	11	19	19	7
Inocybe geophylla (Sow.:Fr.) Kumm. var. *geophylla*		-	-	1	12	14	5
Suillus punctipes (Peck) Singer		2	1	-	9	17	8
Hygrophorus camarophyllus (Alb.&Schw.:Fr.) Dume et al.		1	1	1	8	13	12
Laccaria laccata (Scop.:Fr.) Cooke		15	21	25	20	44	38
Cortinarius undet. spp. (primarily Subg. *Telamonia*)		52	69	66	57	61	64
Additional species observed in plots but not recorded in circles							
Tricholoma sp. RP 10		+	+	-	-	-	-
Amanita frostiana Peck		+	+	-	-	-	+
Russula aurantialutea Kauffman		+	-	-	+	-	-
Hygrophorus cf. *erubescens* Fr.		-	+	+	-	-	-
Lactarius repraesentaneus Britzelmayr		-	+	-	-	-	-
Lactarius cf. *scrobiculatus* (Scop.:Fr.) Fr.		-	+	-	-	-	-
Tricholoma virgatum (Fr.:Fr.) Kumm.		-	+	-	-	-	-
Tricholoma davisiae Peck		-	+	-	+	-	+
Hebeloma sp. RP 03		-	-	+	-	-	-
Russula modesta Peck		-	-	+	-	-	-
Tricholoma focale (Fr.) Ricken		-	-	+	-	-	-
Tricholoma sp. RP 14		-	-	+	-	-	-
Lactarius deterrimus Gröger		-	-	+	-	+	-
Cortinarius sp. RP 36		-	-	-	+	-	-
Inocybe sp. RP 04		-	-	-	-	-	+
Russula aeruginea Lindblad		-	-	-	-	-	+
Russula sp. RP 42		-	-	-	-	-	+
Tricholoma sp. RP 15		-	-	-	-	-	+
Albatrellus confluens (Alb. & Schw.:Fr.) Kotl. & Pouz.		-	-	-	+	-	+
Tricholoma portentosum (Fr.) Quél.		-	-	-	+	+	+

[a] Percentage of circles occupied for the two years; 100 4-m^2 circles/plot. Species and plots are ordered by the corresponding eigenvectors of principal component 2.

[b] + species present in plot.

TABLE 4. Potential indicator species of mature and old-growth red pine forest in Minnesota. Average yearly density, fb./ha.[a], for each plot, 1994-1995.

	Mature			Old-growth		
Species Plot:	1	2	3	1	2	3
Mature						
Cortinarius mucosus	13	+[b]	+			
Cortinarius trivialis	+	25	13			
Hygrophorus sordidus	+	38	+			
Inocybe sp. RP 05	38	113	13			
Suillus luteus	88	+	13			
Tricholoma intermedium	25	+	13			+
Tricholoma sp. RP 09	25	13	13			
Old-growth						
Chroogomphus flavipes				25	38	
Cortinarius cf. *strobilaceus*				13	75	38
Hygrophorus fuligineus				+	+	75
Hygrophorus piceae				275	213	363
Inocybe geophylla var. *geophylla*		13		1013	325	800
Tricholoma portentosum				+	+	+
Hygrophorus camarophyllus[c]	38	13	25	750	563	312

[a] Estimated from number of fruitbodies in 400 m^2 (100 circles), averaged for the two years.
[b] Present in plot but not found in circles.
[c] Significantly greater presence in old-growth plots.

Yearly variation and fruiting phenology

Diversity was greater in 1994 than in 1995. Of the two-year species total, 94% were observed in 1994 and 74% in 1995. Low numbers for the first two visits in 1995 were due to initial dry conditions (Fig. 3). Variation in total fruitbody density varied more between years than between age classes; densities for mature and old-growth plots in 1995 were very close for each of the four visits (Fig. 3). In both years, 83% of the total species observed were recorded in sampling circles. Partial old-growth sampling in 1993 yielded 63% of the species for this age class. For red pine forest the fruiting season extended from late June to early October. The latest sampling visit was October 5, 1993; the final visits in 1994 and 1995 were made the last week in September before the decline in fruiting occurred (Fig. 3). Fruiting patterns varied between species and genera (Fig. 4). A few taxa (e.g., *Cantharellus cibarius* and *Russula*) showed two peaks in fruiting, although not in

both years. *Gomphus flocossus*, *Russula silvicola*, and some *Suillus* spp. were noticeable fruiters in late June and July. Most species exhibited greatest fruiting sometime in September. *Tricholoma* spp. and *Hygrophorus* spp. as a whole were abundant at the end of the season (Fig. 4).

TABLE 5. Range in the number of species per circle and percent of circles occupied.

No. of species/ circle	Number of circles					
	Total 1994-1995		Sep. 23-28, 1994[a]		Aug. 17-18, 1995[b]	
	Mature	Old-growth	Mature	Old-growth	Mature	Old-growth
0	5	8	36	13	174	175
1	40	38	69	53	5	5
2	64	62	47	58	1	
3	63	59	22	32		
4	62	42	5	12		
5	33	49	1	10		
6	18	21		1		
7	4	11		1		
8	7	7				
9	0	2				
10	1	1				
11	3					
Mean no. of spp./circle	3.35	3.47	1.41	2.09	0.04	0.03
SD	1.89	1.94	1.06	1.33	0.22	0.16
Total circles	300	300	180	180	180	180
Total occupied	295	292	144	167	6	5
% occupied	98.3	97.3	80.0	92.7	3.3	2.8

[a] Maximum occupied circles for one visit.
[b] Minimum occupied circles for one visit.

TABLE 6. Yearly variation in abundance of the major species for red pine forest.
The seventeen most frequent species plus a minimal estimate for *Elaphomyces* based on *Cordyceps* presence.

Species	Total freq. 1994–1995[c]	Yearly frequency[a]				Yearly density, fb./ha.[b]			
		Mature		Old-growth		Mature		Old-growth	
		1994	1995	1994	1995	1994	1995	1994	1995
Russula silvicola	33.2	31	27	21	11	1567	1375	842	392
Laccaria laccata	27.2	16	7	27	14	933	625	2125	833
Lactarius vinaceorufescens	13.7	7	4	14	5	367	150	458	183
Lactarius vietus	13.5	9	12	7	8	483	625	308	458
Gomphus flocossus	12.8	16	7	3	3	692	292	117	75
Hygrophorus pudorinus	12.3	6	7	8	9	425	567	517	1208
Entoloma cf. *sericatum*	11.5	5	5	11	6	192	317	608	217
Laccaria bicolor	9.2	2	5	3	9	67	300	92	417
Cantharellus cibarius	6.2	4	4	4	4	275	367	408	525
Cortinarius semisanguineus	6.2	7	1	5	0.3	575	50	325	42
Russula puellaris	6.2	5	2	5	2	175	83	167	83
Suillus punctipes	6.2	0.3	0.6	6	6	8	42	200	175
Hygrophorus camarophyllus	6.0	1	–[d]	11	+[e]	50	–	1083	+
Hebeloma sp. RP 01	5.8	3	3	4	3	92	83	117	92
Inocybe geophylla var. *geophylla*	5.3	+	0.3	8	7	+	8	908	517
Suillus spraguei	5.0	1	1	7	1	50	42	200	58
Cortinarius cf. *anomalus*	4.8	6	2	2	–	242	50	67	–
Data for the 123 species combined	97.8	93	71	90	66	14000	8700	16100	9025
Cordyceps capitata	6.0	4	1	3	5	217	75	117	175
Cordyceps ophioglossoides	9.3	12	3	4	2	725	350	108	108
Minimal estimate for *Elaphomyces*[f]	12.6	13	4	6	6	333	92	158	158

[a] Percentage of 300 circles occupied, rounded to whole number (if greater than one).
[b] Estimated from number of fruitbodies in 1200 m^2 (300 circles).
[c] Combined frequency for both age classes and both years.
[d] Not present in plots at time of visits that year.
[e] Present in one or more plots but not found in circles.
[f] Combined freq. for the parasitic *Cordyceps*. Density estimated from the minimum of one fb. per circle occupied by *Cordyceps*.

Fig. 1. Species-area curve for red pine forest.

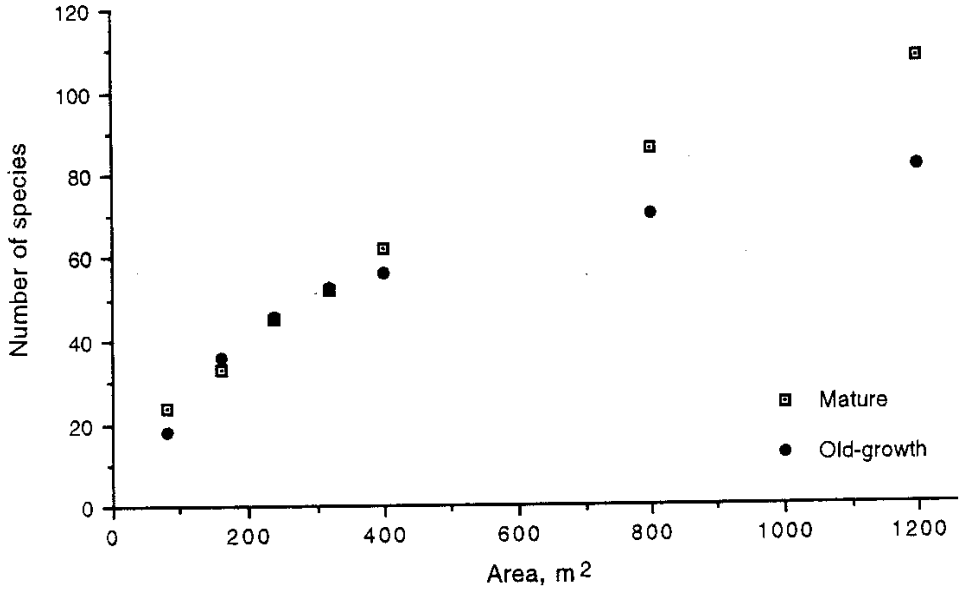


Fig. 2. Dominance-diversity curves for red pine forest.
Scales standardized to a maximum of 100 (see methods).
For clarity, only alternate datapoints are shown for species sequence above 30.
(Group of unidentified *Cortinarius* spp. omitted.)

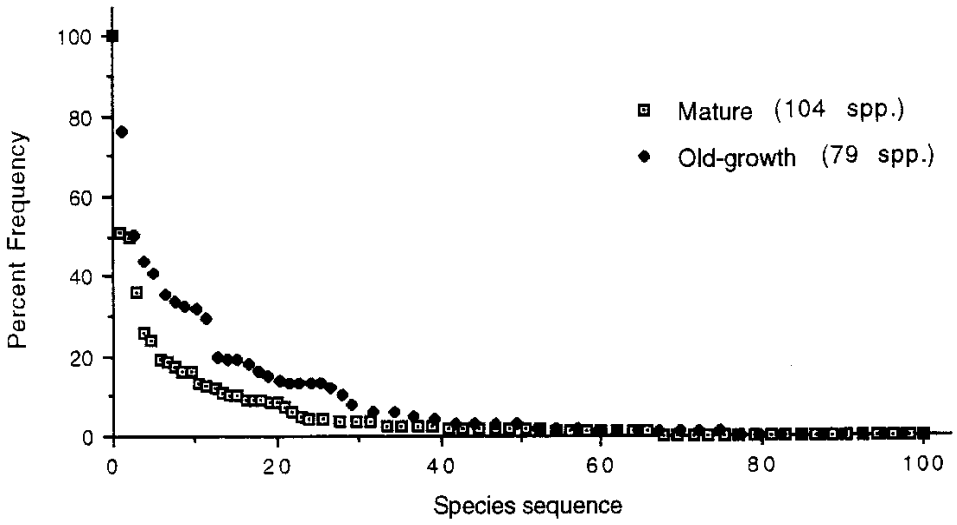


Fig. 3. Yearly variation in fruitbody densities for red pine forest.

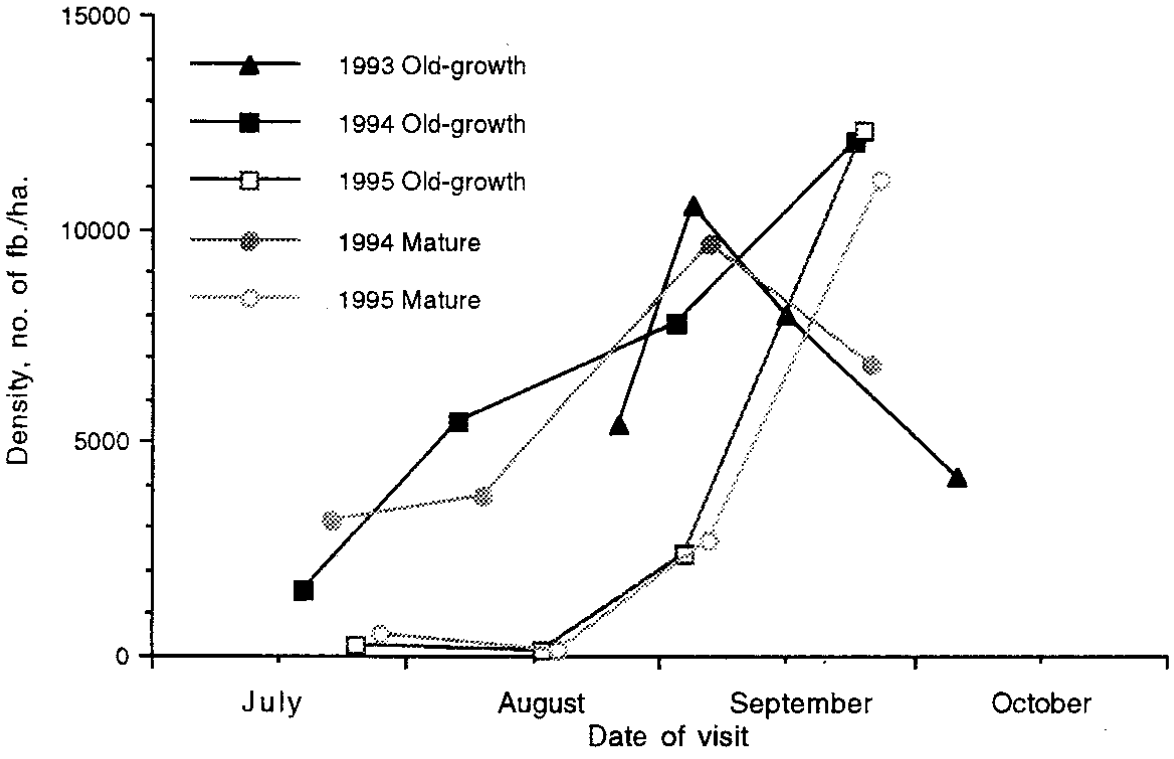
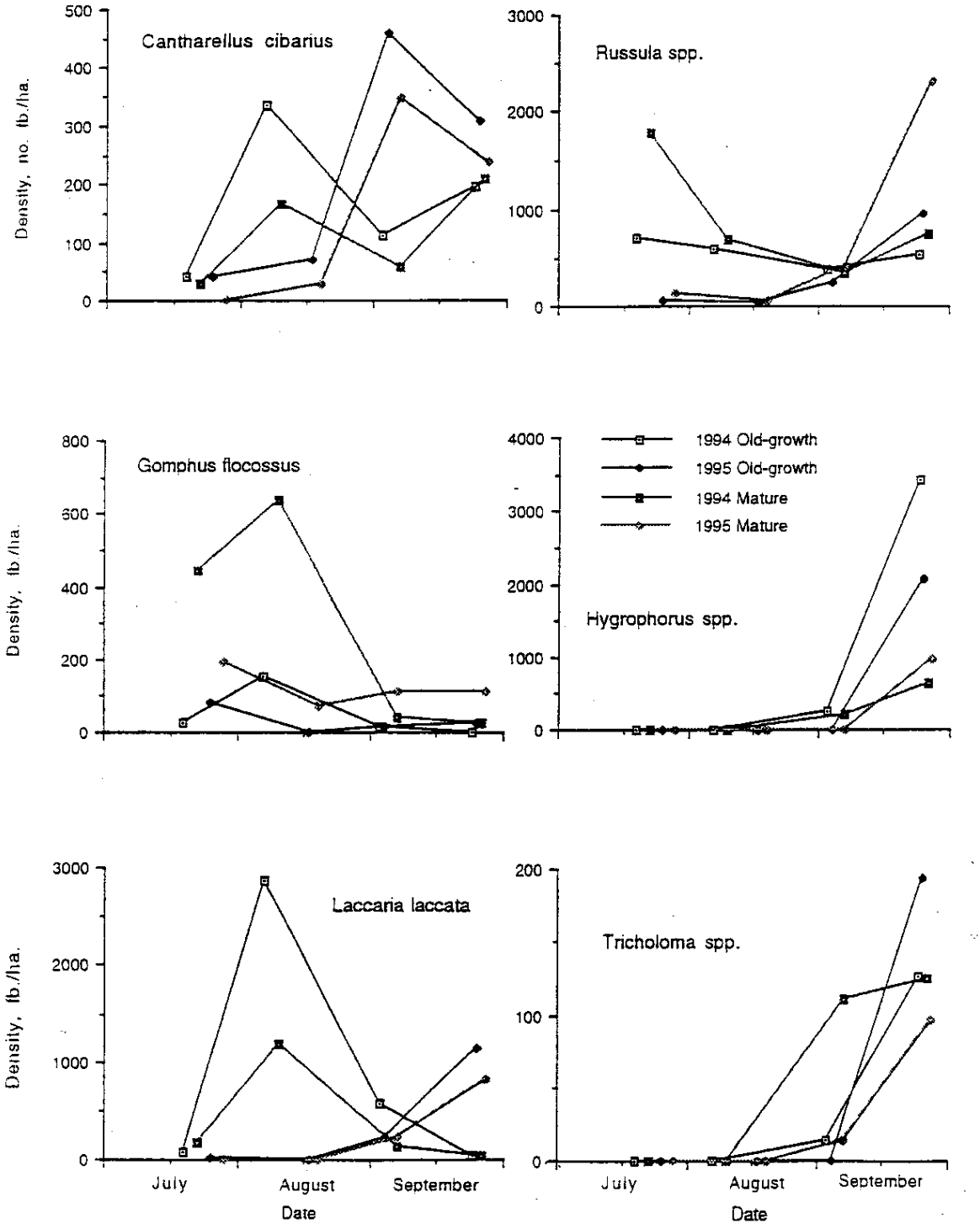


Fig. 4. Fruitbody densities in 1994 and 1995 for mature and old-growth red pine forest.



DISCUSSION

Richness, total frequency, and density estimates were notable for both mature and old-growth red pine forest. Although the mature stands had greater species diversity, the old-growth stands showed greater abundance of fruitbodies and total frequency was the same as in mature stands. Both age classes support diverse fungal communities with unique species despite their proximity to each other. The fungal diversity of old-growth stands is not simply a reduced subset of the mature red pine forest species. In terms of species richness, my findings parallel the high diversity found by O'Dell and Ammirati (1994) for old-growth coniferous forests in the Pacific Northwest (further discussion in Chapter IV).

Total species richness, 125 spp., and the mean number of species per plot, 90 spp., was greater for the mature age class, whereas total richness was 104 spp. (in 1994 1995) for old-growth plots with a mean of 77 species. Twenty-seven percent of the total species were found only in mature stands, 13% only in old-growth, and a majority of 60% were common to both age classes. Overall, the mature and old-growth red pine plots showed a large degree of similarity in their species composition (Table 2). Thirty-six of the 146 species (25%) were common to all six red pine plots. Similarly 54 species were common to all three mature plots and 53 species to all three old-growth plots.

The somewhat lower diversity for old-growth plots correlates with some pine forest studies that indicate a decline of diversity with stand age, although in the present study it is not a major decrease (Hintikka 1988). Miller (1983) reported that diversity increased for western white pine from 34 37 spp. in ca. 37 year-old plantations to 78 spp. in a mature stand 175 215 years old. Higher diversity of root ectomycorrhizae was found for 65- and 122-year-old jack pine than that present on roots of 6- and 41-year-old jack pine, while the 65-year-old stand had the highest number of species fruiting (Visser 1995).

Large numbers of infrequent or rare species occur in both mature and old-growth red pine forests. This high diversity made it difficult to document the total species diversity for this forest and several taxa, in addition to many *Cortinarius* spp., are undocumented (i.e., without voucher specimens and not included in Table 3). Most *Hebeloma*, *Inocybe*, and *Ramaria* are unidentified. Identifications of *Entoloma* require confirmation. I am confident of the identifications of species in the other

genera, with a few exceptions. The separation of *Hygrophorus erubescens* and *H. purpurascens* was unclear. The separation in the field of *Laccaria bicolor* from *L. laccata* was improved in the second year (1994) with continued observation. The *Lactarius mucidus* group needs a systematic analysis. *Russula* was problematical in terms of separating taxa in the field, and adequately documenting this abundant genus. *Russula* species listed in Table 3 were believed to be correct identifications. Several *Russula* taxa were not found in the literature and are possibly undescribed species. I estimate 50% of the species to be new state records.

Cortinarius and Russulaceae accounted for the greatest diversity in these red pine stands. This has also been found in other studies of conifer dominated forests, i.e., red spruce (Bills et al. 1986), Norway spruce (Gulden et al. 1992), heath forest with pine, spruce, birch (Ohenoja 1978), and boreal coniferous and mixed forests (Salo, 1993).

The sampling design of O'Dell and Ammirati (1994) differed from the one used in this study in that they laid out new transects during each visit to avoid sampling areas that had been walked through previously. They reported low recurrence of the same species with subsequent visits. The method of revisiting the same transects provided data showing that certain fungi, common or rare, can be found two or three times in one season or from one year to the next in the same circle. *Russula silvicola* showed a high rate of recurrence; for all 121 circles occupied in mature plots during the two years it occurred within 44% of those circles both years. Several species, including *Boletus edulis* and *Cortinarius traganus*, were each found in a single circle. Data on percent recurrence is given by Tables 9, 10 of Chapter III.

All plots displayed a remarkably high percentage of occupied circles for 1994 1995 (Table 5); only 13 out of 600 circles were unoccupied. Three of these circles, sampled in 1993, contained two species each and other circles did contain saprobic basidiomycetes (data not shown). Two factors account for the high percentage of occupied circles. Most importantly, high abundance during peak fruiting periods resulted in the presence of fruitbodies in 80 93% of the sampled circles in one visit (Table 5). Secondly, these same circles were visited either four or eight times during 1994 1995. The findings of Bills et al. (1986) of a mean of 2.4 species per 4 m^2 quadrat and 85% occupation of quadrats for red spruce forest is similar; 24 30 visits were made over three years in their study. The higher mean

of 3.4 species per 4 m^2 circle for red pine forest is likely due to the variety of tree hosts present versus essentially the one species red spruce plots.

Frequency and density are two different measures of abundance. When based on presence of fruitbodies, they are obviously correlated with intensity of fruiting. The frequency of fruitbodies is a minimal estimate for frequency of the species belowground. Density of fruitbodies relates to biomass production, in this case limited to that above ground. Studies that compared fruiting with belowground presence on root tips do not always find a direct correlation between the two (Egger 1995). Gardes and Bruns (1996) report that the dominant fruiters are not the dominant species on roots. I did not examine below ground activity in this study, but observations of *Cordyceps* abundance provided a minimum estimate of *Elaphomyces* frequency. Surprisingly *Elaphomyces* ranked as the sixth most frequent taxon for red pine forest. This gives some indication of the possible magnitude of hypogeous species abundance.

The high total frequencies for both mature (98%) and old-growth (97%) red pine forest were essentially the same. Although species diversity was lower in old-growth plots the total fruitbody density (> 25,000 fb./ha for 1994 and 1995 combined) was actually greater than that of mature stands (22,700 fb./ha.). Productivity of the old-growth stands was therefore as great as or greater than mature stands.

Some species appear to be characteristic of red pine forest (Table 6). While the data comes from only two years of study, and an additional 1 2 years of study is desirable because of year to year variability in fruiting, some species can be tentatively listed as indicator species that are primarily restricted to stands of a specific age (Table 4). Several species were decidedly more abundant in one age class, e.g., *Hygrophorus camarophyllus* in old-growth and *Gomphus floccosus* in mature stands (Tables 4, 6).

Total fruitbody production varied between years and was greatest in September (Figs. 3, 4), indicating the need to extend the collecting period into October until weather conditions become limiting to better document the abundance of species fruiting in late fall. Drier conditions in 1995 appeared to be the cause for low fruiting in July and August (Fig. 3) and shifting of the peak fruiting for *Cantharellus cibarius* and *Laccaria laccata* (Fig. 4). Some species, e.g., *Laccaria bicolor* and

Lactarius vietus, were more abundant in 1995 (Table 6). The apparent absence of *Hygrophorus camarophyllus* in 1995 (Table 6) was due to its very late fruiting, which occurred in early October after the last sampling visit. Fruiting phenology was variable among genera and species (Fig. 4). Some taxa were abundant in summer, e.g., *Gomphus* and *Russula*, while *Hygrophorus* and *Tricholoma* were more typical of late fall. *Lactarius thejogalus* attained the maximum production of 87 fruitbodies for one 4-m^2 circle in two years.

As discussed by Bills et al. (1986), it is difficult to determine the adequate sampling area required to capture the fungal species diversity of a particular vegetation type. In the two age classes of red pine forest, numbers of species was notably increased from a sampling area of 800 m^2 to 1200 m^2 (Fig. 1). Non-intensive searching of plot area between circles and transects yielded 20 additional species (16%) for mature and 24 additional species (23%) for old-growth red pine forest in two years. When only one plot and one year of sampling is examined, up to 47% of the species in the plot are found outside of circles. From the opposite perspective, the sampling circles, totaling 400 m^2, cover only 8% of a half hectare plot yet 63 80% (mean 72%) of the species documented for a plot are recorded within sampling circles (in 1994 1995). The sampling circles were effective in analyzing mushroom diversity in red pine forest when fruitbody production was high, but less so during periods of poor fruiting. When a greater number of species is observed, sampling efficiency can be increased (Parker-Rhodes 1951).

Obtaining the full diversity for a fungal community does not appear to be possible (Christensen 1981, Bills et al. 1986), and the results obtained in this study show variability similar to that in other studies (Bills et al. 1986). I observed *Leccinum niveum* (Fr.) Rauschert (= *L. holopus* (Rostk.) Watl.), *Tylopilus felleus* (Bull.:Fr.) Karst., and additional species of *Ramaria*, *Russula*, and *Tricholoma* within 10 50 meters of the plots. *Lactarius pseudodeceptivus* Hesler & A. H. Smith and abundant *Hydnellum* spp. were found in a nearby red pine stand of age intermediate between the studied stands.

Several factors in addition to finding fruitbodies in poor condition affected abundance estimates. Counts were made of *Hypomyces lactifluuorum*, an Ascomycete that commonly parasitized certain Russulaceae, in this case *Russula brevipes* and possibly *Lactarius deceptivus*. Total frequencies for *H. lactifluuorum*

were 1.6% for mature and 0.3% for old-growth plots. Other infrequent *Hypomyces* species obscured the identity of *Russula* and bolete species. Another factor that decreased fruitbody counts was mushroom consumption by animals. I found remaining stipe bases as evidence that white-tailed deer ate several taxa including *Amanita porphyria*, *Hygrophorus camarophyllus*, *H. pudorinus*, and *Lactarius affinis* var. *viridilactis*. Red squirrels in old-growth plot 2 had hung several *Russula* spp. in branches to dry for later use. A hunter in the area repeatedly found ruffed grouse crops full of mushroom pieces, including an unexpanded *Amanita* pileus, and I saw evidence of *Hygrophorus piceae* consumption. Most of these mushroom species were more abundant in old-growth plots.

The data indicate that two years sampling with four visits per year is not adequate for a complete community analysis. Three species observed in old-growth plots in 1993, *Cortinarius* sp. RP20 (Sect. Sericeocybe), *Inocybe* sp. RP03, and *Tricholoma argenteum* Ovrebo, were not seen the two subsequent years even though data collecting was more extensive in 1994 1995. Thirty species were found only in 1994 and sampling in 1995 added seven species not seen previously. More intensive sampling is desirable for recording full diversity. However, for red pine forest where abundance is high, two years of sampling provides a valuable initial estimate of diversity.

Some possible variation between plots due to edaphic factors is reduced by having all six red pine forest plots situated close to one another. Old-growth and mature red pine stands differing in tree composition, as well as age, varied only slightly in soil characteristics.

All of the trees in these plots, and the shrub *Corylus* (Table 1), are ectomycorrhizal, except *Acer* and *Amelanchier* which are endomycorrhizal. This gives a ratio of potentially ectomycorrhizal fungi to host plants of 16:1 for both mature (126 fungi : 8 plants) and old-growth (109:7) red pine forest. These ratios would be reduced if any ectomycorrhizal fungi are restricted to associations with the low understory shrubs of Ericaceae: *Epigaea*, *Gaultheria*, and *Vaccinum*. This suggests that each tree species potentially has a large number of associated fungi. These findings disagree with the observations of Dighton and Mason (1985) of lower fungal diversity with increasing stand age; however, their study did not include data from naturally occurring old-growth forests.

Because of the mixture of tree species it was not possible to determine specific mycorrhizal associations in most cases. However, one of the most obvious was that of *Albatrellus ovinus*, which was always in the vicinity of white spruce, *Picea glauca*. The presence of the 5-needle pine *Pinus strobus* accounted for the presence of several host-specific taxa, notably in *Suillus*: *S. spraguei*, *S. punctipes*, *S. placidus*, and *S. granulatus* (Kretzer et al. 1996); likewise *S. intermedius* and *S. luteus* were likely *P. resinosa*, or *P. banksiana*, (2-needle pine) associates. Other species are generalists such as *Lactarius aquizonatus*. This species in Northern Europe is found with many hardwoods and conifers under conditions ranging from dry, upland forests to swampy depressions (Kytövuori 1984); its presence in North America is now confirmed by this study.

A comparison of mature and old-growth red pine forest showed that each has a different fungal composition with much overlap in species. To obtain baseline data for any old-growth forest will probably require an intensive, multiyear analysis. Forests on poor or sandy soils may need study more urgently as they are most susceptible to alteration by acidification or nitrogen deposition. Baseline data is also desirable for long term monitoring of environmental change. Eight species of Thelephorales (*Hydnellum*, *Sarcodon*, *Thelephora*, and *Phellodon*) were encountered in the red pine forest stands. In nearby stands they exhibited high localized abundance. Arnolds (1989) reports that six of the nine species showing the strongest decline in The Netherlands are hydnaceous fungi including *Phellodon melaleucus* (going from rather common to very rare). Their presence here in Minnesota forests is a positive indication of the health of the fungal community.

In conclusion, the ectomycorrhizal mushroom flora is extremely rich in both mature and old-growth red pine forests. It is apparent, at least for some forest types, that older stands can contribute a notable portion of the total diversity. Study of mature and old-growth stands in close proximity to one another has shown consistent differences in abundance of groups of species for the two age classes. One or two additional full years of sampling is desirable to further establish baseline data for this forest, and examination of red pine forest stands at more distant sites will add to our understanding of the fungal community diversity for this forest type.

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III. Diversity of Ectotrophic Mycorrhizal Fungi in Ancient and Successional Northern Hardwood-conifer Forests and Comparison to Red Pine Forest

Introduction

There have been several investigations in Eastern North America of ectomycorrhizal fungi communities. Bills et al. (1986) examined second-growth northern hardwood forest in West Virginia. Nantel and Neumann (1992) studied 11 plots of varying tree composition along a vegetation gradient in Quebec. The study of Villeneuve et al. (1989) included old-growth sugar maple yellow birch forest in Quebec. This study will address the difference in fungal communities between undisturbed ancient northern hardwood-conifer forest and post-logging, second-growth northern hardwood stands in northeastern Minnesota. Findings for the old-growth and successional forests will be compared to those of red pine forest (Chapter 2).

Although aboveground methods for estimating ectomycorrhiza presence have disadvantages, quantitative observations of fruitbodies provide a minimal estimate of fungal diversity and abundance with additional information on phenology of fruiting and community interactions. The same sampling procedure as that used for red pine forest (Chapter II) involving six half-hectare plots containing permanent transects was employed to quantify the ectomycorrhizal fungi composition. Plots were visited during the fruiting seasons of 1993, 1994, and 1996 for frequency measurements and fruitbody density estimates.

Materials and methods (see also Appendix I)

Location and description of the study sites

Using the same plot design as for red pine forest (Chapter II), six half-hectare (50 × 100 m) plots were established in 1993 and 1994. The plots were in three locations in the North Shore Highlands near Finland, Lake County, Minnesota. Within Tettegouche State Park, the three successional northern hardwood plots, spaced 75 175 m apart (47°21126"N, 91°15133"W), elevation 440 470 m, were

2.5 km from adjacent plots 1 and 2 of old-growth northern hardwood-conifer forest (47°20108"N, 91°14110"W), elevation 385 m. George H. Crosby-Manitou State Park contained the additional old-growth stand with plot 3 (47°28124"N, 91°06111"W), elevation 415 m, at a distance of 18 km from the other five plots. The two old-growth stands were a natural assemblage of seven hardwood and conifer tree species of mixed age, from seedlings to well over 200 years old. Sugar maple, yellow birch, and northern white cedar were dominant. Plots 1 and 2 were undisturbed; some minimum selective cutting (mainly northern white cedar) had occurred in a portion of plot 3. The three successional stands originated after logging and consisted of five to nine tree species, dominated by seedling to mature sugar maple and an overstory of aspen, paper birch, and basswood. Conifers (mainly seedlings or saplings) were a minor component, mainly occurring in plot 3. A few old-growth trees were present in the plots and in nearby stands; surrounding stands were of similar composition. Trees and shrubs with diameter at breast height greater than or equal to 2 cm were measured and mapped (the large number of maples with DBH less than 25 cm were not mapped). The basal areas were computed for each plot (Table 1). Twenty to 40 vascular plant species were present in each plot.

Successional plot 1 was the only plot in which the entire 0.5 ha. was fairly level. The remaining five plots contained both level and moderate to strongly sloped areas with total relief per plot varying from seven to twelve meters. The stands are located on gravelly sandy loam, well drained soil with a few pockets of less drained soil. Soil samples were collected and analyzed as for the red pine forest (Chapter II). Soil pH ranged from 4.0 to 4.7 for old-growth plots and 4.0 to 5.2 for the successional plots. The organic matter content of the old-growth plots did not differ significantly, 14.4 % ± 3.1 % (mean ± 1 SD, n = 15), from that of the successional plots, 15.0 % ± 4.9 % (n = 15).

TABLE 1. Basal areas (square meters per hectare) of woody plants for northern hardwood-conifer forest plots.[a]

		Successional[b]			Old-growth[c]		
Species	Plot[d]:	1	2	3	3	1	2
Tilia americana L.		4.70	6.13	– –	– –	– –	– –
Populus tremuloides Michx.		7.69	1.90	– –	– –	– –	– –
Acer saccharum Marsh.		20.24	22.12	18.59	20.14	19.70	9.72
Populus balsamifera L.		1.97	– –	4.50	– –	– –	– –
Quercus rubra L.		– –	– –	0.11	– –	– –	– –
Prunus serotina Ehrh.		0.02	– –	– –	– –	0.01	<0.01
Prunus virginiana L.		– –	– –	<0.01	0.01	– –	– –
Acer rubrum L.		– –	<0.01	– –	– –	0.02	– –
Betula papyrifera Marsh.		2.08	7.98	5.32	– –	2.24	4.94
Corylus cornuta Marsh.		0.01	– –	0.04	<0.01	0.02	0.09
Abies balsamea (L.) Mill.		0.09	– –	<0.01	0.24	0.08	0.11
Picea glauca (Moench) Voss		<0.01	– –	0.60	0.94	1.34	0.50
Pinus strobus L.		– –	– –	– –	– –	– –[e]	0.99[f]
Acer spicatum Lam.		0.02	<0.01	0.12	0.21	0.87	1.15
Betula alleghaniensis Britt.		0.25	– –	1.06	9.40	11.69	7.27
Thuja occidentalis L.		– –	– –	– –	5.47	4.20	15.01
Total basal area/ha		37.08	38.13	30.34	36.41	40.18	39.79

[a] Actual basal area multiplied by 2 to give basal area per hectare. Species and plots are ordered by the corresponding eigenvectors of principal component 2 (see methods).

[b] Successional, overstory of *Populus, Betula, Tilia*.

[c] Mixed age, old-growth stands; oldest trees 200- to 300-years-old.

[d] 50 × 100 m (0.5 ha).

[e] Single old-growth tree 8 m outside of plot with 111 cm dbh.

[f] Single old-growth tree in plot with 79.5 cm dbh.

Sampling procedures

Old-growth plots 1 and 2 were partially sampled the initial year (1993) on three visits (August 28 September 19). During four visits, July through September, in both 1994 and 1996, the transects of all plots were visited on a rotating basis every 3 wk employing the same sampling procedure as for red pine forest. As before, several infrequent unidentified taxa with insufficient material or fruitbodies in poor condition were not included in the analysis. Damage by slugs was especially common on *Russula*. Presence of the ectomycorrhizal fungi-associated plants *Monotropa* and *Corallorhiza* was recorded.

Voucher specimens are deposited in the Herbarium of the University of Minnesota (MIN). Identification of certain taxa was made by consultation with J.

Ammirati, University of Washington, Seattle (*Cortinarius*), G. Mueller, The Field Museum, Chicago (*Laccaria*), T. Baroni, State University College at Cortland, New York (Entolomataceae), and C. Ovrebo, University of Central Oklahoma, Edmond (*Tricholoma*). Genera present in northern hardwood-conifer forest plots, for which some data were collected, but excluded from the analysis because of doubtful mycorrhizal status include *Camarophyllus, Clavulina, Clavulinopsis, Hygrocybe, Lyophyllum,* Entolomataceae other than *Entoloma s.s.,* and the species *Clavaria vermicularis* Fr., *Ramaria concolor* (Corner) Petersen, *R. stricta* (Fr.) Quél., and *Ramariopsis kunzei* (Fr.) Donk (Arnolds 1989, 1991; Gulden et al. 1992; Singer 1986; Villeneuve et al. 1989).

Data Analysis

Methods for the analysis of quantitative data were the same as in Chapter II with the following exception. Generation of species-area curves did not involve a random addition of transects and plots because plot order would greatly affect curve appearance due to the variability in diversity among plots. Instead, the average number of species per transect was used for the first pair of data points (area of 80 m^2), then averages computed from all possible combinations of two, three, and four transects within each plot for the successive three data point pairs (160, 240, 320 m^2). The average number of species per plot, the average number for possible combinations of two plots, and the total for three plots were used for the final data points (400, 800, 1200 m^2).

RESULTS

Diversity of species

Sixty-two taxa of ectomycorrhizal fungi in 21 genera were documented for the six plots, totaling three hectares, of northern hardwood-conifer forest (Tables 2, 3). *Russula* was the most diverse genus with at least 12 species. The genus *Lactarius*, 7 species, was the most abundant, largely due to *L. thejogalus*. Other taxa with high numbers of species were Boletaceae with 6 species and *Cortinarius* with at least 9 species. Not all *Cortinarius* were separable in the field and several species of subgenus *Telamonia* have been combined here for purposes of analysis. A few *Russula* taxa were undocumented because of inadequate material; most

Russula occurred singly and slug damage was common. The *Trichopilus* species found in two old-growth plots was determined to be an undescribed species (Baroni, personal communication). The presence of the ectomycorrhizal ascomycete *Elaphomyces* is inferred by a single occurrence of *Cordyceps capitata* (Holmsk.:Fr.) Link in old-growth plot 1.

TABLE 2. Number of ectomycorrhizal basidiomycetes recorded from six plots of northern hardwood-conifer forest in northern Minnesota in 1993 – 1996.

Area:	No. of taxa[a] in circles, 400 m^2/plot						No. of taxa[a] in plots of 5000 m^2					
Age:	Successional			Old-growth			Successional			Old-growth		
Plot:	1	2	3	1	2	3	1	2	3	1	2	3
1993[b]				11	10					22	13	
1994	4	5	8	4	9	11	5	7	15	10	14	13
1996	1	5	11	3	1	6	2	5	21	6	2	13
1994,1996	5	7	17	7	9	12	7	8	26	14	14	18
1993-1996				15	17					29	23	

	Successional	Old-growth	Shared[c]	Successional	Old-growth	Shared[d]
Total 1993		16			28	
Total 1994	12	19	4	20	28	10
Total 1996	15	9	7	22	15	9
1994,1996	23	22	9	31	33	14
1993-1996		32			48	

	Successional and old-growth	Successional and old-growth
Total 1994	27	38
Total 1996	17	28
1994,1996	36	50
1993-1996	44	63

[a] Numbers are underestimates because several unidentified *Cortinarius* are collectively counted as one taxon. Additional undocumented taxa are omitted.

[b] Only two plots of old-growth were sampled in 1993.

[c] Number of species found in circles of both age classes.

[d] Number of species found in plots of both age classes.

Successional and old-growth northern hardwood-conifer forest stands displayed similar species richness overall for 1994 plus 1996 athough diversity varied among plots and years (Table 2). For old-growth plots, 31% (15 spp.) of the species were recorded only in 1993, 23% (11 spp.) only in 1994, and 6% (3 spp.) only in 1996. A total of 32 species, or 51%, were found only in old-growth, 19 species in 1994 plus 1996 and 13 additional in 1993. For the two years of sampling successional stands, 29% (9 spp.) were observed only in 1994 and 35% (11 spp.) only in 1996. Of the 31 species found in successional plots, 15 were not observed in old-growth stands. Two species from successional plots were found in old-growth in 1993 making a total of 16 species shared between the two age classes for the three years. No species were frequent enough or consistent enough in occurrence among the plots to be considered indicator species of either age class. Even so the two age classes exhibited only a 25% overlap in species composition (16 shared of a total of 63). The percent overlap is the same if only those species recorded in sampling circles are considered. This low overlap in species was also found for old-growth plots 1 and 2, which were sampled all three years. Even though these two plots are adjacent, sharing a common border, the overlap in species was only 28% (11 out of 39). *Lactarius thejogalus* was the only species found in all six plots. *Amanita sinicoflava* and *Paxillus involutus* were observed in five of the six plots. Successional plot 1 had the lowest diversity with only 5 species. The difference in range of the number of species per plot was somewhat greater among the successional plots (5 17 spp. in circles) than among the old-growth plots (7 12 species) (Table 2). Ordination of the plots using frequencies of the 35 species did not separate the two age classes. Plots and species in Table 3 are therefore unordered

Presence of the achlorophyllous plants *Corallorhiza* and *Monotropa*, associated with ectomycorrhizal fungi (Cullings et al. 1996, Taylor and Bruns 1997), was recorded (Table 4). *Corallorhiza maculata* was observed in two successional plots. *Monotropa hypopithys* and *M. uniflora* were noticeably more frequent in and representative of old-growth northern hardwood-conifer stands.

TABLE 3. Ectomycorrhizal species in Minnesota successional and old-growth northern hardwood-conifer forest.

Age class:	Successional			Old-growth		
Species Plot:	1	2	3	1	2	3
A. List of species present in circles with frequency[a] for 1994, 1996						
Amanita sinicoflava Tulloss	-	2	1	+	3	1
Amanita vaginata (Fr.) Vitt.	-	-	-	-[b]	1	-
Cantharellus cibarius Fr.	1	-	-	-[b]	-[b]	-
Cortinarius allutus Fr.	-	-	-	1	-	-
Cortinarius cf. *anomalus* (Fr.:Fr.) Fr.	-	-	2	-	1	-
Cortinarius cf. *laniger* Fr.	-	-	1	-[b]	+	-
Cortinarius sp. HC 09 (Subg. *Myxacium*)	1	-	-	-	-	-
Entoloma eulividum Noordeloos	-	-	-	4	-[b]	-
Entoloma sp. HC C1	-	-	-	-[b]	-[b]	1
Entoloma sp. HC 30	-	-	1	-	-	-
Gyroporus cyanescens (Bull.:Fr.) Quél.	-	-	+	1	-	-
Hebeloma sp. HC 02	-	-	1	-	-	1
Hygrophorus paludosus Peck	-	-	-	-	-	2
Inocybe geophylla (Sow.:Fr.) Kumm. var. *geophylla*	-	-	1	-	-	-
Inocybe lanuginosa (Bull.:Fr.) Kumm.	-	1	+	-	-	-
Inocybe sp. HC 01	1	+[c]	-	-	-[b]	-
Laccaria bicolor (Maire) Orton	-	1	1	-	-	-
Laccaria laccata (Scop.:Fr.) Cooke	-	-	1	-[b]	-	7
Lactarius camphoratus (Bull.:Fr.) Fr.	-	-	+	-[b]	2	1
Lactarius glyciosmus (Fr.:Fr.) Fr.	-	-	1	-	-	-
Lactarius thejogalus (Bull.:Fr.) S. F. Gray	+	7	5	2	+	16
Lactarius uvidus (Fr.:Fr.) Fr.	-	-	1	-	-	-
Lactarius vietus (Fr.:Fr.) Fr.	1	-	1	-[b]	-	+
Lactarius sp. HC 01	-	-	-	-	-	1
Paxillus involutus (Batsch:Fr.) Fr.	-	1	+	+	1	1
Rozites caperatus (Pers.:Fr.) Karst.	-	-	-	1	-	-
Russula betularum Hora	-	-	-	+	-	3
Russula silvicola Shaffer	-	-	1	-	-	2
Russula sp. HC 06	-	1	1	+	-	-
Russula sp. HC 08	1	-	2	-	-	-
Russula sp. HC 09	-	-	1	-	-	-
Russula sp. HC 11	-	1	-	-	-	-
Suillus americanus (Peck) Snell ex Slipp & Snell	-	-	-	-	1	-
Tricholoma odorum Peck	-	-	-	-	1	-
Trichopilus sp. (undescribed)	-	-	-	-	1	+
Cortinarius undet. spp. (primarily Subg. *Telamonia*)	-	-	3	1	5	7

TABLE 3. (*concluded*)

Age class:	Successional			Old-growth		
Plot:	1	2	3	1	2	3
B. Additional species observed in plots for 1994 or 1996 but not recorded in circles						
Amanita fulva (Schaeff. ex) Persoon	+	-	-	-	-	-
Cortinarius cf. *septentrionalis* Bendiksen et al.	-	-	-	+	-	-
Hygrophorus tephroleucus (Fr.) Fr.	-	-	-	-	-	+
Leccinum scabrum (Bull.:Fr.) S. F. Gray	-	-	+	+	+	-
Leccinum holopus (Rostk.) Watl. [*L. niveum* (Fr.) Rauschert]	-	-	-	-	-	+
Russula sp. HC 03	-	-	-	-	+	-
Russula sp. HC 05	-	-	-	-	+	-
Russula sp. HC 14	-	-	-	-	-	+
Russula sp. HC 15	-	-	+	-	-	-
Russula sp. HC 16	-	-	+	-	-	-
Russula sp. HC 17	-	-	+	-	-	-
Tricholoma inamoenum (Fr.) Quél.	-	-	+	-	-	-
Tricholoma subresplendens (Murr.) Ovrebo in ed.	-	-	-	+	-[b]	-
Tricholoma sp. HC 09	-	-	-	-	-	+
C. Additional species recorded in plots for 1993 but not observed in 1994 or 1996						
Cortinarius armillatus (Fr.:Fr.) Fr.	-	-	-	x[d]	-	-
Cortinarius pholideus (Fr.:Fr.) Fr.	-	-	-	x	-	-
Cortinarius sp. HC 03	-	-	-	x	-	-
Cortinarius sp. HC 04	-	-	-	x	-	-
Hydnum repandum L.:Fr.	-	-	-	-	x	-
Lactarius cf. *pseudoflexuosus* Hesler & A. H. Smith	-	-	-	x	-	-
Ramaria sp. HC 03	-	-	-	-	x	-
Ramaria sp. HC 04	-	-	-	x	-	-
Tricholoma flavovirens (Pers.:Fr.) Lundell	-	-	-	x	-	-
Tricholoma cf. *fulvum* (DC.:Fr.) Sacc.	-	-	-	-	x	-
Tricholoma sp. HC 05	-	-	-	-	x	-
Tylopilus sp. HC 01	-	-	-	x	-	-
Xerocomus spadiceus (Fr.) Quél.	-	-	-	x	-	-

[a] Percentage of circles occupied for the two years; 100 4-m^2 circles/plot.
[b] Species present in plot in 1993 only.
[c] +, species present in plot in 1994 and/or 1996 but not in circles.
[d] x, species present in plot in 1993 only.

TABLE 4. Frequency[a] of *Corallorhiza* and *Monotropa* in Minnesota successional and old-growth northern hardwood-conifer forest for 1996.

	Age class:	Successional			Old-growth		
Plant species	Plot:	1	2	3	1	2	3
Corallorhiza maculata (Raf.) Raf.		+[b]	-	1	-	-	-
Monotropa hypopithys L.		-	-	-	1	+	+
Monotropa uniflora L.		-	-	1	5	3	5

[a] Percentage of circles occupied for 1996; 100 4-m^2 circles/plot.
[b] +, species present in plot in 1996 but not in circles.

All plots had a low range in the number of species per circle for 1994 plus 1996 (Table 5). Because of the small number of occupied circles (15% total), plots averaged just a fraction of one taxon per 4 m^2 circle. Number of occupied circles ranged from 5% for successional plot 1 to 30% for old-growth plot 3. Old-growth displayed a greater overall percentage of occupied circles (18% vs. 12%) and a greater mean number of species per circle (although neither difference is significant). Species-area curves (Fig. 1) for old-growth and successional are very similar. Both demonstrate a strong increase in number of species recorded with the addition of a second and third plot.

TABLE 5. Range in the number of species per circle and percent of circles occupied.

No. of species/ circle	Number of circles	
	Total 1994, 1996	
	Successional	Old-growth
0	264	246
1	30	41
2	4	10
3	2	3
Mean no. of spp./circle	0.15	0.23
SD	0.44	0.55
Total circles	300	300
Total occupied	36	54
% occupied	12	18

TABLE 6. Yearly variation in abundance of the eleven most frequent species for northern hardwood-conifer forest.

Species	Total freq. 1994, 1996[c]	Yearly frequency[a]				Yearly density, fb./ha.[b]			
		Successional		Old-growth		Successional		Old-growth	
		1994	1996	1994	1996	1994	1996	1994	1996
Lactarius thejogalus	5.00	1.7	3.0	4.0	3.0	50	233	225	117
Laccaria laccata	1.33	+[d]	0.3	1.7	0.6	+	25	92	58
Amanita sinicoflava	1.17	1.0	0.3	1.0	0.3	33	8	25	8
Entoloma eulividum	0.67	-[e]	-	1.3	-	-	-	42	-
Cortinarius cf. *anomalus*	0.50	0.7	-	0.3	-	67	-	8	-
Lactarius camphoratus	0.50	+	-	1.0	-	+	-	33	-
Lactarius vietus	0.50	-	0.7	-	0.3	-	33	-	8
Paxillus involutus	0.50	+	0.3	0.7	+	+	8	17	+
Russula betularum	0.50	-	-	0.7	0.3	-	-	17	8
Russula silvicola	0.50	+	0.3	0.3	0.3	+	8	8	8
Russula sp. HC 08	0.50	1.0	+	-	-	25	+	-	-
Data for the 36 species combined	15.0	7.3	7.0	15.0	5.3	342	592	842	875

[a] Percentage of 300 circles occupied.
[b] Estimated from number of fruitbodies in 1200 m^2 (300 circles).
[c] Combined frequency for both age classes and both years.
[d] Present in one or more plots but not found in circles.
[e] Not observed in plots.

Abundance of fruitbodies, frequency and density

A similar low range in abundance was displayed by species in both age classes. The dominance-diversity curves (Fig. 2) show that 60% of the species had the lowest possible frequencies, i.e. only one occurrence out of 300 circles, or 0.3 frequency. The highest frequencies recorded were 6% in old-growth plots and 4% in mature plots for *Lactarius thejogalus* (Table 3). The total frequency of all species for 1994 plus 1996 was 15% (Table 6) and equals the percent of occupied circles (Table 5). The (cumulative) total frequency as defined by Villeneuve et al. (1989), i.e., the sum of frequencies for all species, was 15% for the successional plots and 23% for the old-growth plots.

Production of fruitbodies varied between plots and years (Table 6). Total density was greatest in 1996 with an estimated 875 fruitbodies per hectare (fb./ha.) in old-growth and 592 fb./ha. in successional stands. Density for individual visits ranged from 736 fb./ha. for September 13 17, 1994 down to 0 fb./ha. for September 1 3, 1996. On this latter visit *Amanita sinicoflava* was observed five times in six plots and *Lactarius thejogalus* once but no ectomycorrhizal species were present within sampled circles. Species with the greatest estimated total densities for 1994 plus 1996 were *Lactarius thejogalus*, 342 fb./ha. for old-growth, 283 fb./ha. for successional, and *Laccaria laccata*, 150 fb./ha. for old-growth, 25 fb./ha. for successional plots. Although total yearly frequency for old-growth in 1996 was only a third that of 1994, the density was as great. This indicates that of the fewer circles occupied in 1996 on average three times as many fruitbodies were present in these circles. In contrast, frequency for successional plots was similar both years with density also greater in 1996.

Yearly variation and fruiting phenology

Diversity was greater in 1994 than in 1996. Of the two-year total, 76% of the species were observed in 1994 and 56% in 1996. In 1994 71% and in 1996 61% of the total species observed in plots were recorded in sampling circles (Table 2). Partial old-growth sampling in 1993 yielded 58% of the species for this age class. The fruiting season extended from June to early October. Abundance was relatively high in 1993 and the peak in fruiting was in late August rather than September (Fig. 3). Total fruitbody production during 1994 and 1996 was very similar between years and between age classes with the exception of the higher density recorded for old-growth in mid September, 1994. The latest sampling visit was through September 27, 1996; the final visits in 1994 and 1996 were made before the decline in fruiting occurred (Fig. 3). Because of the low abundance of the majority of species, little can be inferred on phenology of fruiting. *Lactarius*, largely *L. thejogalus*, displayed greatest fruiting in September (Fig. 4).

Some observations on other fungi

Saprobic fungi were as diverse and abundant or in some cases more abundant than ectomycorrhizal fungi in the northern hardwood-conifer stands; these included Entolomataceae and Tricholomataceae, particularly *Mycena* (data not shown). *Hygrocybe* was also abundant in most plots and 16 taxa were recorded (Table 7). Ordination of the *Hygrocybe* frequency data clearly separated successional from old-growth plots along principal component 2, accounting for 26% of variation in the data; this proportion was reduced to 20% when these data were combined with those of the ectomycorrhizal species (from Table 3A). *Hygrocybe ceracea* and *H. conica* were present in all three old-growth stands but absent from successional plots. Several others were also found in old-growth only. The taxon found in successional stands only was the single occurrence of *H. conica* var. *atrosanguinea*. *Hygrocybe* fruiting varied between years and age classes but was higher overall in July and the first half of August than in September (Fig. 4).

TABLE 7. *Hygrocybe* species in Minnesota successional and old-growth northern hardwood-conifer forest.

Age class:	Successional			Old-growth		
Species Plot:	1	2	3	2	1	3
Ordination of species present in circles by frequency[a] for 1994, 1996						
Hygrocybe flavescens (Kauffman) Singer	1	9	13	2	3	1
Hygrocybe conica var. *atrosanguinea* Grund and Harrison	-	-	1	-	-	-
Hygrocybe laeta (Pers.:Fr.) Kumm.	-	-	1	x[b]	x	-
Hygrocybe sp. HC 06	-	-	1	+[c]	x	+
Hygrocybe sp. HC 21	-	-	-	-	1	+
Hygrocybe psittacina (Schaeff.:Fr.) Wunsche	-	-	+	1	x	-
Hygrocybe reai (Maire) Lange	-	-	-	1	1	-
Hygrocybe punicea (Fr.) Kumm.	-	9	10	2	7	1
Hygrocybe cf. *miniata* (Fr.) Kumm.	-	1	-	4	1	+
Hygrocybe ceracea (Wulf.:Fr.) Karst.	-	-	-	3	1	2
Hygrocybe parvula (Peck) Murr.	-	-	4	3	4	7
Hygrocybe cantharellus (Schw.:Fr.) Murr.	-	+	5	1	6	9
Additional species observed in plots for 1993, 1994, 1996						
Hygrocybe conica (Scop.:Fr.) Kumm.	-	-	-	x	x	+
Hygrocybe marginata (Peck) Murr.	-	-	+	+	+	-
Hygrocybe sp HC 07	+	-	-	x	-	-
Hygrocybe unguinosa (Fr.) Karst.	-	-	-	x	x	-

[a] Percentage of circles occupied for 1994, 1996; 100 4-m^2 circles/plot. Species and plots are ordered by the corresponding eigenvectors of principal component 2.

[b] x, species present in plot in 1993 only.

[c] +, species present in plot in 1994 and/or 1996 but not in circles.

Fig. 1. Species-area curve for northern hardwood-conifer forest based on computed averages (see methods).

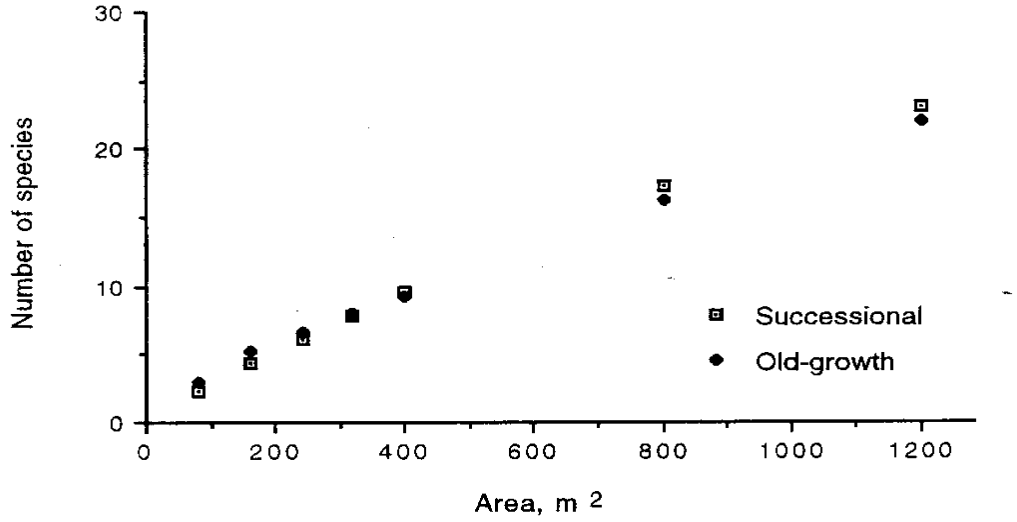


Fig. 2. Dominance-diversity curves for northern hardwood-conifer forest. Scales standardized to a maximum of 100 (see methods). (Group of unidentified *Cortinarius* spp. omitted.)

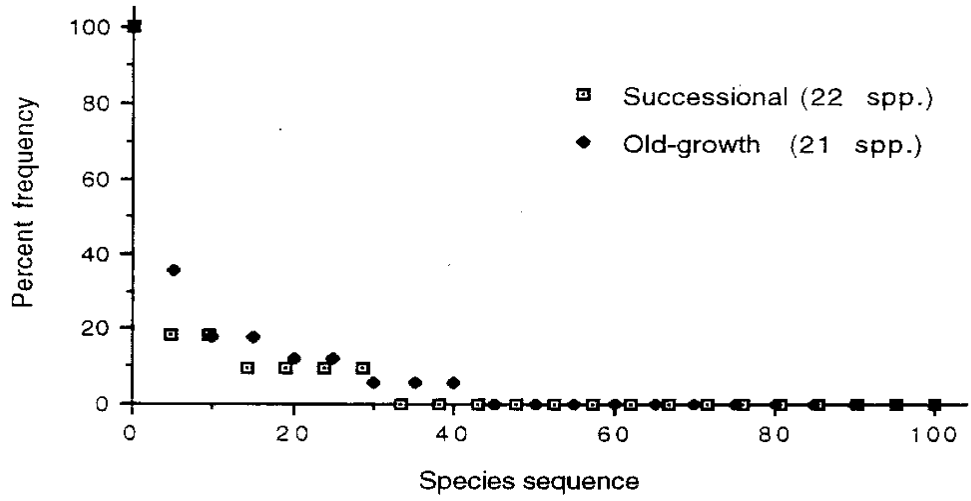


Fig. 3. Yearly variation in fruitbody densties for northern hardwood-conifer forest.

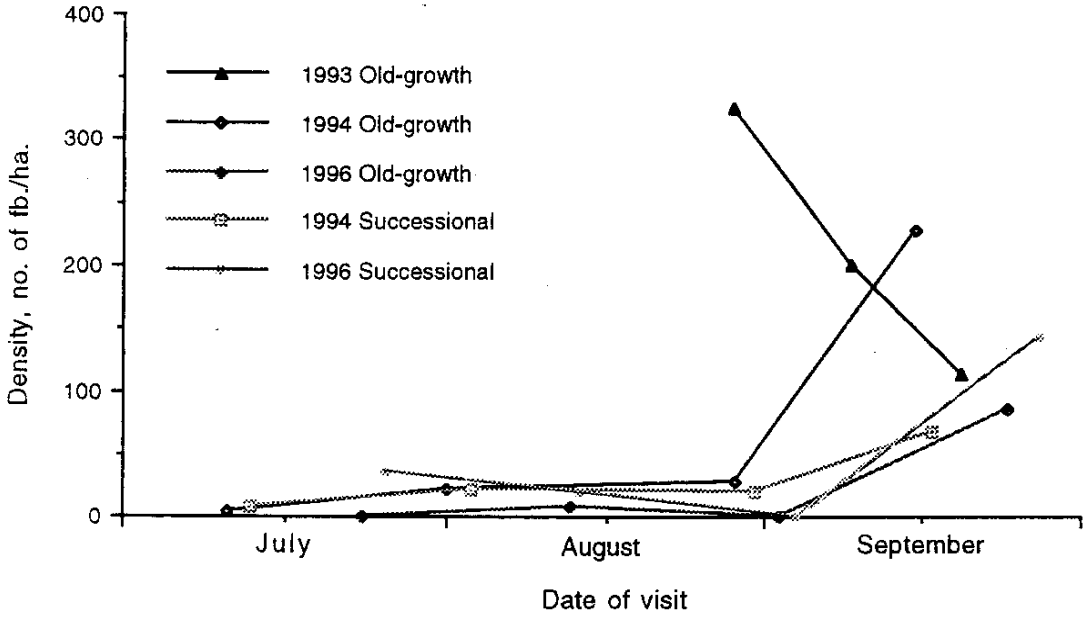
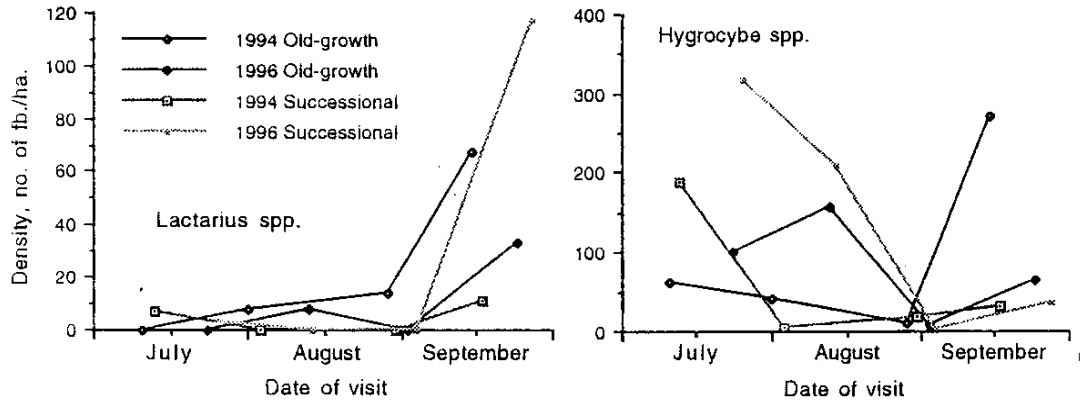


Fig. 4. Density of fruitbodies in 1994 and 1996 for two genera from northern hardwood-conifer forest.



DISCUSSION

Comparison of successional and old-growth northern hardwood-conifer forest

Species richness was nearly the same for successional and old-growth stands of northern hardwood-conifer forest for the 1994 plus 1996 data. Old-growth plots showed greater abundance both in total frequency and total density. Because of low overlap (9 to 28%) in species composition among any combination of plots and incomplete separation of age classes by ordination, it is not clear whether successional and old-growth stands harbor distinct communities of ectomycorrhizal fungi. Of the total species for 1994 and 1996 only one third (16 out of 50) were found both years.

Old-growth stands showed greater fruitbody production than successional stands. In 1994 abundance (frequency and density) was more than double that of successional plots (Table 6). Total estimated density for 1994 plus 1996 was 1717 fb./ha. in old-growth versus 934 fb./ha. for successional stands. This may be explained by the greater component of ectomycorrhizae-associated tree species, particularly conifers (Vogt et al. 1992). Partial sampling in 1993 of two old-growth plots showed high diversity (Table 2) and high abundance (Fig. 3) yielding 13 species not seen in 1994 or 1996. Several additional years of sampling would be required at a minimum to more completely characterize these two communities and determine possible indicator species for either age class. Species-area curves (Fig. 1) show little indication of levelling off, indicating the need for more extensive sampling of existing plots.

Species diversity was similar between age classes. For 1994 plus 1996 total richness was 31 taxa for successional plots with mean of 13.7 species per plot compared to 33 taxa and a mean of 15.3 species per plot for old-growth stands. For each plot, 50 88% (mean 68%) of the taxa were recorded within sampling circles, with a total of 72% for all plots combined. A low proportion, 28%, of the species were shared between age classes for the two years. Only one species, *Lactarius thejogalus,* was common to all six plots. No species were common to the three successional plots. Four taxa, *Amanita sinicoflava*, *Entoloma* sp. HC C1, *Lactarius camphoratus*, and *Paxillus involutus*, were shared among the three old-growth plots.

Examination of 1994 plus 1996 species shows that overlap in species composition is not directly correlated with proximity of plots. The adjacent old-growth plots 1 and 2 share 17% of their species while they in turn share 14% and 19%, respectively, with old-growth plot 3 located 18 km away. Recurrence of fruitbodies within the same circle for 1994 plus 1996 was displayed by three species. *Amanita sinicoflava* recurred in one out of seven circles and *Russula* sp. HC 11 fruited in a single circle both years. *Lactarius thejogalus* recurred in five out of 30 circles and was also found three times in the same circle the same year. The "early-stage" fungus (Dighton and Mason 1985) *Laccaria laccata* was six times more abundant in old-growth plot 3 (150 fb./ha.) than in successional plot 3 (25 fb./ha.), was observed once in old-growth plot 1, and not seen in the remaining three plots. The presence of host trees, particularly conifers, may partially explain this pattern of distribution.

Most species occurred at minimal frequencies which caused difficulty with adequately documenting unknown taxa. Of the 63 species recorded in three years of sampling, 39 or 62% were not observed in more than one plot. Low frequencies were correlated with very low mean number of 0.15 to 0.23 species per circle (Table 5) and a fairly low percentage of total occupied circles for each plot; old-growth ranged from 10 to 30% (mean 18%) and successional from 5 to 18% (mean 12%) circles occupied. The study of Bills et al. (1986) on northern hardwood forest (beech present; conifers absent) found 41% occupied 4 m^2 quadrats and a mean of 1.2 species per quadrat. Abundance was augmented by the high frequency of *Boletinellus merulioides* and *Scleroderma citrinum* in one plot, associated with ash and oak, respectively.

Villeneuve et al. (1989) examined five 400 m^2 plots of old-growth sugar maple yellow birch at approximately 47° north latitude in Quebec. Each plot was divided into 100 contiguous quadrats. Beech and red spruce were also present; one stand had black ash. Soils were described as extremely acidic. In 38 visits (7 10 days apart) over two years, 37 ectomycorrhizal fungi were observed; 27 or 73% of the species were recorded within the plots. The two most frequent species were *Lactarius rufus*, 11% frequency (54 quadrats out of 500), and *Amanita fulva*, 10% frequency. Species richness is comparable to this study. Their higher species

frequencies were possibly due to the greater number of visits, 19 per year versus my 4 per year. Comparison of dominance-diversity curves indicates somewhat less equitability among species frequencies for the Quebec stands. Only five species are shared with the Minnesota stands: *A. fulva*, *Gyroporus cyanescens*, *Laccaria laccata*, *Paxillus involutus*, and *Rozites caperata*. Computing Jaccard's coefficient of similarity (no. of shared spp./no. of total spp.), for comparing species composition, yields a low 0.06 for both successional and old-growth stand similarity with the Quebec stands.

The achlorophyllous plants *Corallorhiza maculata*, *Monotropa hypopithys*, and *M. uniflora* serve as additional indicators of ectomycorrhizal activity. Taylor and Bruns (1997) determined by molecular methods from numerous collections from California, Oregon, Washington, Wisconsin, and Ohio that the orchid *C. maculata* associates specifically with members of Russulaceae. This "cheater" species, which taps into the carbon flow of the mycorrhizae, was observed twice in two successional plots. Its association with Russulaceae may in large part explain this plant's preference in Minnesota for somewhat acidic soils that are often nutrient-poor (Smith 1993), conditions which favor ectomycorrhizae.

Monotropa was frequent in old-growth (Table 4) and nearly absent from successional stands. Both species occurred in all three old-growth plots. *Monotropa hypopithys* was found to be an associate of the suilloid group of Boletales, at least for samples from Wyoming (Cullings et al. 1996). The single occurrence of *Suillus americanus* (the only suilloid taxon found) was near a white pine. The *M. hypopithys* in old-growth plots 1 and 2 were located 28 to 65 meters from the nearest pine (old-growth white pine) which implies that the fungal taxon involved is itself likely associated with other conifers or hardwoods in the plots, especially in old-growth plot 3 where pine is absent. *Monotropa uniflora* had a frequency in old-growth plots of 4.3%, which would rank its fungal host(s) up to second most frequent. Russulaceae were found to be the hosts for six samples from Minnesota, Oregon, and Oregon/Washington border (Cullings et al. 1996); the species are unknown. In the old-growth plots, a third of the circles in which *M. uniflora* was found also included various *Lactarius* and *Russula* taxa.

The ectomycorrhizal fungi composition varied among plots of successional and old-growth northern hardwood-conifer forest but a clear separation of age classes was not possible. Older stands harbor a sizeable portion of the total diversity. Comparison of adjacent and distant old-growth stands reveals that similarity in species composition is correlated more with age class than plot proximity. Low species frequencies and little overlap among plots indicates the need for several years additional sampling; otherwise, an analysis of the saprobic fungi may further reveal differences between the two age classes. A partial investigation of the abundance of certain saprobic taxa, i.e., *Hygrocybe*, has shown that they can be utilized to characterize and separate two age classes.

Comparison of northern hardwood-conifer forest with red pine forest

The red pine forest harbored a larger community of ectomycorrhizal fungi with two to three times more species and abundance an order of magnitude greater than observed for northern hardwood-conifer forest. Factors that correlate with this difference in fungal diversity and abundance between forest types are the composition of host trees and soil characteristics. Numerous fungi of the red pine stands showed distinct affinities for either mature or old-growth forest. Separation of successional and old-growth northern hardwood-conifer ectomycorrhizal communities was poor. More abundant saprobic taxa, e. g., *Hygrocybe* spp., provided further data for an initial segregation of fungal communities between age classes.

Diversity of ectomycorrhizal host trees for the two forests was comparable, eight tree species for both red pine and northern hardwood-conifer; however, the composition and total basal areas of tree species were significantly different. Red pine forest plots were composed of nearly 100 percent host trees (by basal area) with a dominant *Pinus* component (83%). The host tree portion of total basal area was less for northern hardwood-conifer forest (42% in successional; 34% in old-growth plots) because of the dominance of the endomycorrhizal *Acer* in all plots and the addition of *Thuja* in old-growth plots. The conifers *Abies* and *Picea* were present in both forest types. *Betula* was a larger component of the northern

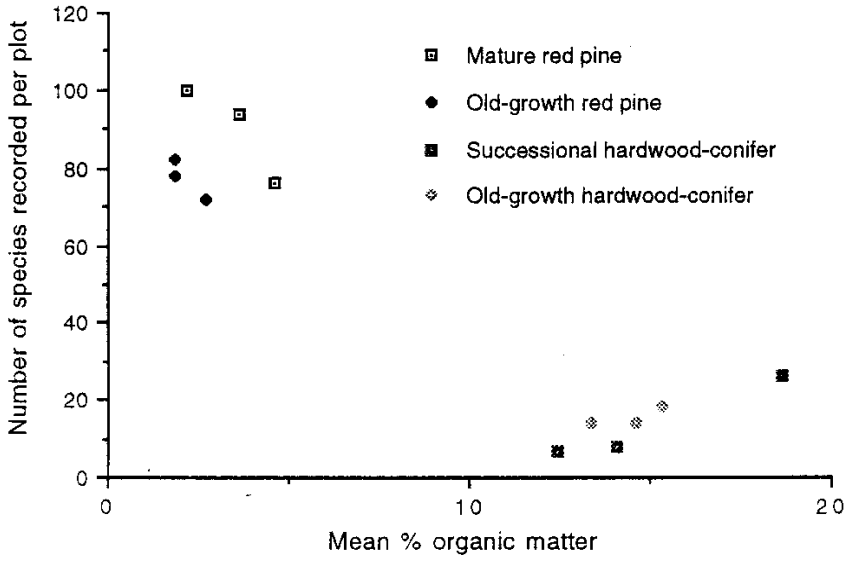
hardwood-conifer forest, particularly *B. alleghaniensis* in old-growth plots. The forests also differed in age composition. The red pine stands were dominated by even-aged 100- or 230-year-old pine with young *Pinus resinosa* extremely rare, while *P. strobus* seedlings and saplings were common. Most of the tree species in the northern hardwood-conifer stands ranged in age from seedlings to mature or old-growth trees. The exceptions being the two old-growth *P. strobus* at old-growth plots 1 and 2, and the *Quercus rubra* saplings in successional plot 3. Old-growth trees formed a partial upper canopy in the old-growth stands with many younger trees filling in the gaps. During the four year study several old-growth trees died, primarily as windfalls.

The red pine stands with a major conifer component had an average yearly ectomycorrhizal fruitbody production that was 18 times that of the northern hardwood-conifer plots. Other studies have also found coniferous forests to be more productive seemingly related to the greater proportion of ectomycorrhizal associations (Vogt et al. 1992). Villeneuve et al. (1989) found greater abundance of ectomycorrhizal fungi in closed coniferous forests, with greater dominance of host trees, than in either deciduous or open coniferous stands. A study of 11 stands, ranging from sugar maple through various hardwood-conifer compositions to primarily coniferous, showed an increase in diversity of ectomycorrhizal species in stands with a greater number of host tree species (Nantel and Neumann 1992). Bills et al. (1986) found greater total fungal diversity in six hardwood plots containing *Fagus, Betula, Fraxinus,* and *Quercus* in addition to *Acer* and *Prunus,* than for six spruce plots having only the one host *Picea rubens.*

Percent organic matter content was five times greater for northern hardwood-conifer stands than for red pine forest soils (Fig. 5). This difference may provide some basis for the lower species richness in the former forest type. Studies have shown a negative correlation between fungal species diversity and amount of soil organic matter in *Pinus sylvestris* forests (Baar 1996, Shaw and Lankey 1994). In contrast, Tyler (1985) found greater diversity of ectomycorrhizal fungi for beech forest plots with increased organic matter content and soil acidity. A negative correlation with percent organic matter is displayed among plots of both the mature and old-growth red pine stands (Fig. 5) but not for the northern hardwood-

conifer plots where a positive correlation is suggested. Species richness can also show a negative correlation with higher nitrogen levels and lower pH in soils (Baar 1996). Data on nitrogen levels was not obtained for the two forests. The range in pH of all twelve plots overlapped, although the three successional northern hardwood-conifer plots had somewhat less acidic soil; no correlation with diversty was apparent.

Fig. 5. Soil organic matter content in relation to species richness for two forest types.



Russulaceae was a major component in both forest types and contained the most abundant taxon of each forest: *Russula silvicola* for red pine forest, and *Lactarius thejogalus* for northern hardwood-conifer forest. *Cortinarius* was a dominant group in the red pine forest with an unknown number of species. The red pine forest had greater diversity of genera and higher taxa in addition to species richness. Red pine forest taxa not observed for northern hardwood-conifer forest included *Albatrellus, Boletus, Chroogomphus, Clitopilus, Coltricia,*

Gomphus, the Thelephoraceae, i.e., *Hydnellum*, *Phellodon*, *Sarcodon*, *Thelephora*, and the near absence of *Suillus*. Other genera, such as *Hygrophorus*, were very infrequent in the northern hardwood-conifer stands. Some of these absent taxa can be explained by the scarcity of *Pinus*; other taxa, e.g., Thelephoraceae, are also likely absent because of the higher organic matter content (Baar 1996). Hardwood-conifer genera not recorded in red pine stands were *Gyroporus*, *Paxillus*, and *Trichopilus* (Entolomataceae); all but *Paxillus* were infrequent. The majority of northern hardwood-conifer forest species were at minimal frequencies, thus sampling likely captured less of the total species richness than was obtained for red pine forest.

Laccaria laccata was the second most frequent species and had higher densities in old-growth stands of both forest types. *Laccaria bicolor* was somewhat more abundant in old-growth plots. This higher abundance in older established forests is unexpected for *Laccaria*, an "early-stage" taxon identified as characteristic of young forest plantations by Dighton and Mason (1985), who also stated, "The phenomena occurring under unmanaged forests or woodlands, where natural regeneration occurs in the understorey, may be different. Here we expect a stable and possibly declining microflora of mainly 'late-stage' fungi." Data from red pine forest does not completely agree with this hypothesis. Even though the "late-stage" taxa *Russula*, *Lactarius*, and *Cortinarius* were dominant the "early-stage" taxa were not a minor component. Of the 18 species of *Hebeloma* and *Inocybe*, nine were found only in mature plots, three only in old-growth, and six shared between the two age classes. Species within these genera differed in distribution and abundance. *Inocybe geophylla* var. *geophylla* was overall the second most abundant indicator species of old-growth red pine forest while *Inocybe* sp. RP 05 was a potential indicator species for mature stands (Chapter II, Table 4). The high species diversity and abundance for both mature and old-growth red pine forest disagrees with the often published diagram indicating a distinct decline in fungi after canopy closure (Dighton and Mason 1985); this model may better apply to monoculture plantations.

Low abundance and low similarity between plots and more variable fruiting from year to year prevented the estimation of indicator taxa for successional and old-growth northern hardwood-conifer forest. Greater sampling intensity, i.e.,

more visits per year for more years may reveal these taxa. The potential indicator taxa for mature and old-growth red pine forest (Chapter II, Table 4), though not exclusive to that forest type, were not recorded for the northern hardwood-conifer stands except for a single occurrence of *Inocybe geophylla* var. *geophylla* in successional plot 3. The near linear species-area curves for northern hardwood-conifer forest also point to the need for more sampling. Data from increased sampling intensity may also improve the separation of successional and old-growth stands if such differences exist, as are suggested by the *Hygrocybe* data. It is possible that because of the higher soil organic matter the more abundant terrestrial saprobic taxa are better suited for characterization of these stands. Villeneuve et al. (1991*a*) found that dividing fungi into trophic groups improved community definition; the ectomycorrhizal trophic group was preferable to the total mycoflora and splitting saprobes into terricolous and lignicolous groups improved community analysis.

The red pine forest and northern hardwood-conifer forest plots shared 23 ectomycorrhizal fungi and six host tree species. In addition, *Elaphomyces*, abundant in red pine stands (as inferred by *Cordyceps capitata* and *C. ophioglossoides* presence) was recorded for one northern hardwood-conifer plot. *Lactarius thejogalus* was the only species and *Betula papyrifera*, a common host for this species, the only tree common to all twelve plots. Both fungus and tree had fairly similar abundances between the two forest types and both appear to be generalists, adapted to a wide range of community types (personal observation). *Monotropa uniflora* was present in both forest types and, as discussed above, characteristic of old-growth northern hardwood-conifer stands and infrequent in successional plot 3. This plant was present and had a frequency of 4 or less in each of the three mature red pine forest plots in 1995; in contrast, *Monotropa* was not recorded in any old-growth red pine plots. *Corallorhiza* sp. was observed in several red pine plots. The distributions of both *Monotropa* and *Corallorhiza* warrant further investigation as well as the identification of their Russulaceae associates by molecular methods (Cullings et al. 1996, Taylor and Bruns 1997).

Fig. 6. Distribution of the number of species per circle for red pine forest.

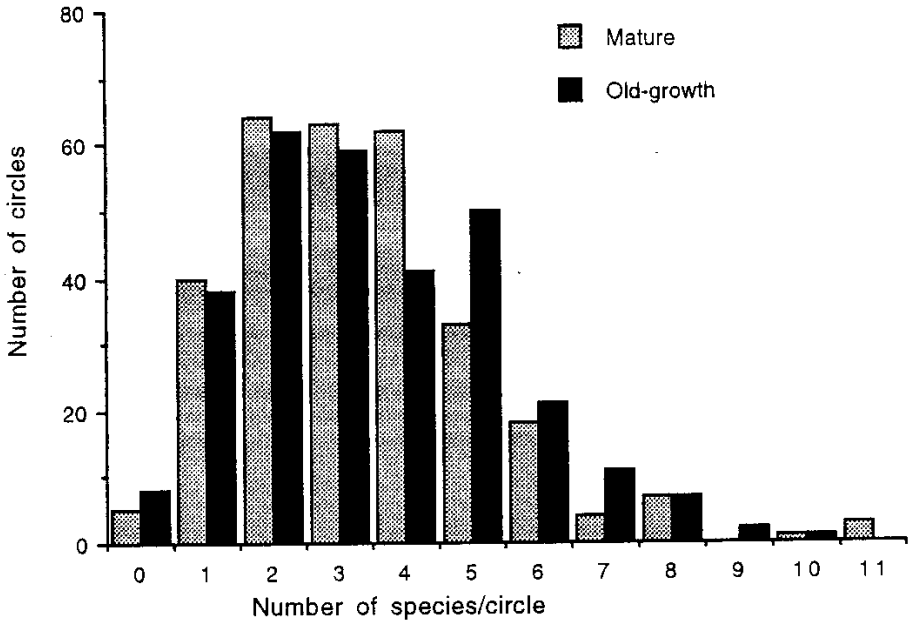


Fig. 7. Distribution of the no. of species per circle for northern hardwood-conifer forest.

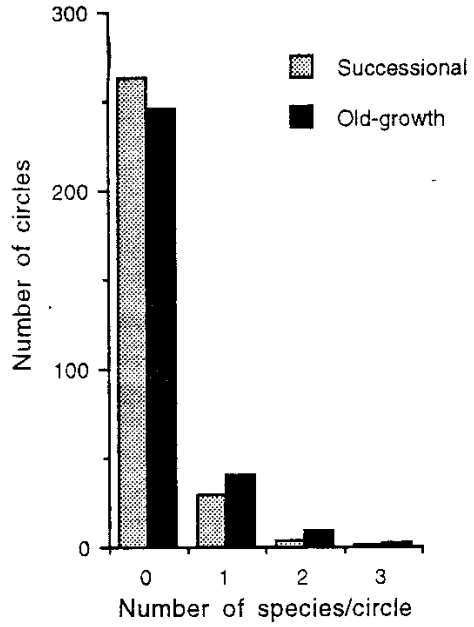


Table 8. Jaccard's coefficient of similarity between Minnesota forest stands and other compilations of ectomycorrhizal fungi.[a]

	Minnesota forest types				Other forest communities, Eastern North America										
Minnesota forest types	A	B	C	D	1	2	3	4	5	6	7	8	9	10	11
A. Mature red pine	1.00				0.04	0.03	0.04	0.03	0.03	0.09	0.04	0.07	0.05	0.06	0.06
B. Old-growth red pine	0.60	1.00			0.05	0.04	0.04	0.03	0.03	0.07	0.05	0.09	0.06	0.07	0.07
C. Successional n. hardwood-conifer	0.09	0.11	1.00		0.10	0.09	0.06	0.07	0.08	0.11	0.06	0.10	0.08	0.10	0.11
D. Old-growth n. hardwood-conifer	0.06	0.08	0.28	1.00	0.07	0.09	0.06	0.05	0.05	0.07	0.11	0.10	0.12	0.14	0.15

	North America				Other forest communities, Europe						
Minnesota forest types	12	13	14	15	16	17	18	19	20	21	22
A. Mature red pine	0.07	0.04	0.01	0.07	0.22	0.15	0.10	0.03	0.02	0.01	0.04
B. Old-growth red pine	0.06	0.04	0.01	0.08	0.23	0.16	0.12	0.03	0.03	0.02	0.05
C. Successional n. hardwood-conifer	0.01	0.04	0.04	0.04	0.14	0.12	0.05	0.04	0.02	0.06	0.09
D. Old-growth n. hardwood-conifer	0.00	0.03	0.04	0.05	0.09	0.06	0.06	0.04	0.05	0.06	0.06

[a] Minnesota forest types (A–D) are compared with other communities from: 1) red spruce, 2) northern hardwood (Bills et al. 1986); 3) sugar maple-yellow birch, 4) balsam fir-birch, 5) black spruce-feather moss, 6) black spruce-cladina (Villeneuve et al. 1989); 7) paper birch-maple (station 2), 8) birch-fir-aspen (station 8), 9) balsam fir-birch (station 11), 10) white cedar-fir-birch (station 9), 11) white cedar-fir-spruce (station 5) (Nantel and Neumann 1992); 12) jack pine (Danielson 1984); 13) aspen (Cripps and Miller 1993); 14) alder-birch (Brunner et al. 1992); 15) black spruce in Minnesota (Doudrick et al. 1990); 16) boreal mixed forests (Salo 1993); 17) pine-spruce-birch (Ohenoja 1978); 18) Scots pine (Hintikka 1988); 19) Scots pine (Hora 1959); 20) Scots pine (Richardson 1970); 21) beech (Tyler 1985); 22) deciduous forests (Tyler 1989). Underlined indicates communities with greatest similarity.

Correlated with greater diversity and abundance in red pine forest was an order of magnitude greater mean number of species per circle, 3.4, as compared to the overall mean of 0.2 species per circle for northern hardwood-conifer plots. The distribution of the number of species/circle showed very few unoccupied circles (Fig. 6) for red pine plots and a preponderance of empty circles for northern hardwood-conifer stands (Fig. 7). Within either forest type the distribution is remarkably similar between age classes. Even though percent frequencies and number of species were similar, or less than mature forest, old-growth stands of both forest types had greater total production of fruitbodies: 1.1 times greater for red pine and a significant 1.8 times greater for northern hardwood-conifer forest. This means that on average there were a greater number of fruitbodies produced per species and in most cases per circle for old-growth stands of both forest types. This greater productivity in these two old-growth forests is a factor to consider in their value for ecosystem function and also conservation. Natural old-growth or ancient forests do not necessarily show a decline in diversity with tree age. Greater overall fungal diversity (ca. 40% ectomycorrhizal) has been documented for old-growth stands in comparison to mature stands of spruce-hemlock forest in Washington (Walker et al. 1994).

Calculations for Jaccard's coefficient of similarity between plots were 0.60 for mature and old-growth red pine forest, 0.28 for successional and old-growth northern hardwood-conifer forest, and 0.06 to 0.11 for combinations of red pine and northern hardwood-conifer forest age classes (Table 8), indicating greatest similarity within the red pine forest. Coefficients of similarity, computed for comparisons to other studies were, in most cases low (Table 8). These computations are affected by lists containing unidentified species and authors possibly having differing species concepts. Greatest values were found with comparison of red pine forest to boreal mixed forests of Finland, 0.22 0.23 (Salo 1993), and with heath forests (containing pine, spruce, birch) of Finland, 0.15 0.16 (Ohenoja 1978), both sets of forests composed of four or more host tree species. The ectomycorrhizal composition of old-growth northern hardwood-conifer forest had greatest similarity to two forest stations of southern Quebec, 0.14 0.15 (Nantel and Neumann 1992, with incomplete species list). Both stations contained northern white cedar, balsam fir, and paper birch; in addition, station 9 had trembling aspen and station 5 had black spruce and red maple.

Except for aspen and black spruce, these tree species were components of the old-growth northern hardwood-conifer stands. Forests with one or a few tree hosts tended to have lower coefficients of similarity with the two mixed forest types of Minnesota. These data suggest that similarity and diversity of tree host composition may be as great a factor as geographic location. Villeneuve et al. (1991*b*) found greatest similarities with studies from the same phytoclimatic region in Quebec, citing geographic segregation as the primary factor involved.

My sampling methods were modeled after those of O'Dell and Ammirati (1994) who also used transects of 4-m^2 circles spaced 5 m apart totaling a sampling area of 400 m^2. My methods differed in that only 60 of the 100 circles (240 m^2) were sampled during each visit and that permanent transects were reused each visit. Diversity of epigeous ectomycorrhizal fungi in red pine forest, with 72 to 100 species per plot (55-73 in circles) or 143 total for the six plot site, equaled that found for the 250- to 300-year-old Douglas fir-hemlock forests (O'Dell and Ammirati 1994), with 40 to 100 species per site (two or 3 stands sampled for each site) and over 200 species total for three sites. Ten to twelve visits were made in two years as compared to my eight visits in two years. O'Dell and Ammirati (1994) found between years a 35% recurrence of species in total for stands, most species being observed in a single stand in a single year. In sampling the same transects for red pine forest, recurrence of species between years averaged 46% for plots (range 41 53%) and 63% in total for the site. Recurrence of individual species in the same circle(s) ranged up to 100% for infrequent species and up to 44% for the most frequent species, *Russula silvicola* (Table 9). Percent recurrence of species in old-growth red pine plot 2 for three successive years was less (Table 10). The degree of recurrence varied greatly between species and is likely a reflection of many factors involved with yearly variation in fruiting, including mycorrhizal activity, climatic conditions, and timing of visits.

TABLE 9. Recurrence of red pine forest species within same circles for two successive years

	Mature[a]			Old-growth[a]		
Species	R[b]	C[c]	%R[d]	R	C	%R
Boletus edulis	1	1	100	–[e]	–	–
Cantharellus cibarius	4	20	20	7	17	41
Chroogomphus rutilis	0	6	0	1	7	14
Coltricia perennis	1	2	50	0	4	0
Cortinarius armillatus	3	12	25	1	7	14
Cortinarius semisanguineus	2	23	9	1	14	7
Cortinarius traganus	1	1	100	–	–	–
Entoloma aff. *politum*	1	13	8	0	4	0
Entoloma cf. *sericatum*	6	24	25	5	45	11
Gomphus flocossus	9	62	15	2	15	13
Hebeloma sp. RP 01	2	16	13	2	19	11
Hebeloma sp. RP 04	0	1	0	1	2	50
Hydnum repandum	0	3	0	1	6	17
Hygrophorus piceae	–	–	–	1	13	8
Hygrophorus pudorinus	8	32	25	8	42	19
Inocybe geophylla var. *geophylla*	0	1	0	12	31	39
Inocybe sp. RP 02	1	12	8	2	14	14
Inocybe sp. RP 07	–	–	–	1	1	100
Inocybe sp. RP 10	1	1	100	–	–	–
Laccaria bicolor	3	20	15	1	35	3
Laccaria laccata	7	61	11	19	102	19
Lactarius affinis var. *viridilactis*	1	12	8	0	9	0
Lactarius deceptivus	0	3	0	1	11	9
Lactarius thejogalus	1	5	20	3	14	21
Lactarius vietus	18	44	41	9	37	24
Lactarius vinaceorufescens	4	30	13	6	52	12
Phellodon niger	0	3	0	1	2	50
Ramaria sp. RP 06	1	2	50	1	2	50
Russula puellaris	4	17	24	3	20	15
Russula silvicola	53	121	44	18	78	23
Russula sp. RP 08	0	2	0	1	3	33
Russula sp. RP 11	1	3	33	0	1	0
Russula sp. RP 16	0	4	0	2	14	14
Russula sp. RP 19	1	5	20	0	20	0
Russula sp. RP 41	1	5	20	0	1	0
Suillus granulatus	2	15	13	0	4	0
Suillus intermedius	0	11	0	1	8	13
Suillus punctipes	0	3	0	4	34	12
Tricholoma flavovirens	1	7	14	0	5	0
Tricholoma saponaceum	1	2	50	1	16	6
Cortinarius undet. spp.	54	187	29	55	182	30

[a] Three plots, 300 circles total.
[b] R, number of circles in which species recurs for both years.
[c] C, total number of circles in which species occurs.
[d] %R, percent recurrence = R/C × 100.
[e] Species not recorded in circles for this age class.

TABLE 10. Recurrence of red pine forest species within the same circles for three successive years, 1993-1995.

	Old-growth[a]		
Species	R[b]	C[c]	%R[d]
Cantharellus cibarius	4	11	36
Chroogomphus rutilis	1	12	8
Hygrophorus pudorinus	1	27	4
Inocybe geophylla var. *geophylla*	1	8	13
Laccaria bicolor	1	11	9
Laccaria laccata	6	53	11
Lactarius vietus	5	23	22
Lactarius vinaceorufescens	1	32	3
Phellodon niger	1	2	50
Russula silvicola	1	18	6
Suillus punctipes	1	16	6
Cortinarius undet. spp.	11	71	15

[a] Plot 2, 100 circles total.
[b] R, number of circles in which species recurs for all three years.
[c] C, total number of circles in which species occurs.
[d] %R, percent recurrence = R/C × 100.

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IV. SUMMARY DISCUSSION

In 1993 only two old-growth plots were sampled for both of the forest types. Much time that initial season was spent locating suitable sites, establishing plots and transects, and beginning documentation of the surprisingly abundant and diverse fungal species. Delimitation of mature and successional plots was completed in 1994. Complete sampling of both forests was made in 1994. Due to limited resources 1995 sampling was done for red pine forest only and 1996 sampling carried out for northern hardwood-conifer forest. The benefit of multiple years of observation can be assessed by comparison between the three years for two of the old-growth plots. Figure 1 shows the annual and cumulative numbers of species recorded within sampled circles and the total numbers observed within the plot for old-growth red pine plot 2. Figure 2 shows the comparable data for old-growth northern hardwood-conifer plot 1. In both cases, species diversity (and abundance) were high in 1993 and lowest in the last year. Even though yearly diversity decreased, new species were added each year. The difference in abundance between 1993 and other years was greatest for the northern hardwood-conifer plots; 31% of old-growth species were seen only in 1993.

Villeneuve et al. (1988) found that coniferous forests showed a clear dominance of ectomycorrhizal fungi from the Cortinariaceae, Russulaceae, and Boletaceae, while deciduous forest had higher diversity of saprobes. This was also observed for red pine forest versus northern hardwood-conifer forest, the latter being dominantly deciduous. Data were collected for a limited number of saprobic (and some questionably ectomycorrhizal) taxa. *Hygrocybe* (16 taxa) was diverse and abundant in northern hardwood-conifer forest (see Chapter III) and much less so in red pine forest (seven spp.). Entolomataceae were also more diverse in northern hardwood-conifer forest (data not shown) and other saprobic taxa were more frequently observed, e.g, *Mycena* and other Tricholomataceae. Villeneuve et al. (1988) also found a significant positive correlation between the number of ectomycorrhizal fungi and the percent cover of host trees, whereas the shrub and lichen cover showed a negative relationship.

Fig. 1. Annual number of species and cumulative number of species of fungi in plot 2 of old-growth red pine forest; numbers given for circles (400 m^2) and plot (5000 m^2).

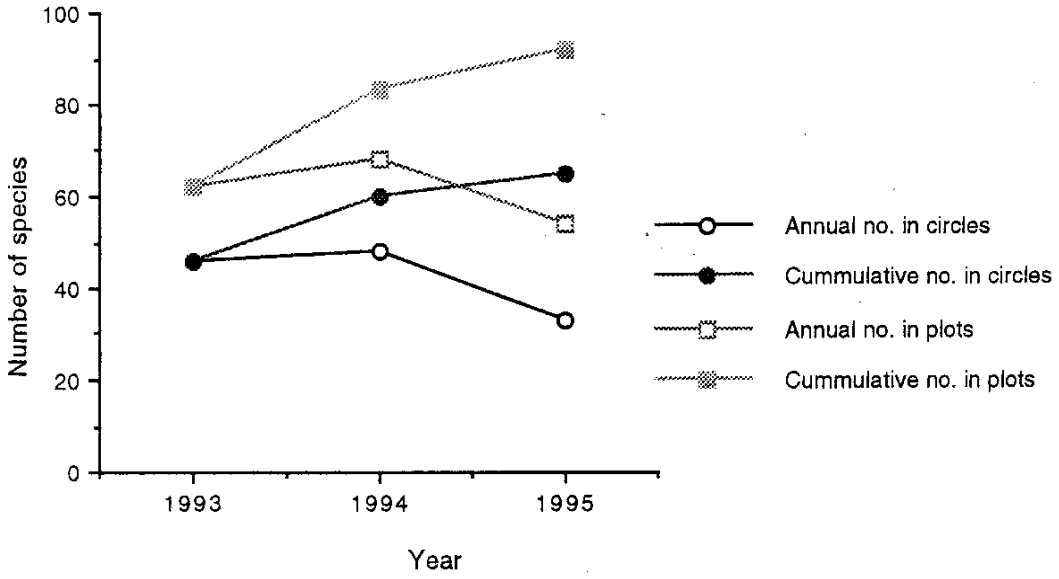
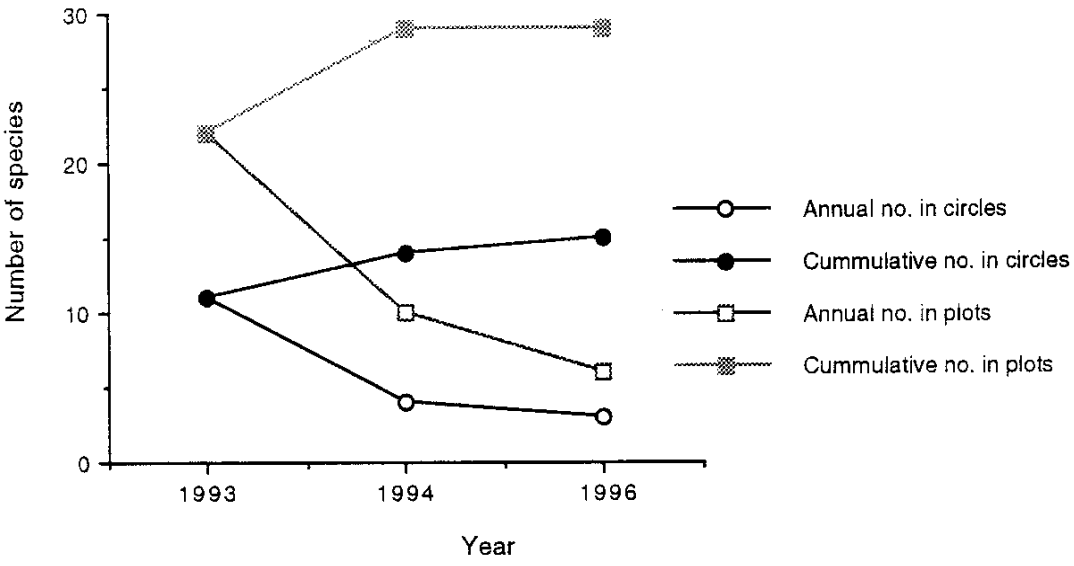


Fig. 2. Annual number of species and cumulative number of species of fungi in plot 1 of old-growth northern hardwood-conifer forest; numbers given for circles (400 m^2) and plot (5000 m^2).



Numerous species recorded in these two forests, particularly for the red pine forest, are showing marked declines in areas of northern Europe. Those listed as extirpated (E) or threatened with extinction (T) in The Netherlands (Arnolds, 1988, 1989) include *Cantharellus cinereus* (E), *Cortinarius allutus* (E), *C. mucosus* (T), *C. traganus* (E), *Hydnellum aurantiacum* (E), *H. caeruleum* (E), *Hygrophorus pudorinus* (E), *Phellodon melaleucus* (T), *P. niger* (T), *Rozites caperata* (T), *Suillus placidus* (E), *Tricholoma focale* (T), and *T. virgatum* (T). In addition to these species, the presence of a total of 10 species of hydnaceous fungi (*Hydnum*, *Hydnellum*, *Phellodon*, and *Sarcodon*), and a high frequency of *Cordyceps* *Elaphomyces* (in the red pine forest plots), is a strong indication of the health of these forest communities in Minnesota. Arnolds (1989) reported that six of the nine species showing the strongest decline were hydnaceous fungi including *Phellodon melaleucus* (going from rather common to very rare); another was the polypore *Coltricia perennis* (was very common, now rather rare). *Elaphomyces* was considered strongly threatened in The Netherlands as well as its parasites *Cordyceps capitata* and *C. ophioglossoides* (Arnolds 1989).

In Europe, a prime threat to macrofungi, especially ectomycorrhizal species, is increased air pollution, particularly nitrogen deposition (Arnolds and Jansen 1992, Floravårdskommittén för svamper 1991). Rassi and Väisänen (1987) cited the single most important cause of the decline of animals (especially invertrebrates), plants, and fungi in Finland to be intensive forest management with its effects on microclimate, reduction of decaying wood, and changes in species ratios and age structure of tree stands; loss of habitat due to logging accounted for almost one-third of the extirpated species. A Swedish red data list of endangered fungi stated that many wood decaying species require large substrates and old-growth trees and that mycorrhizal fungi are at risk due to the cutting of stands before they mature and due to the use of fertilizers (Florasvårdskommittén för svamper 1991). Ectomycorrhizal fruitbody production was somewhat reduced by phosphorus addition and greatly reduced by nitrogen fertilization for 11-year-old *Pinus taeda* plantations in North Carolina (Menge and Grand 1978). With simulated nitrogen deposition, the mean number of ectomycorrhizal fruitbodies dropped after three years to one percent or less of pretreatment levels in Swedish beech forest (Rühling and Tyler 1991). The red pine and northern hardwood-conifer stands used in this study were located in Minnesota state parks where observed disturbances have been minimal. The

disturbances include the current use of trails and a few very limited incidences of selective cutting in the past.

These old-growth forests appear to have a significant role in harboring fungal diversity. Twenty-one species of red pine forest (14%) and 32 species of northern hardwood-conifer forest (51%) were not observed in the younger stands and additional species showed significantly greater abundance in the old-growth stands. As discussed previously (Chapter III), the idea that diversity declines in forests following canopy closure (Dighton and Mason 1985) needs to be discarded for naturally occurring forests where community dynamics are more complex than for monoculture plantations. Newton (1992) discusses the limitations of the concept of "early-" and "late-stage" fungi, one being that the original observations were made from trees planted in agricultural soils; seedlings planted in forest soils near mature trees acquired predominantly late-stage taxa. Some species of late-stage genera have been described as early-stage types and some species, e.g., *Lactarius pubescens*, have been classified as "intermediate" (Newton 1992). Newton (1992) argues for an alternative classification based on the characteristics which determine competitive ability, e.g., the term "pioneer" fungi for the early-stage species which are easily established via spores; the large number of remaining species have varying characteristics for colonization by mycelial inocula, such as mycelial strands, mycelial branching, host preference, response to abiotic factors, and ability for nutrient capture and transfer. In terms of nutrient capture, evidence from field and laboratory studies shows that many ectomycorrhizae have the biochemical potential for direct access to organic residues in the soil, the predominant source of nitrogen in forest systems, allowing them to be effective competitors (Read 1992).

Bruns (1995) discusses the importance of understanding ectomycorrhizal diversity and community structure on a localized scale in order to reveal the functional significance of this diversity. A portion of ectomycorrhizal diversity can be explained by the fungal resources, carbohydrates supplied by host short roots and nutrients captured from soil and other substrates, being partitioned in time (e.g., season and host age) and space (e.g., distance of short roots from stem, soil depth, and litter heterogeneity) (Bruns 1995). Patterns of local diversity can be influenced by small scale disturbances (e.g., rodent burrows), nutrient enrichment, presence of other soil organisms, and competition (Bruns 1995). Further studies on life histories (Bruns 1995) may help explain why *Hygrophorus piceae* was restricted to old-

growth red pine and *Albatrellus ovinus* was more frequent in mature red pine stands, yet neither of these two spruce associates was found in northern hardwood-conifer forest. There is great diversity in ectomycorrhizal fungi, with well over 5,000 species reported, species with narrow (e.g., *Suillus*) to broad (e.g., *Laccaria*) host preferences (Allen et al. 1995). Species distributions range from circumglobal (e.g., *Laccaria laccata*) to locally endemic (e.g., *Suillus weaverae* in Minnesota). Allen et al. (1995) argue in support of the hypothesis that distribution and diversity of arbuscular mycorrhizae and ectomycorrhizae may regulate the diversity of different plant communities and that loss of fungal species could produce a measurable effect. The diversity and abundance of ectomycorrhizal fungi documented for Minnesota red pine and northern hardwood-conifer stands shows them to be a major component in these forests.

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V. APPENDIX I

ADDITIONS TO MATERIALS AND METHODS AND SOIL ANALYSIS

Site selection, location and sampling --

Twenty sites were visited during 5 weeks in July and August, 1993, to choose stands for sampling ectomycorrhizal fungi. Sites were chosen based on vegetation characteristics, size, low disturbance, understory density, absence of edge effects, status of protection, and accessibility. For quantitative data collection permanent transects were established during an additional 2 weeks in an old-growth red pine stand (Scenic State Park) and two old-growth northern hardwood-conifer stands (Tettegouche and George H. Crosby-Manitou State Parks). These plots originally had twice as many transects spaced 5 m apart instead of 10 m as used in the final sampling design. The plot at Crosby-Manitou was originally a double plot, 100 × 100 m, with 20 transects; the half on the far side from the trail was not sampled. These sites and transects were renumbered in 1994. During 2 weeks in June and early July, 1994, permanent transects were located in one old-growth and three mature red pine stands (Scenic State Park) and in three successional northern hardwood stands (Tettegouche State Park).

The accompanying figures show locations of stands within the State Parks and the orientation of the plots. For the red pine site, the three old-growth plots and mature plot 1 are located east of Pine Lake in the NW¼ of NE¼ of Sec. 32, T61N R25W. Mature plots 2 and 3 are in the SE¼ of SW¼ and SW¼ of SE¼ of Sec. 29, T61N R25W. For the northern hardwood-conifer sites, old-growth plots 1 and 2 are in Tettegouche State Park, Lake County, mostly in the NW¼ of SE¼ of SW¼ of Sec. 9, T56N R7W, and the adjacent area to the south and west. Old-growth plot 3 in George H. Crosby-Manitou State Park, Lake County, is mostly in the NE¼ of SE¼ of SW¼ of SW¼ of Sec. 28, T58N R6W, and the adjacent area to the north and west. The three successional plots in Tettegouche State Park are west of Nipisiquit Lake in the NW¼ of NW¼ of Sec. 8, T56N R7W, and the adjacent portion of sec. 5 to the north. All plots are located near hiking trails.

The ends and the midway point (50m) of the center and outside pair of

transects of each plot were marked long term (visually) with heavy duty PVC (polyvinyl-chloride) pipe hammered vertically into the ground and extending about 2 feet out of the ground. These pipe are gray with white caps. Some of these pipe locations were marked permantly with one foot stainless steel pipe sunk completely underground (see maps at end of appendix). Each plot had 5 transects (plot 1: transects numbered 1 5; plot 2: no. 6 10; plot 3: no. 11 15). The center transect of each plot was sampled every visit. If I were to redo this I would consider setting out an even number of transects for each plot.

For establishing the circles along the transects, a good compass with sighting mirror and a 100-m measuring tape were used at the start of each season to place a wire flag at the center of each circle. Different colored flags were used for alternate transects. Each flag is numbered with its distance along the transect (i.e. 5, 10, 15,...100 m). Flags are removed at the end of the season prior to winter. During sampling a light plastic pole is used. The pole is marked in from both ends a distance of 1.1284 m. This indicates the radius of the area to be surveyed. A person can place one end of the pole at the flag in center of circle and travel around the circle to determine which fungi fall within the 4-m^2 area. The pole comes in very handy to part the ground vegetation when looking for fungi and also as a walking stick.

When fruiting is moderate to high, six days in the field were required to complete each visit, particularly for the red pine forest. The optimum situation for efficient sampling (in our case) involved a team of three persons: a student assistant, and a dedicated volunteer (often from the Minnesota Mycological Society), and myself: two persons to look for and count fungi inside circles, while I recorded species and fruitbody numbers on the data sheets and also looked for additional taxa between circles.

Fungal identification --

Detailed descriptions of fruitbody morphology were written, spore prints made, and color photographs taken on Kodak Ectachrome slide film, ASA 100, with a daylight flash. Specimens were dried for later microscopic examination. Much time for microscopic examination and literature review is required for identifications, particularly taxonomically difficult genera for which there is incomplete knowledge in North America. The primary references that I (and my advisor) used for species identifications were as follows.

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Voucher specimens --

Collections were deposited in the University of Minnesota Herbarium (MIN). Voucher specimens cite "Old-growth Forest Project" and give plot location. Data were also entered into the MIN herbarium fungi collections database. Supporting documentation, filed in the Herbarium, included specimen descriptions, photographic slides, and collection list.

Database description --

I used R:BASE version 4.5++ (Microrim, Bellevue, WA) on a Zeos Desktop Model 486DX-33DT (IBM compatible) computer. R:BASE is a relational database program employing full ANSI Level II Structured Query Language. Program access is via graphic-style menu systems or direct command

language. The database for this project consisted of several tables, some linked by common fields. The data could be selected/sorted on any field and simple to complex queries made to extract information. Forms, views, and reports provide for entering, editing, viewing, and compiling data. Additional tables were generated to calculate different summations of species occurrences and fruitbody counts to determine frequencies and estimate densities.

The main tables of sampling data for the fungi in the forest plots included fields for forest type code, age class code, replicate plot no., visit no., sampling date, transect no., sampling circle no., no. of fruitbodies in circle, presence outside circle, genus code and species code for original field determination, original degree of taxon certainty, genus code and species code for current determination, current degree of certainty, note field for comments on each record, and a field for collection no. when a voucher was saved. A taxon table consisted of fields for genus code, species code, genus name, species name, author citation, variety/form/synonym, status of species determination, and a note field. A third table contained locality data for each forest plot: forest type code, age class code, replicate plot no., county, name of management unit, township and section, and a note field for habitat information. For vouchers the relevant data from these three tables were combined into a fourth table that had the same structure as the main collections table in the University of Minnesota Herbarium (MIN) database. This fourth table was used to generate herbarium labels and to transfer collection data to the MIN database.

Soil analysis --

Methods for soil collection was described in Chapter II. The Research Analytical Laboratory, University of Minnesota dried the soil samples and used a portion to determine percent organic matter by the loss on ignition method.

The $CaCl_2$ method was used by a student assistant to determine pH (McKeauge 1978). In 100 ml beaker was put 60 ml of 0.001 M $CaCl_2$ and 30 grams of dry soil (2 parts $CaCl_2$ solution to 1 part soil). Soil slurry was mixed and allowed to sit for at least one minute; mixing up 15 samples took about 20 minutes. Slurry was stirred prior to taking the pH reading with a gel pH probe.

Results for each soil sample.

Forest type	age class	Plot	transect and circle	pH	% organic matter (L.O.I.)
Red pine	mature	1	1-15	4.34	2.5
			1-65	4.12	5.0
			2-15	3.74	3.6
			3-25	4.26	5.4
			5-75	3.95	6.4
		2	6-25	4.42	3.1
			7-5	4.46	2.2
			8-25	4.84	1.9
			8-70	4.62	2.1
			10-75	4.47	1.7
		3	11-15	4.41	1.7
			11-60	4.36	3.8
			12-95	4.19	3.8
			13-80	4.09	4.6
			15-20	4.35	4.4 / 4.3
Red pine	old-growth	1	1-45	4.25	5.3
			1-95	4.00	2.7
			2-35	4.65	1.8
			4-80	4.48	1.8
			5-45	4.21	1.9
		2	6-75	4.40	2.4 / 2.7
			6-85	4.45	2.0
			7-60	4.32	1.7
			8-100	4.26	1.5
			10-45	4.49	1.3
		3	11-55	4.54	1.4
			11-85	4.25	1.8
			13-15	4.48	2.4
			14-25	4.26	1.8
			15-60	4.26	1.8
Northern hardwood-conifer	Successional	1	2-30	4.96	13.7
			3-30	5.14	13.0
			3-45	4.82	11.7
			4-30	4.88	11.8
			4-95	5.21	11.9
		2	7-35	4.98	14.6
			7-85	4.70	14.2
			9-25	4.05	13.2
			10-10	4.23	16.0
			10-60	4.26	12.4
		3	11-45	5.12	21.1 / 21.7
			11-55	4.62	24.4

Forest type	age class	Plot	transect and circle		pH	% organic matter (L.O.I.)	
Northern hardwood-conifer	Successional continued	3	12-95		4.77		10.2
			13-85		4.60		11.0
		14-75		5.14		26.1	
Northern hardwood-conifer	old-growth	1	1-20		4.46		11.0 / 9.9
			1-65		4.36		9.7
		1-95		4.36		14.5	
			3-20		4.17		13.6
			5-30		4.06		18.5
		2	6-40		4.26		19.2
			7-85		4.73		13.6
			8-65		4.43		13.3
			9-25		4.05		13.5
			10-30		4.31		13.3
		3	12-100	4.39		18.9	
			13-35		4.34		12.6
			14-95		4.06		14.6
			15-5		4.14		19.0
			15-90		3.96		11.5

Some samples had two measurements made for organic matter content.

Figure 1. Plot design used for red pine and northern hardwood-conifer forests. Half-hectare plot contained five 100 m transects spaced 10 m apart. Twenty 4-m^2 sampling circles were spaced 5 m apart along each transect. Nine PVC pipe marked each plot location; some pipe locations marked by sunken one foot stainless steel pipe.

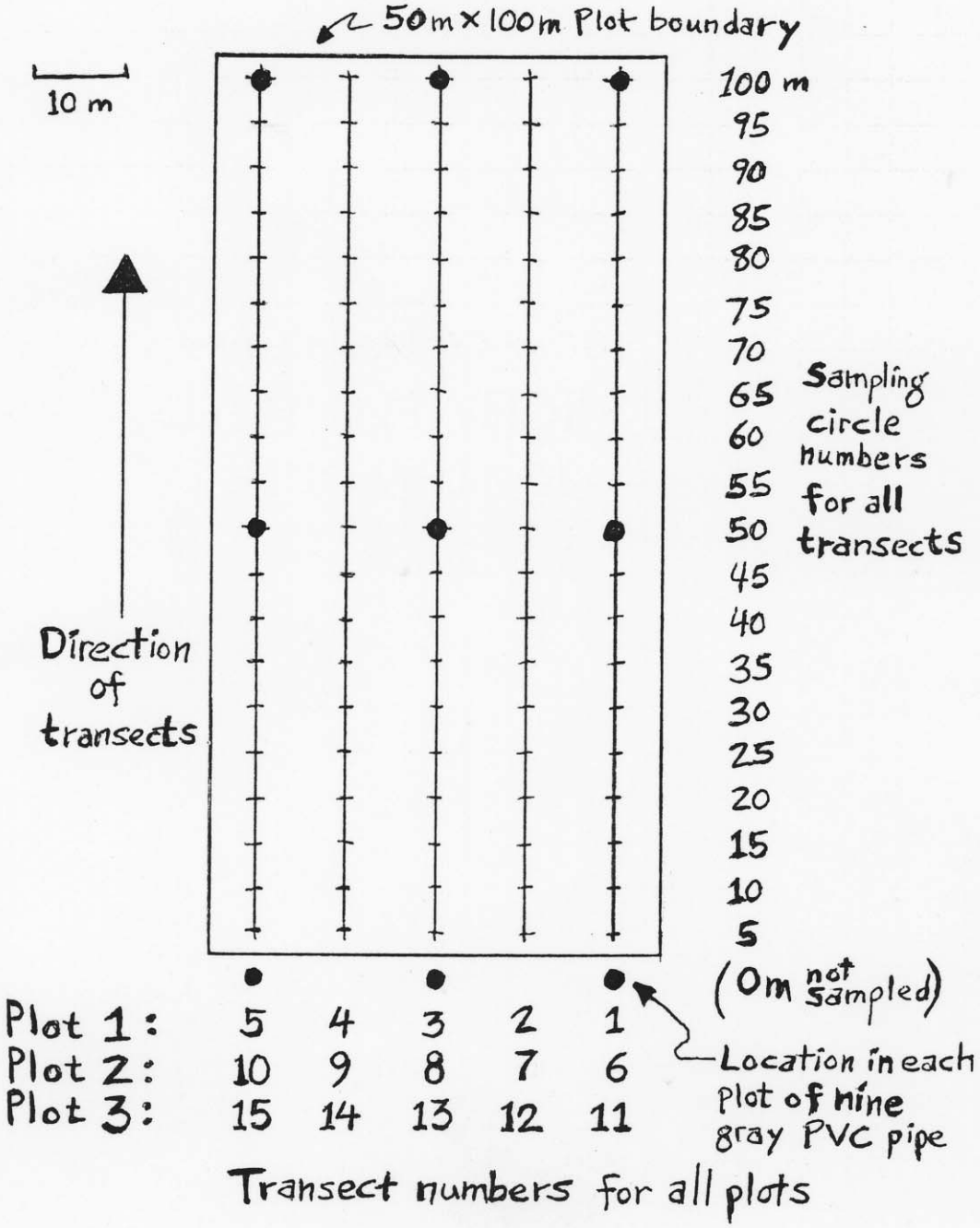

Figure 2. Northern part of Scenic State Park, Itasca Co., Minnesota, showing locations of mature and old-growth red pine forest stands.

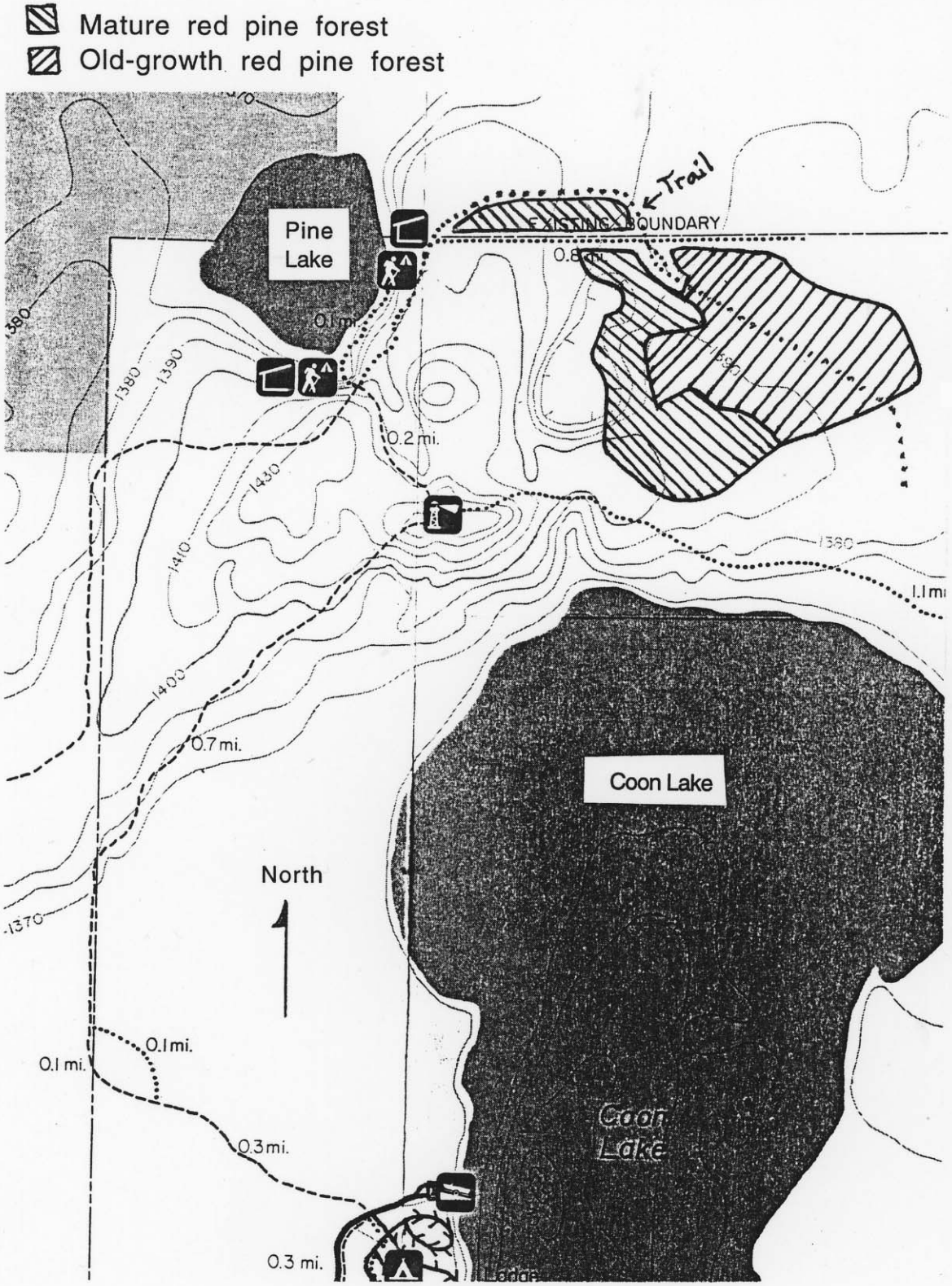

Figure 3. Mature and old-growth red pine forest stands showing locations of plots.

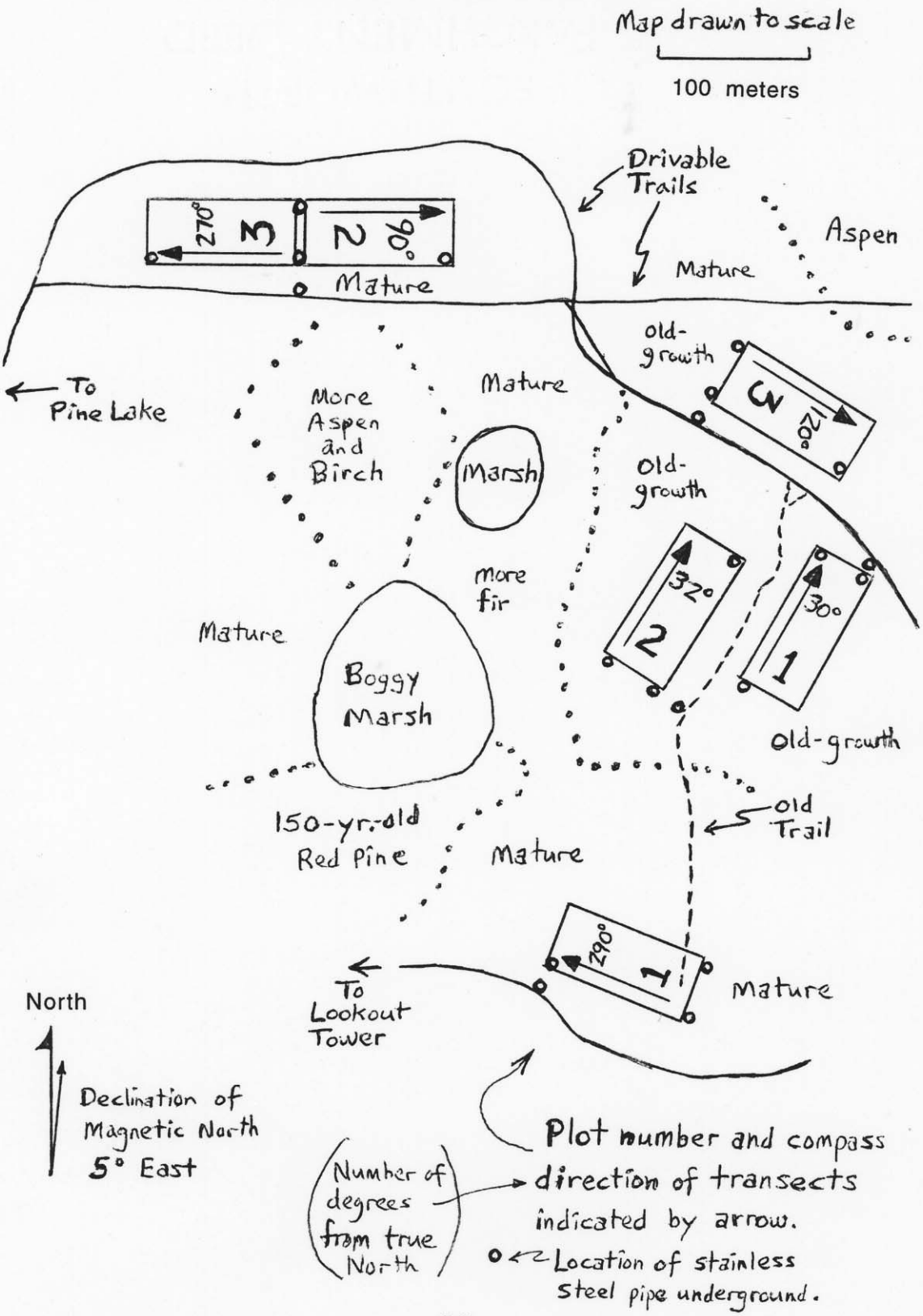

Figure 4. Detail of mature red pine plots 2 and 3 showing location of section marker.

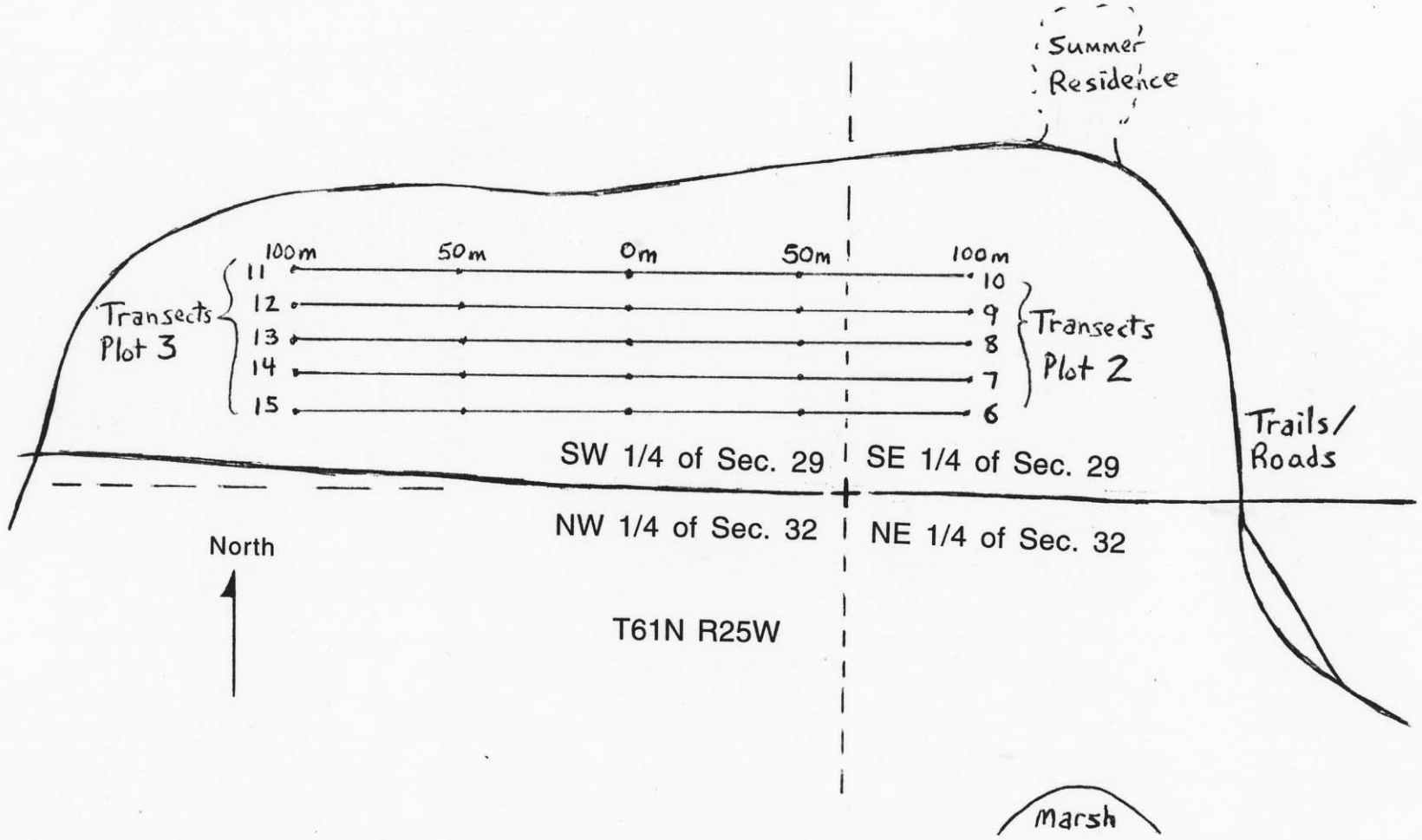

Figure 5. Detail of red pine forest plots showing placement of supplementary stainless steel pipe outside of mature plots 1-3 and old-growth plot 2. Maps A-C drawn to the same scale as Fig. 1. Distance and compass direction between pipes is indicated. Section marker indicated in Fig. 5-B (see Fig. 4).

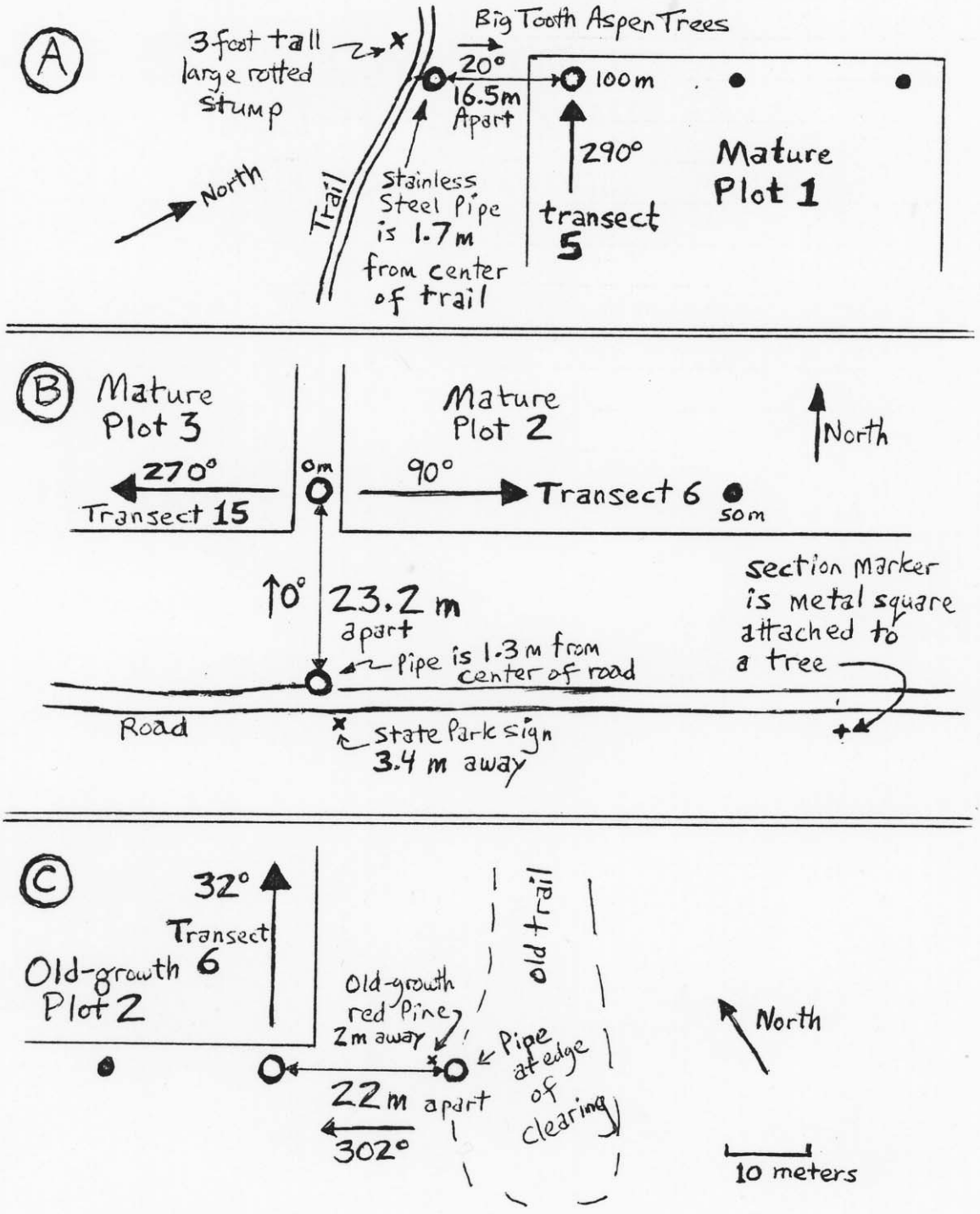

Figure 6. Detail of old-growth red pine plots 1-3 showing placement of stainless steel pipe outside of plots. Same scale as Fig. 1. Roads and old-growth pine trees served as reference points.

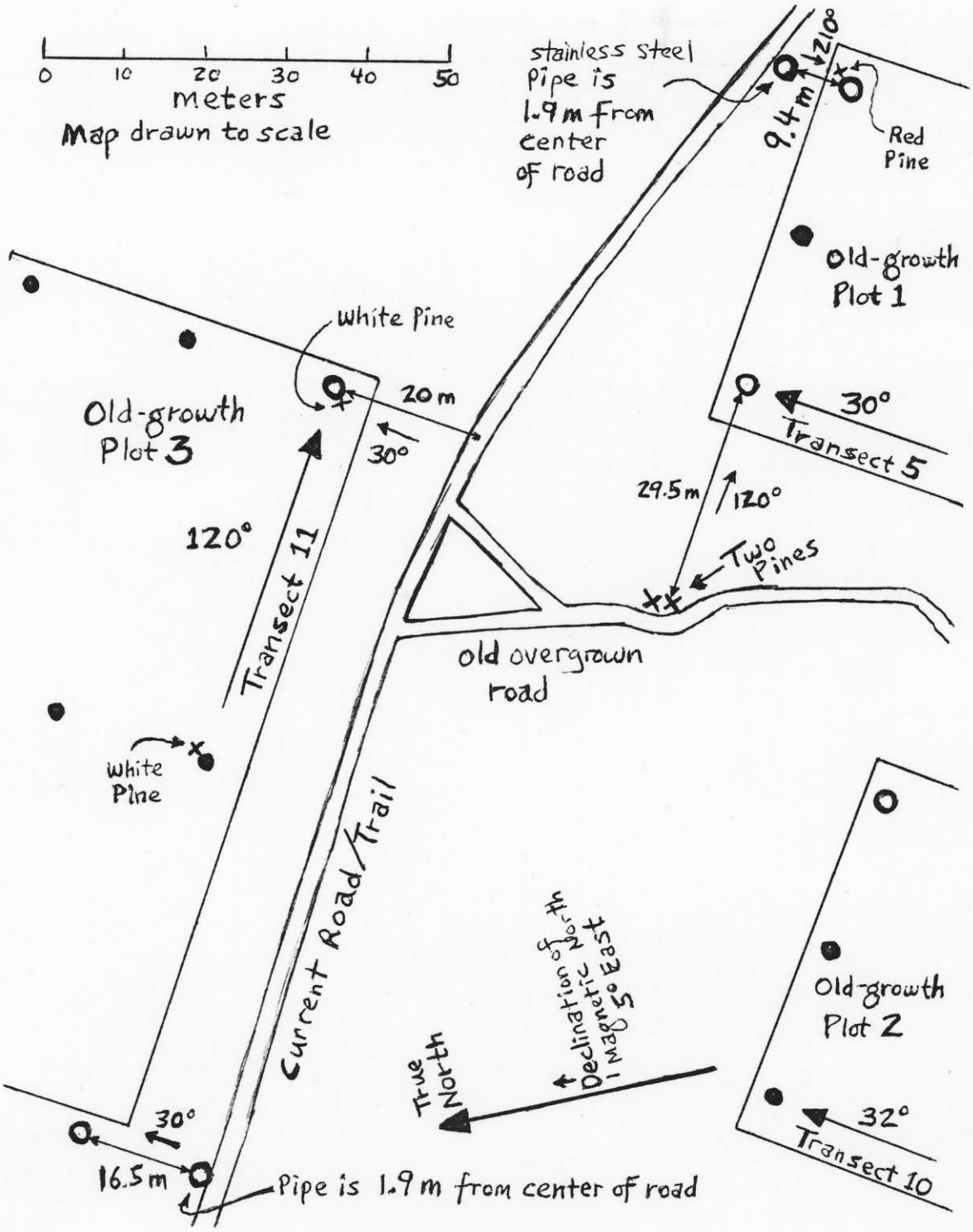

Figure 7. Tettegouche State Park, Lake Co., Minnesota, showing locations for old-growth northern hardwood-conifer plots 1 and 2 and successional plots 1-3.

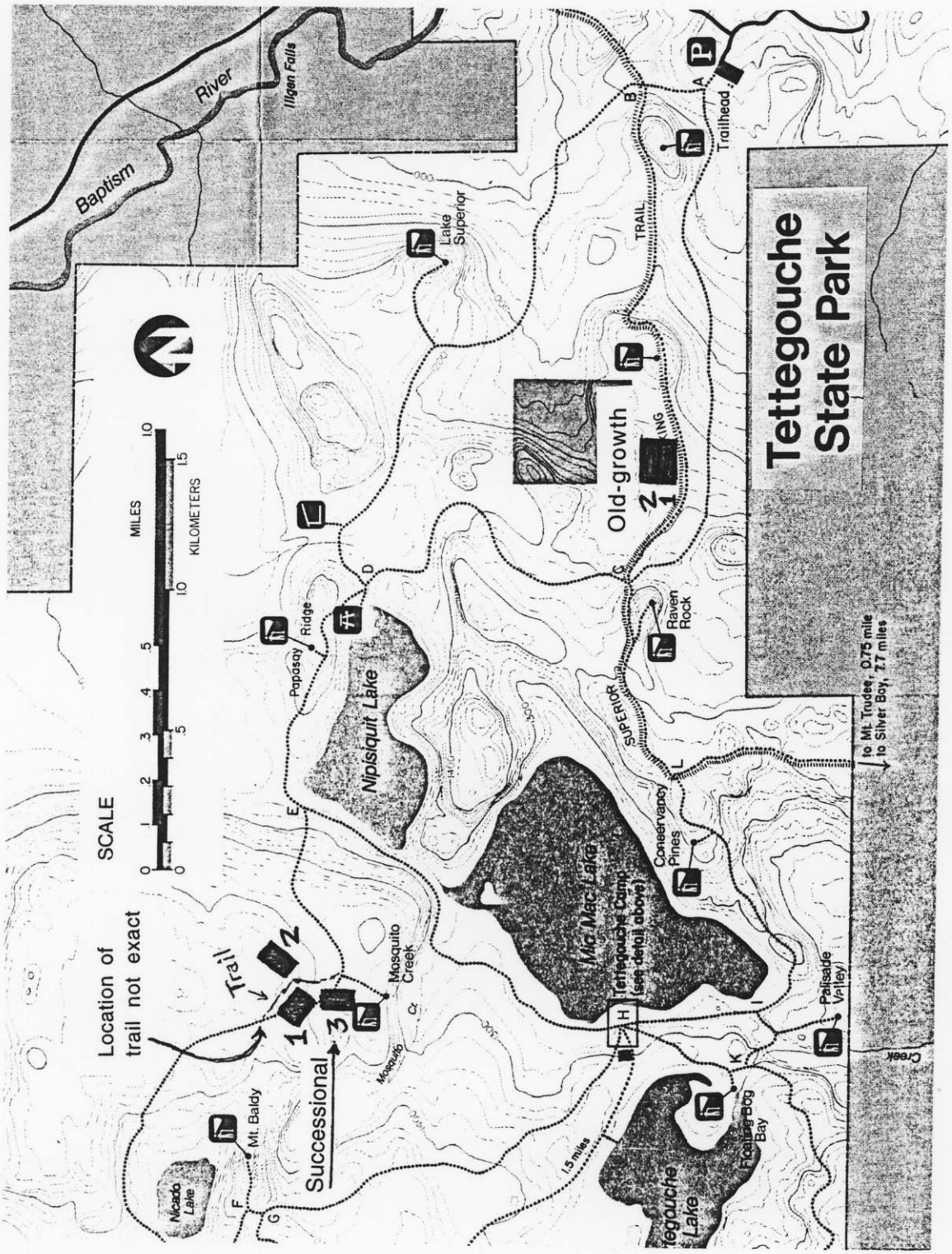

Figure 8. Approximate location of successional plots 1-3 in Section 5 and 8 of T56N R7W. There was no section marker at the NW corner of Section 8, just to the west of plot 1. Access was by trail from Tettegouche Camp, at end of service road from County Road 4.

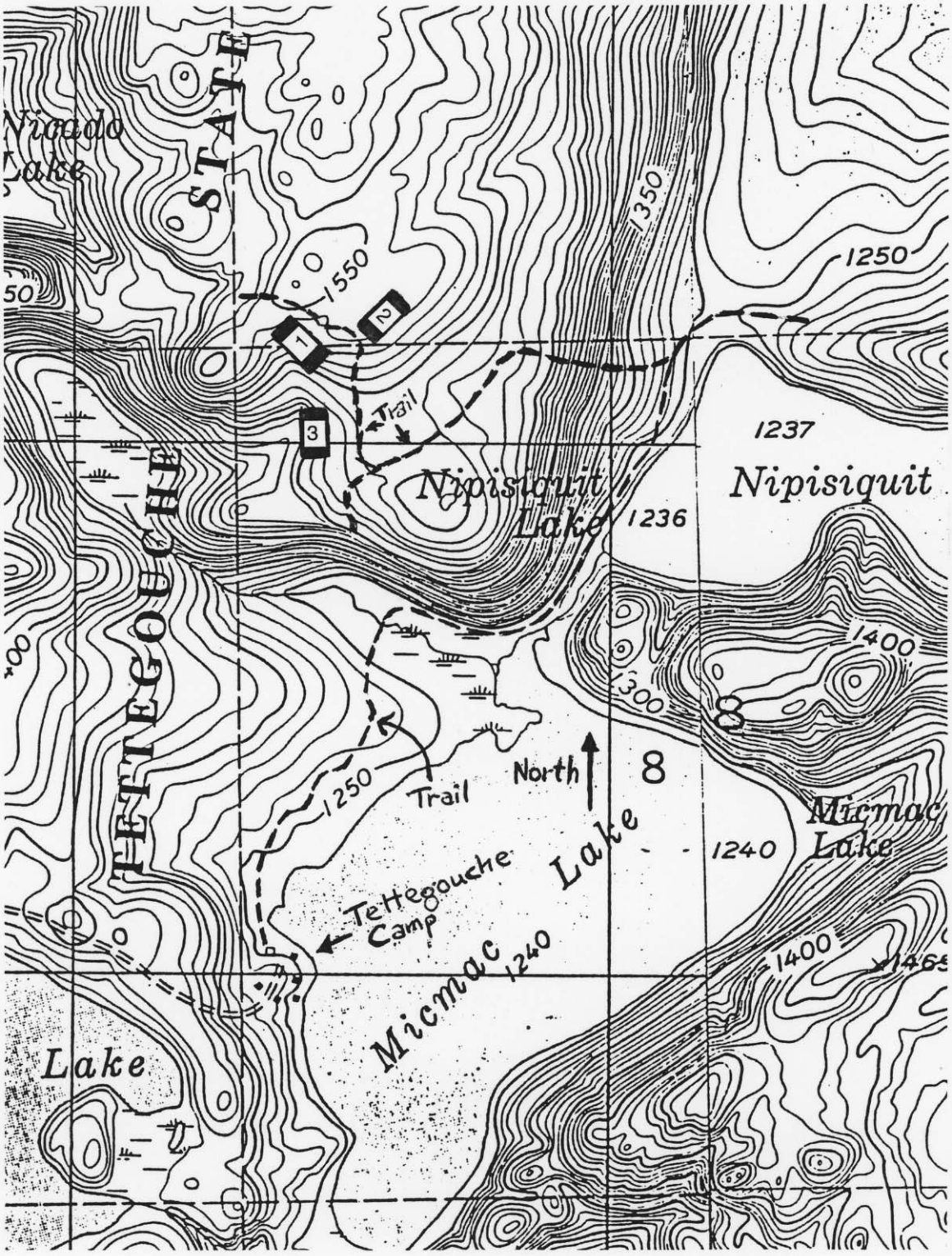

Figure 9. Detail of successional northern hardwood-conifer plots showing orientation of plots and locations of stainless steel pipe. Distances and compass direction to plots from pipes on trail are indicated. Plot 3, the hardest to locate, was 65 m down a slope.

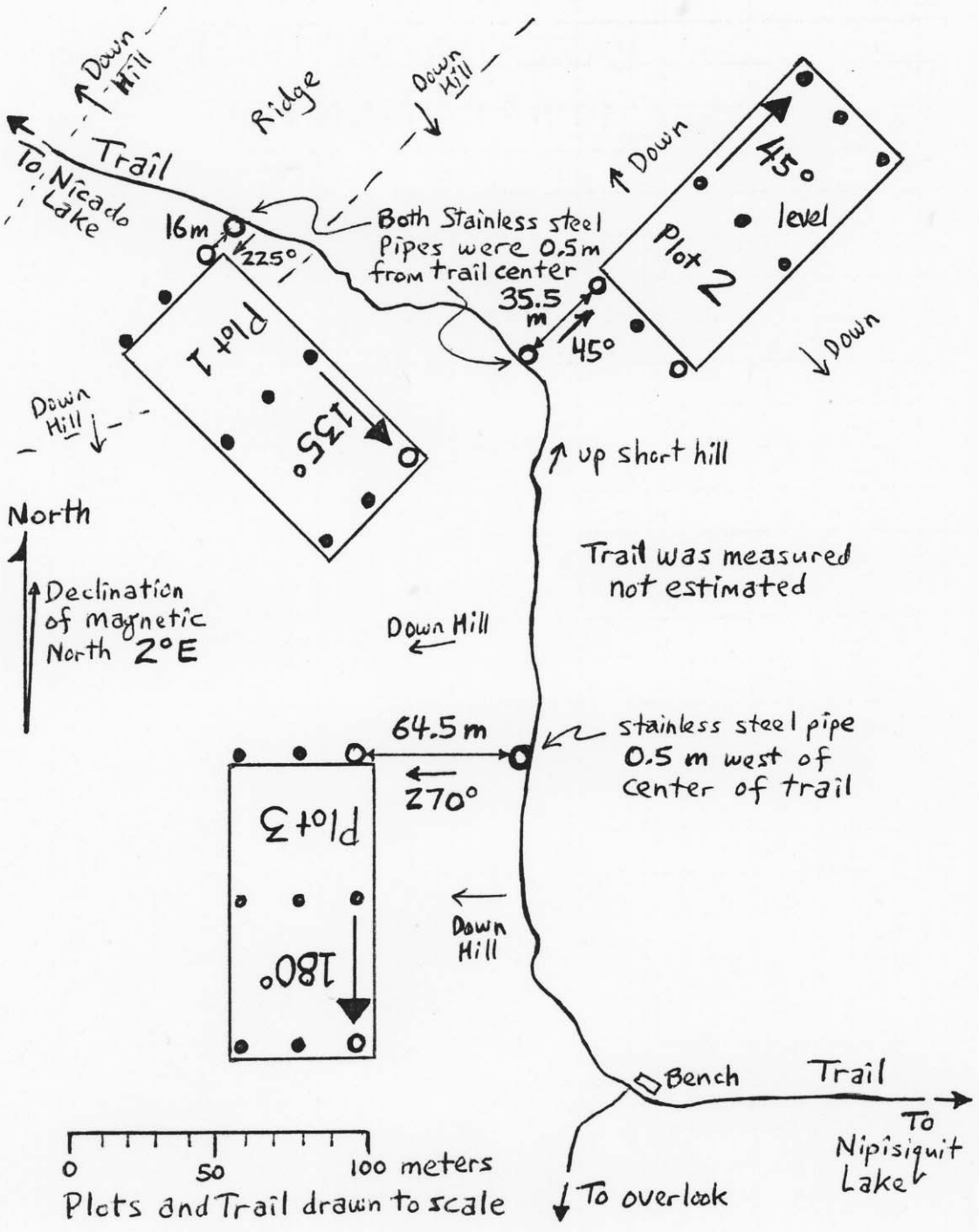

Figure 10. Location of old-growth northern hardwood-conifer plots 1 and 2 in Section 9 of T56N R7W. Plots are adjacent to the Superior Hiking Trail with trailhead located to the east (see Fig. 7).

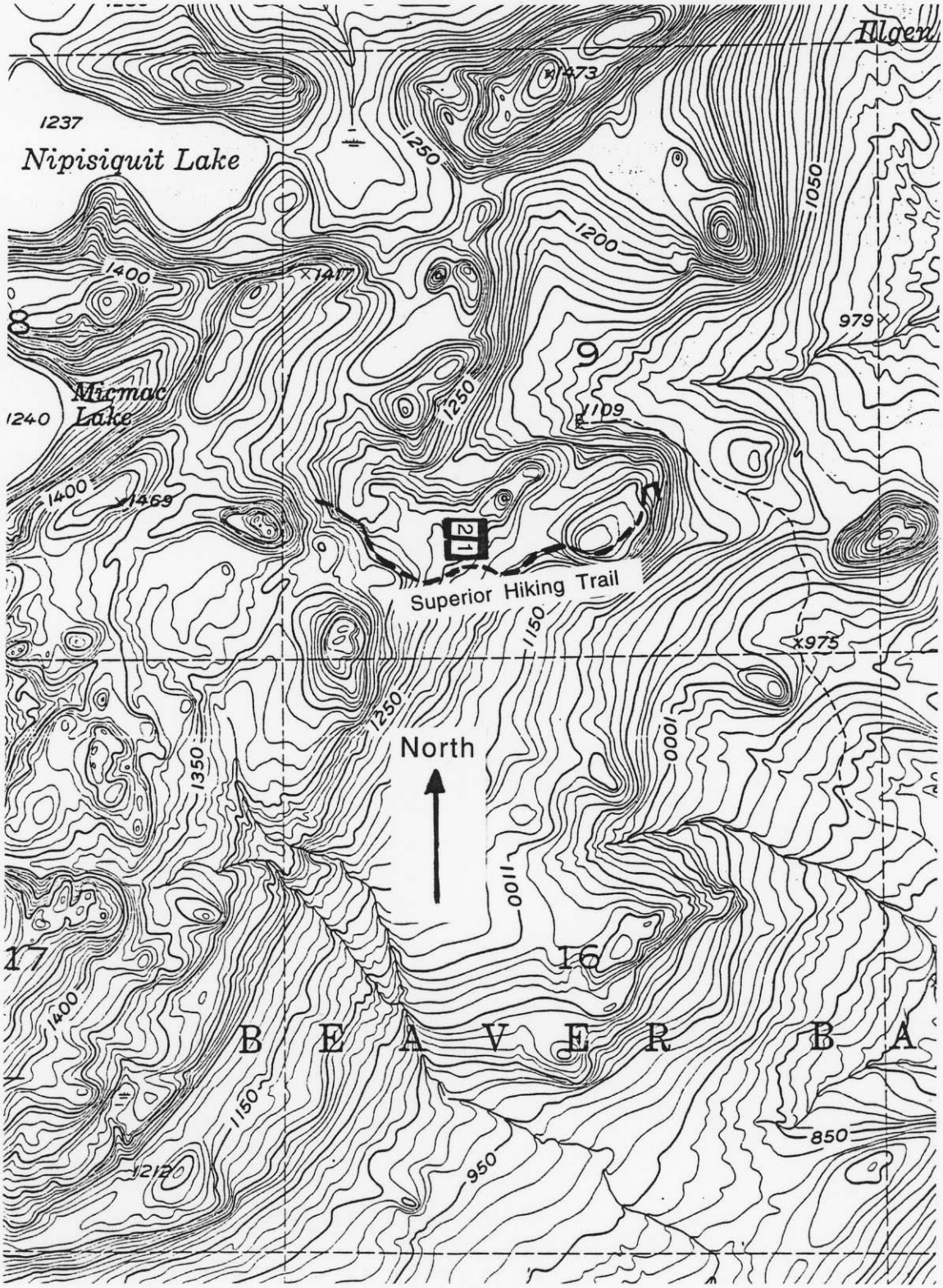

Figure 11. Detail of old-growth northern hardwood-conifer plots 1 and 2 showing orientation of plots and locations of stainless steel pipe. Distance and compass direction from large rock outcrop is indicated. Two old-growth white pine trees were in area of plot.

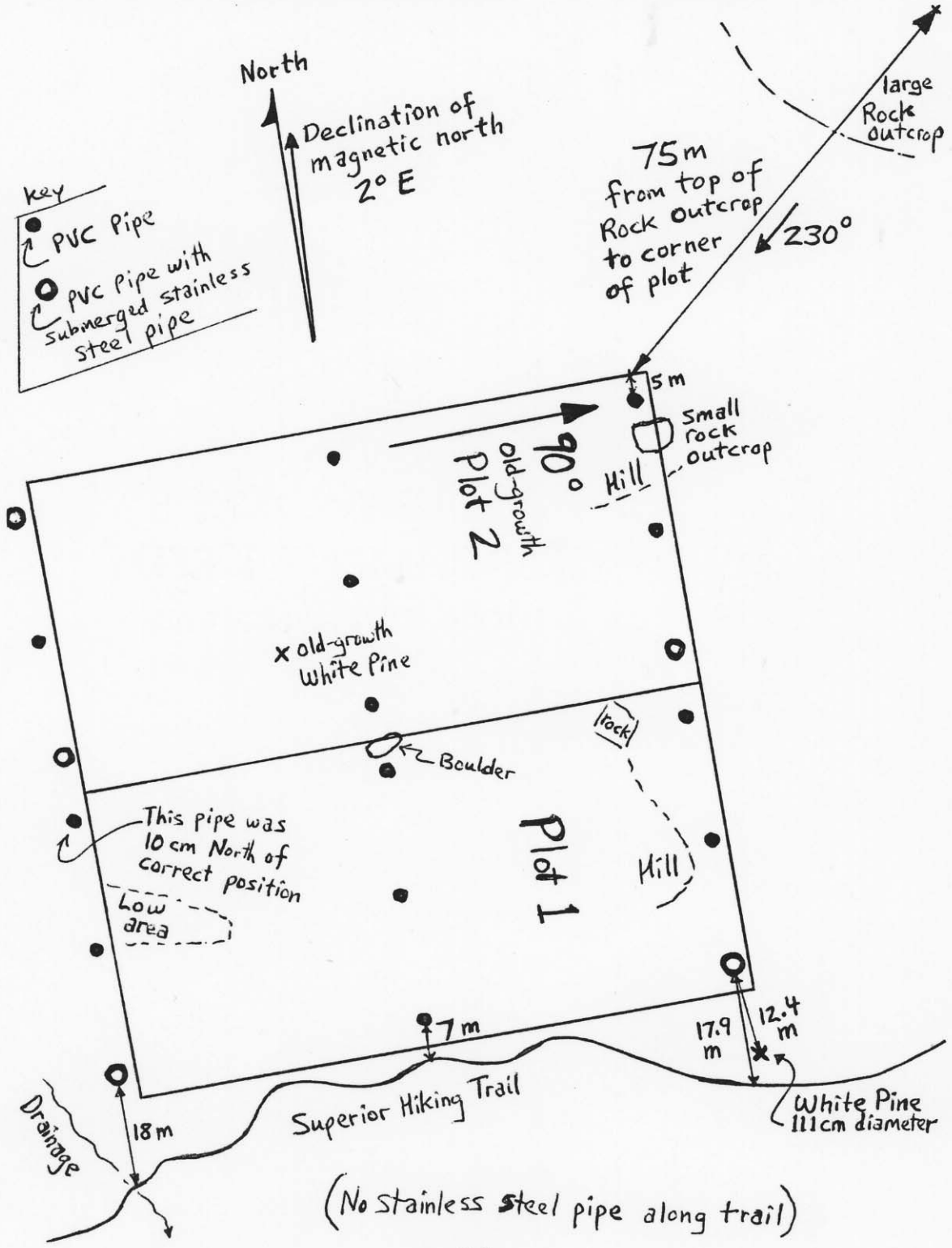

Figure 12. George H. Crosby - Manitou State Park, Lake Co., Minnesota, showing location for old-growth northern hardwood-conifer plot 3. Trailhead is north of Bensen Lake. Plot is on west side of "Cedar Ridge Trail" (trail location inaccurate on park map).

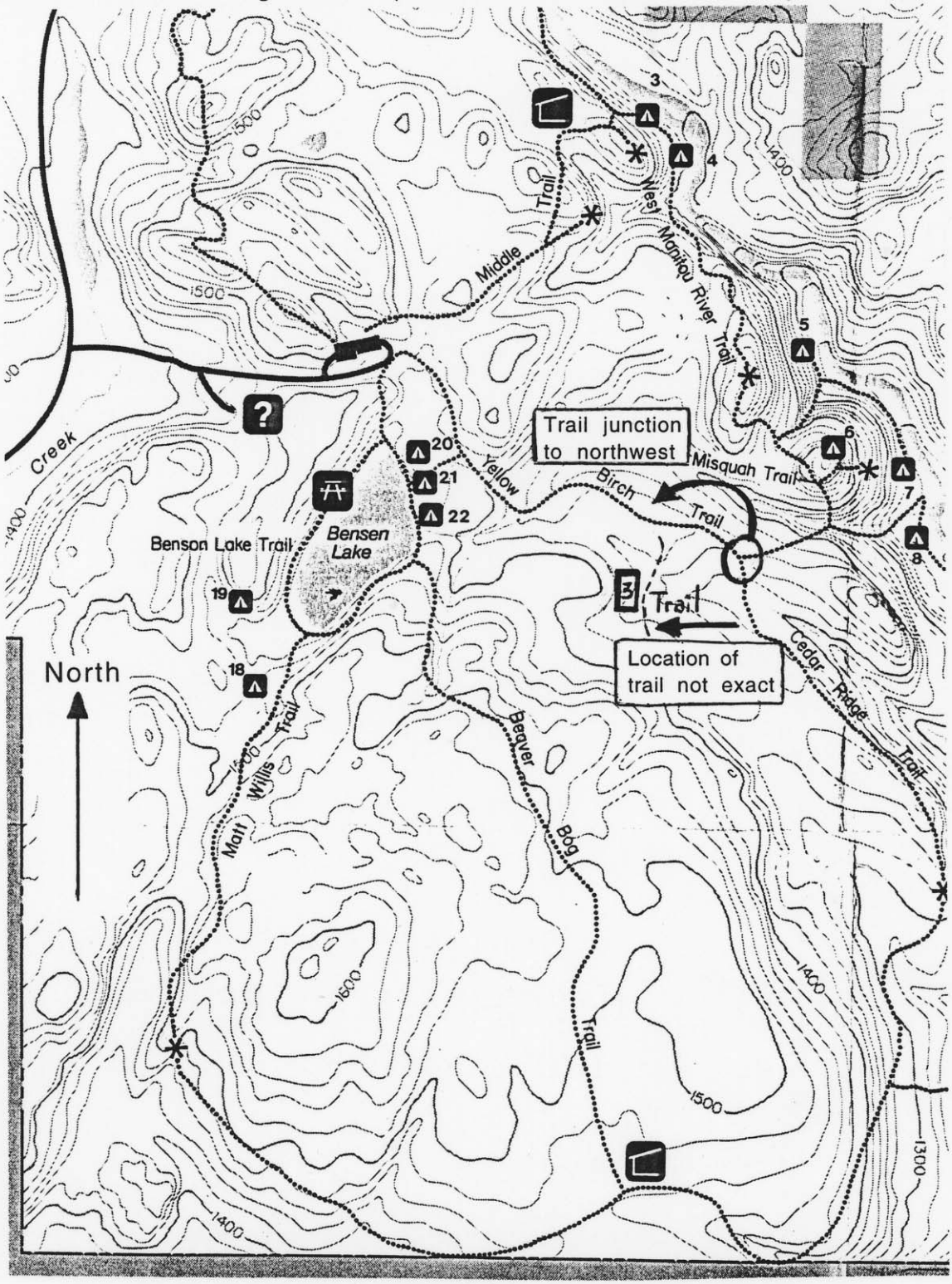

Figure 13. Location of old-growth northern hardwood-conifer plot 3 in NE1/4 of SE1/4 of SW1/4 of SW1/4 of Section 28, T58N R6W. Plot is east of a hill (east of Bensen Lake), between two drainages.

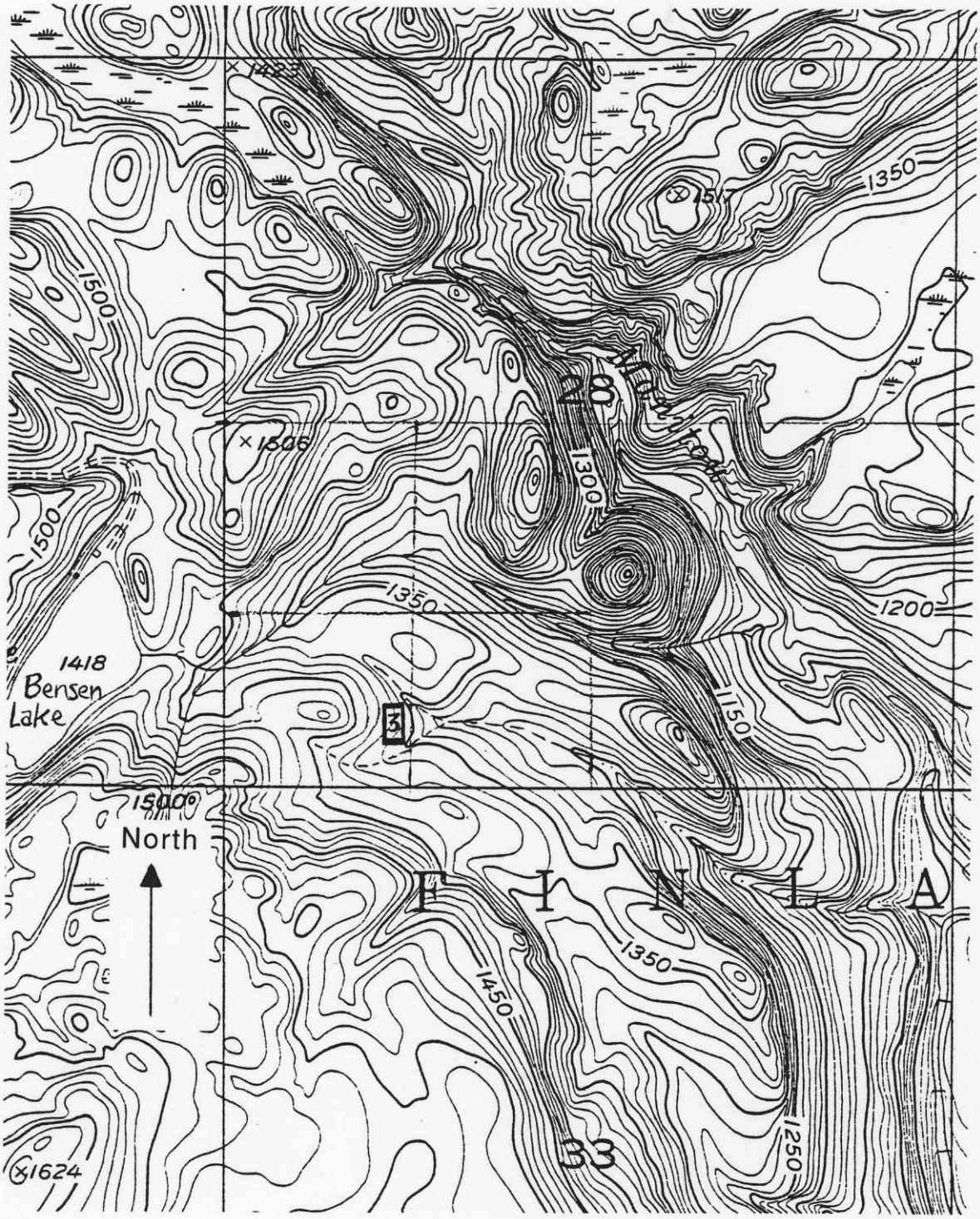

Figure 14. Detail of old-growth northern hardwood-conifer plot 3 showing orientation of plots and locations of stainless steel pipe. Plot was adjacent to two boardwalks of "Cedar Ridge Trail." An additional plot, marked with PVC pipe, was not used. The magnetic north declination used for this plot was 3° East.