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A study of basidiomycetes isolated from coarse woody debris and contiguous soil horizons in a mixed deciduous-conifer forest in New Hampshire, United States of America

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Ph.D. DISSERTATION

A STUDY OF BASIDIOMYCETES ISOLATED FROM COARSE WOODY DEBRIS AND CONTIGUOUS SOIL HORIZONS IN A MIXED DECIDUOUS-CONIFER FOREST IN NEW HAMPSHIRE, USA

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DISSERTATION

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DEDICATION

This dissertation is dedicated to my advisor, Dr. Robert O. Blanchard, and my husband, Dr. Kevin Thompson.

Dr. Blanchard's unending support during my MS and PhD degrees and his patience with my many life's up and downs has been greatly appreciated. He never pressured me and I am sure he wondered many times whether this dissertation would ever get done. I would NOT be who I am nor be where I am without Dr. Blanchard taking on the un-known student from out of state.

Kevin provided an environment where I could finish both of these degrees in order to pursue a new career in teaching.

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ABSTRACT

A STUDY OF BASIDIOMYCETES ISOLATED FROM COARSE WOODY DEBRIS AND CONTIGUOUS SOIL HORIZONS IN A MIXED DECIDUOUS-CONIFER FOREST IN NEW HAMPSHIRE, USA

by

Therese Ann Thompson
University of New Hampshire, September 2004

The wood decay fungus *Hypholoma sublateritium* (Fr.) Quel., links the mineral (BC & E) and organic (Oe) soil layers to decomposing coarse woody debris (CWD) in a northern hardwood forest. This link supports the possibility that energy stored in woody debris can facilitate the vertical transfer of elements and compounds within the soil profile. This potential transfer implies new pathways for biogeochemical cycling within forests. *H. sublateritium* was isolated from basidiocarp fruiting bodies (October 1997 & 1998), *Acer rubrum* L. bole wood (June 1999), and three soil horizons (October 1999 and October 2000) from one of six research sites in the Bartlett Experimental Forest, New Hampshire. The dominant tree species at these research sites, determined by basal area, were *Acer rubrum* , *Fagus grandifolia* Ehrh., and *Betula papyrifera*

Marshall. The soil type was Monadnock fine sandy loam, which is very stony, with a 3-8 percent slope.

Fungi were isolated from basidiocarp fruiting bodies, bole wood, and soils using 1) selective media containing two fungicides (benomyl and dichloran), three antibiotics (chlortetracycline-HCl, streptomycin sulfate, and penicillin G), selective culture methods for isolating from soil, 2) French square jar wood baits, and 3) soil pit wood baits. Media containing lignin and guaiacol (LGBDA and LGBA) were most effective for isolation of basidiomycetes from organic soil, whereas a malt-yeast medium (MYBDA) was most effective for isolation from mineral soil; all three media were equally effective for isolation from bole wood or fruiting bodies. Fungal isolates were differentiated by texture, color, and microscopic characteristics, and identified by sequencing their nuclear ribosomal DNA (nrDNA) in the ITS1-5.8S-ITS2 region using the primers ITS1-4.

INTRODUCTION

Humans are having a negative effect on our forests. Many areas of North America and Europe have experienced forest decline caused by atmospheric acid inputs that have increased aluminum availability and as a result lowered the availability of calcium (Shortle and Smith 1988, Allen et al. 1997). These forests are in decline as a result of decreased growth and mortality. Clear-cutting of forests drastically changes the soil environment (Chen et al. 1992) and causes dramatic seasonal temperature changes at the forest floor (Marra and Edmonds 1996) when compared to interior natural forests.

Over the past 20 years a major paradigm shift has occurred in commercial forest management after the realization that forest practices intended to protect wildlife and fish habitat were actually having the opposite effect (Hicks & Stabins 2002). Forest management practices do not allow trees to die, fall to the ground, and decay. Timber harvesting affects the soil organic matter, which in turn impacts the chemical, biological, and physical properties of the soil (Jurgensen et al. 1997). Traditional forest management significantly reduces the amount of coarse woody debris (CWD) in a forest (Harmon and Hua 1991). Logging operations may increase biomass at the forest floor in the short term (Harmon et al. 1986), but with the reduction of standing biomass by logging, there will be a reduction in the future potential for CWD on the forest floor (Hodge and Peterken 1998).

Clear-cutting adversely affects the ectomycorrhizal (EM) fungal community through the reduction of species richness (Byrd et al. 2000). As present management practices in the forests decrease the diversity of fungal species primarily responsible for decay (Niemela et al. 1995), organic matter reserves in the soil necessary for maintaining forest health and soil productivity are being compromised (Everett et al. 1994). If replenishment of forest soils also depends upon the translocation of elements (essential plant nutrients) by fungi from the mineral soil to CWD, then this process must be studied.

The forest ecosystem is an association of plants, animals, and microorganisms that is influenced by abiotic environmental factors such as soil and climate. During decomposition, organic substances from dead material (plant and animal) are converted from the organic to the inorganic elemental form, releasing minerals, thereby making these nutrients available for uptake by plants. This completes or creates a closed nutrient cycling system in the forest ecosystem.

The total number of vascular plant species is estimated to be 500,000, with about 50% already described (Stork 1997). However, the total number of fungal species is estimated to be 1.5 million (Stork 1997, Hawksworth 2001), with only about 10% described (Stork 1997). As fungi are intimately involved in nutrient cycling, more work needs to be done to identify species within the fungal community to further understand the complexity of the forest ecosystem.

When considering forest conservation, Ehrlich (1988) and Franklin (1988), have argued for habitat preservation in contrast to the individual endangered

species approach. Describing a forested plant community not only involves the description of the herbaceous, shrub, and tree layers, it also involves the description of the fungal community. The study of plant communities includes synecology, which provides relationships in a habitat. A habitat is defined to include everything relating to the ecological factors in a geographic locality, as well as external factors that influence plants (Nichols 1923). These external factors include biotic factors (influences by microorganisms in the soil and in the decay of dead organic matter) and abiotic factors (mineral content). Fungi are the major microorganisms involved in these processes.

Fungi have many vital roles in the forest ecosystem and every living plant is dependent on fungi either as mycorrhizae in the uptake of nutrients or to provide nutrients through the decay of plant and animal detritus. Fungi are involved in 1) decomposition, 2) elemental storage, transport, and release, 3) mutualistic symbioses, and 4) weathering (Christensen 1989, Harley 1971, Miller 1995, Waksman 1944). Following the decay of dead organic matter, fungi are able to recycle nutrients in the forest by immobilization and accumulation of elements in their fungal tissue, and by mineralization of simple inorganic compounds that are then released into the soil (Dighton 1997). To further understand these roles in the forested ecosystem, identification of the fungal community is a necessary first step.

About 95% of above ground biomass in a temperate forests is wood (Rodin and Basilevic 1967). The greatest volume of CWD is found in the northwestern United States, with estimates being 6-200 t/ha (Grier 1978).

Studies in the Pacific Northwest show estimates of more than 200 t/ha in temperate old growth coniferous forests (Franklin and Waring 1980) and 4-12 t/ha in deciduous forests (Harmon et al. 1986). Forest ecologists met at the Harvard Forest in 1994 to discuss the definition of an old growth forest and what factors are associated with older stands. The volume of CWD in the forest was considered an important ecosystem attribute of an old growth forest (Hunter and White 1997). CWD includes dead branches, whole fallen trees, standing dead trees and stumps (Woldendorp, et al. 2002). CWD on the forest floor, defined as fallen limbs and tree boles having a diameter greater than 10 cm (Harmon, et al. 1986), is a major contributor to habitat structure as nutrient pools (Huston 1993). It is also a nutrient source for fungi, bacteria, invertebrates, and vertebrates.

The decay of CWD occurs in stages and involves interactions between a variety of organisms. These stages include colonization/conditioning, channelization, and successional colonization. Organisms involved in these stages include: 1) colonization/conditioning (zygomycete fungi, mold fungi, bacteria, and invertebrates [diplopods]), 2) channelization (soft-rot fungi and invertebrates [beetles, carpenter ants, and termites]), and 3) successional colonization (brown rot and white rot fungi). Brown rot and white rot fungi are climax organisms that break down the cellulose and lignin found in wood.

Successional replacement and interactions between fungal species have been studied in-vitro (Holmer and Stenlid 1997, Holmer et al. 1997, Rayner and Boddy 1988, Tsuneda and Thorn 1995) and in-vivo (Howard and Robinson 1995, Niemela et al. 1995). Niemela et al. (1995) observed fungal fruiting bodies in a

study of fungal successors and their predecessors varied from tree to tree species. In these studies, progressive changes in species composition occurred over time. Further, Miller (1995) observed seasonality shifts in the appearance of macro-fungal fruiting bodies. In temperate forests, species were more diversified in Spring than in Fall. Moisture windows (after the onset of rainfall) in the forest environment can regulate fungal community activity (Sinsabaugh et al. 1991). Along a moisture gradient, species composition of the soil fungal communities was influenced by the level of calcium found in the litter (Christensen 1969). Harmon and Sexton (1995) concluded that moisture strongly affects decomposition. Gilbertson (1980) concluded that the most critical factors for the development of wood decay fungi were precipitation and temperature. Therefore, comprehensive retrieval of fungal fruiting bodies, soil fungi, and wood fungi will require time-course collections from spring and into fall.

Fungi have an extended hyphal network and are able to translocate elements, and spatially redistribute these elements throughout the forest from one resource into another (Cairney 1992, Jennings 1990). Fungal cord systems can extend over 50 meters (Thompson and Boddy 1983) and can thus redistribute and forage for nutrients over long distances. Fungi also act as accumulators of nutrients (Harley 1971) and are "inherently greedy " (Boddy and Watkinson 1995 p. S1380). Nitrogen and phosphorus concentrations increase in wood during decomposition, as carbon is lost as CO^2 (Boddy and Watkinson 1995). After correcting for carbon loss, increases of base cations were detected in red spruce wood decayed in-vitro (Ostrofsky et al. 1997). Harley (1971)

pointed out that the ability of fungi to accumulate nutrients is important because essential nutrients used as food by other soil organisms is locked up in the fungal network and is their by prevented from leaching. Wood decay basidiomycete fruiting bodies result in a release of nutrients from wood to other organisms. Harley (1971) also stated that fungal fruiting bodies provide a source of food and nesting place for species of Diptera and Coleoptera.

Decay is essential to a healthy forest, as it eventually releases nutrients bound up in plant tissues. CWD has a greater impact on biodiversity than any other manageable property of a forest (Huston 1993). The rate of decomposition of CWD is determined by environmental factors such as rainfall, temperature, soil pH, species of microorganisms, plant community, and the size of the CWD (Harmon et al. 1986, Harmon and Hua 1991, Hutson and Veitch 1985). Decomposition of organic matter is greater in the tropics due to the increased temperature and moisture in the forest. In North American forests, the time required for decomposition of 99% of the CWD found on the forest floor may exceed 100 years (Foster and Lang 1982). Wood decay basidiomycete fungi are the primary organisms involved in decomposition. These fungi rarely figure in lists of fungi isolated from litter or soil, even when their fruit bodies are abundant on and around the bole wood, and we still know little about their involvement in mineral cycling. The stored chemical energy (minerals) in the wood is released into the forest floor and soil during decomposition. Therefore, we must first identify the fungi involved in this process and further our knowledge of nutrient cycling by decay fungi. With this extended understanding, forest conservation

can be advanced by the development and implementation of more effective forest management practices to enhance the health of the forest ecosystem.

As far as is known, no studies have been done to show the similarities or differences in basidiomycete flora in vertical transects through felled logs, organic soil, and mineral soil at the same site. The primary goal of this research was to determine if a hyphal bridge exists to transport nutrients from the mineral soil into the decaying bole CWD and is thereby involved in nutrient cycling on the forest floor. This goal required the selective isolation of wood-decay basidiomycete fungi from contiguous soil horizons, from the mineral soil up to and including the organic soil, and previously felled logs at a given site. Molecular methods were used to determine if some of these fungal isolates were the same species. To further understand the research sites, the plant community composition was cataloged and the soil horizons were classified.

Objectives

1. To selectively isolate basidiomycetes from felled logs and contiguous soil horizons
2. To employ traditional and molecular techniques to identify to species, or place in like groups, basidiomycetes isolated in Objective 1
3. To describe the forest community
4. To classify the soils at each research site.

CHAPTER I

THE FOREST COMMUNITY

Introduction

A description of the plant community may be important in tying together relationships between the wood decay fungal community and the environmental factors of the plant community. For example, Grier and Logan (1977) found it helpful to describe soil types and plant communities in their study while determining biomass of old growth forests in Oregon. Parkinson et al. (1971), described methods that should be used when studying the ecology of soil microorganisms. Factors considered important were soil characteristics such as chemical analysis, pH, temperature, and descriptions of the plant community. In his examination of plant communities in the Bartlett Experimental Forest, Leak (1978, 1982) showed a correlation between plant species composition and the soil parent material. On the other hand, Widden (1979) studied the relationship between fungal communities and their environment in four forest sites. He could not attribute the differences in the soil fungal populations due solely to vegetation type; rather these differences were more influenced by their soil properties and nutrients.

Deforestation in temperate regions equals or exceeds that in tropical regions because of the conversion of the use of the land for pasture, crops, and

timber (Armesto et al. 2001). In Sweden and Great Britain, where forestry has been practiced for many years, the conversion of hardwood to coniferous forests has resulted in a large number of species being listed as endangered. In eastern forests, excessive leaching of calcium from soils has occurred due to acid rain. In addition to the loss from leaching, the removal of above ground biomass (whole-tree harvest) doubles the net loss of calcium (Federer et al. 1989). The combination of these two removal mechanisms could be detrimental to the health of the forest in only 120 years. Federer et al. (1989) did not see as severe a depletion of magnesium and potassium in eastern US forests. In addition to increased leaching of calcium from forest soil, acid rain can mobilize naturally occurring aluminum in forest soils. Aluminum interferes with the uptake of calcium by trees and the storage of calcium in forest soils. Decline and mortality of red spruce (*Picea rubens*) Sarg. was linked to acid rain through an aluminum-induced calcium deficiency syndrome (Shortle and Smith 1988). It has been suggested by Attiwill and Adams (1993) that with the absence of forest decline in the southern hemisphere, studies of long-term trends in nutrient cycling, as opposed to short-term measurements of nutrient cycling, should be a priority.

Graham and Cromack (1982) found it helpful to describe their research sites by indicating the tree size by species. Allen et al. (2000) visually estimated the percent canopy cover above each research log and reported it as an important factor in the diversity of fungi on decaying mountain beech (*Nothofagus solandri* var. *cliffortioides*) in New Zealand. Reiners (1992) saw forest

compositional changes and suppression of plant species after deforestation experiments.

North American eastern deciduous forests are composed of deciduous woody angiosperms and include some evergreen gymnosperms such as species of *Pinus* and *Tsuga*. This type of forest has an understory that is diverse with a rich assemblage of spring ephemeral herbs that are perennials. The changes in the seasonal environment found in these forests include plant survival strategies of avoidance and tolerance of stresses associated with a closed canopy in the Summer. Spring ephemeral herbs complete their annual growth cycle within a few weeks of early Spring before canopy leaf out, taking advantage of the sunlight reaching the forest floor. These plants go dormant to avoid the impact of shading in the Summer months. Hardwoods associated with this forest type include species of *Acer, Fagus, Quercus,* and *Betula*. This mesic canopy has a co-dominant tree composition and as a late-successional (climax) community creates the border zone (ecotone) between temperate deciduous and boreal coniferous forests.

Research Sites

Six research sites were selected in the Bartlett Experimental Forest (BEF) located in the White Mountain National Forest near Bartlett, New Hampshire in Carroll County (Fig. 1). Bartlett Experimental Forest is a U.S. Forest Service long-term ecological and forest management research site, used as a field laboratory for research on the growth, culture, and composition of a northern mixed hardwood-coniferous forest and the study of the ecology and management

of northern forest ecosystems. This 1052 ha forest receives an average of 127 cm of precipitation distributed throughout the year. Commercial logging began in the forest about 1870 and research activities at the Experimental Forest began in 1931.

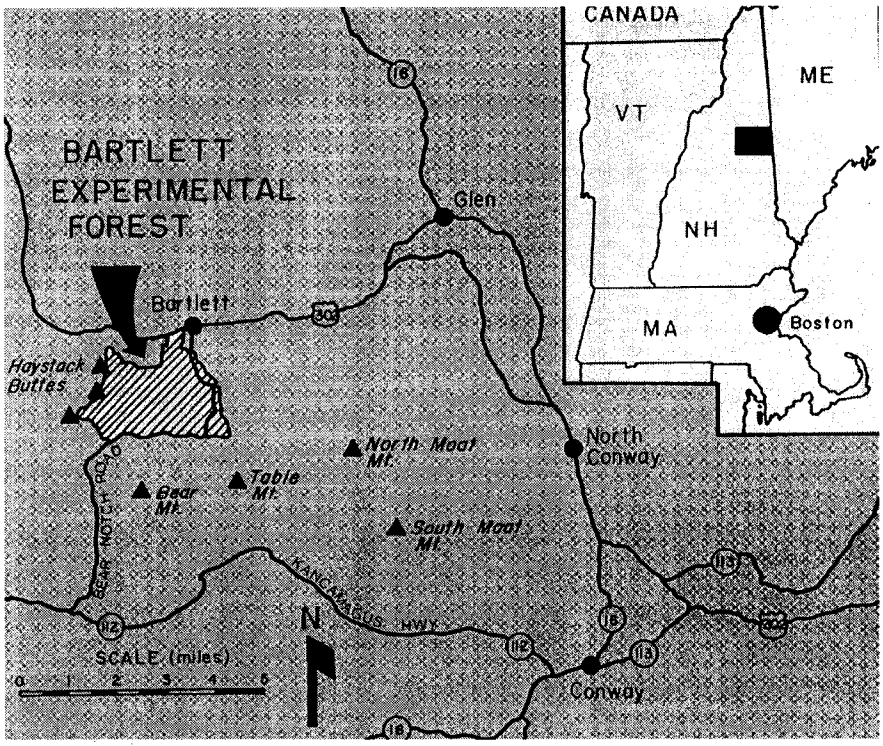


Fig. 1. Location of the Bartlett Experimental Forest in New Hampshire. (BEF, USDA pamphlet NE-INF-78-88)

Bartlett Experimental Forest is located at longitude 44° 03' 23.798" N, 71° 17' 47.396" W, with an elevation of 213-883 (meters) above sea level, just 23 km south of Mt. Washington (Fig. 2). The mean annual temperature is 7.23°C, mean annual precipitation is 1200-1400 mm, the percent mean annual precipitation as snow is 30% and the mean number of frost-free days is 120-140 (BEF, USDA pamphlet). The vegetation is dominated by northern hardwoods (*Fagus grandifolia, Acer saccharum* L., *Acer rubrum, Betula alleghaniensis* Britt., *Betula*

paprifera) with stands of mixed and pure conifers at both low and high elevations (Major species: *Tsuga canadensis* (L.) Carriere, *Picea rubens* Sarg.; Minor species: *Pinus strobus* L., *Abies balsamea* (L.) Mill.). By total area, this forest contains 69.4% northern hardwood, 13.2% red spruce-mix, and 17.4% hemlock-mix. The dominant soil is coarse-loam over sandy or sandy skeletal, mixed, frigid, Typic Haplorthod (Monadnock series). The geology bedrock is granitic with 0-2 meters of till at the surface.

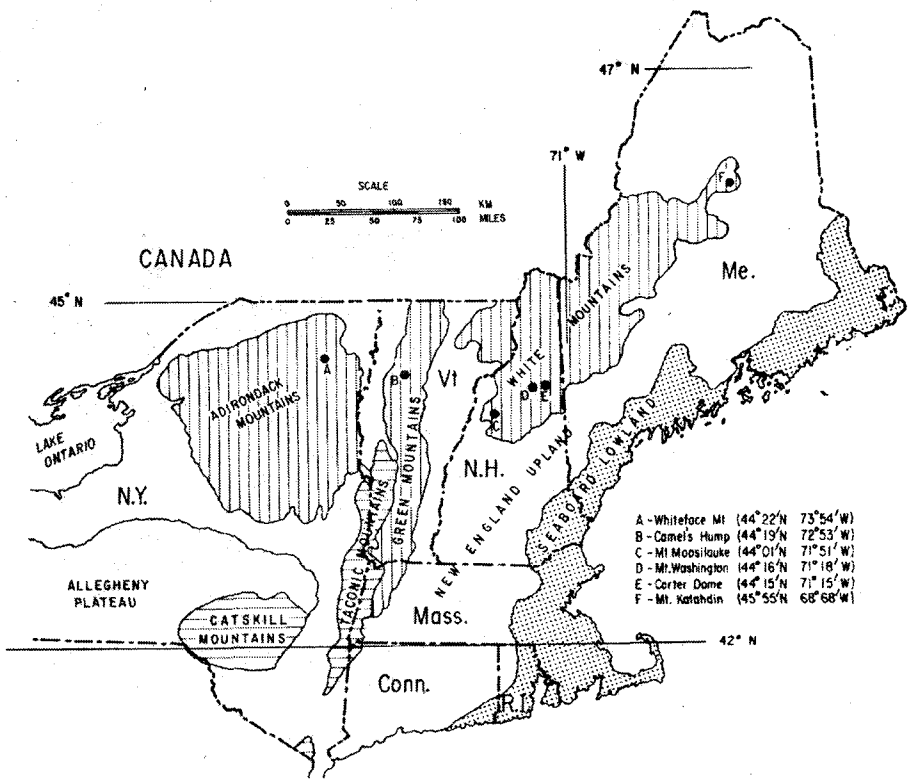


Fig. 2. Map of northeastern United States illustrating the major mountain Ranges (Reiners & Lang 1979).

The research sites (Nos. 19, 21, 22, 26, 28, & 29) are located at an elevation of 124 meters, mean above sea level in compartment number 9 off Bear Notch Road (Fig. 3). *Acer rubrum* trees were felled and numbered by

scientists from the USDA Forest Service in 1990 and left undisturbed until 1997. The soils at these research sites are spodosols, developed on glacial till derived from granite and gneiss. The black humus layer of the soil is nutritionally rich for plant growth, while lower mineral soil layers are nutritionally deficient. In many places the soil mantle is very shallow; boulders and rocks are common.

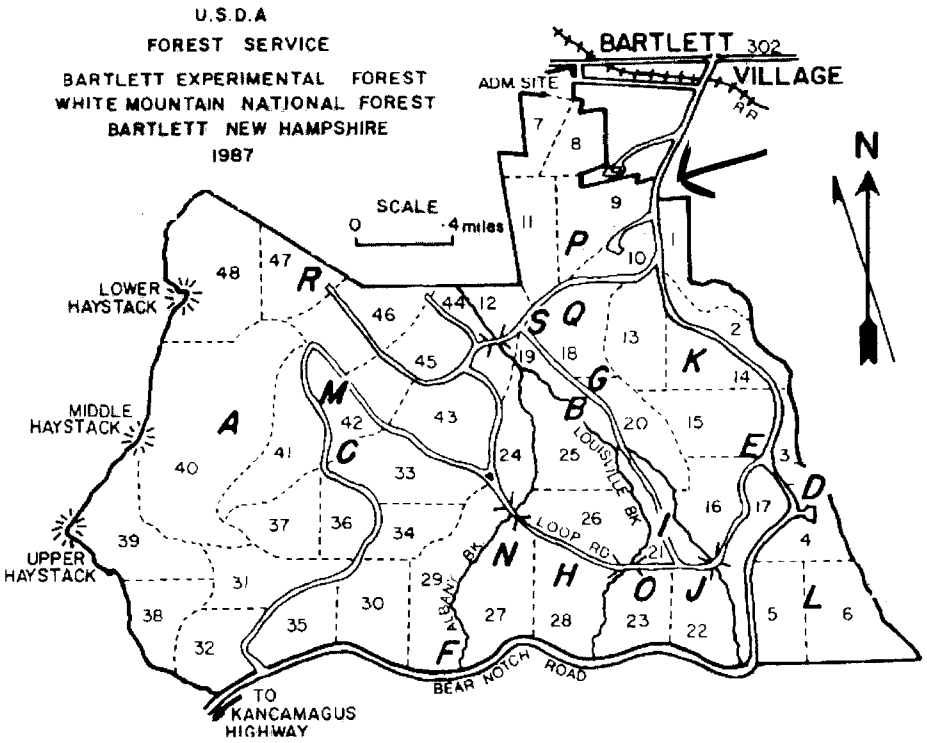


Fig. 3. Bartlett Experimental Forest numbered compartments/study areas. The arrow points at compartment 9 where the six research sites are located. (BEF, USDA pamphlet NE-INF-78-88)

Figure 4 is the USGS topographical map of Bartlett Experimental Forest. Included are the Bartlett Experimental Forest compartments, roads near the six research sites, and part of the town of Bartlett. This map was developed from the GRANIT digital raster graphical database (University of New Hampshire

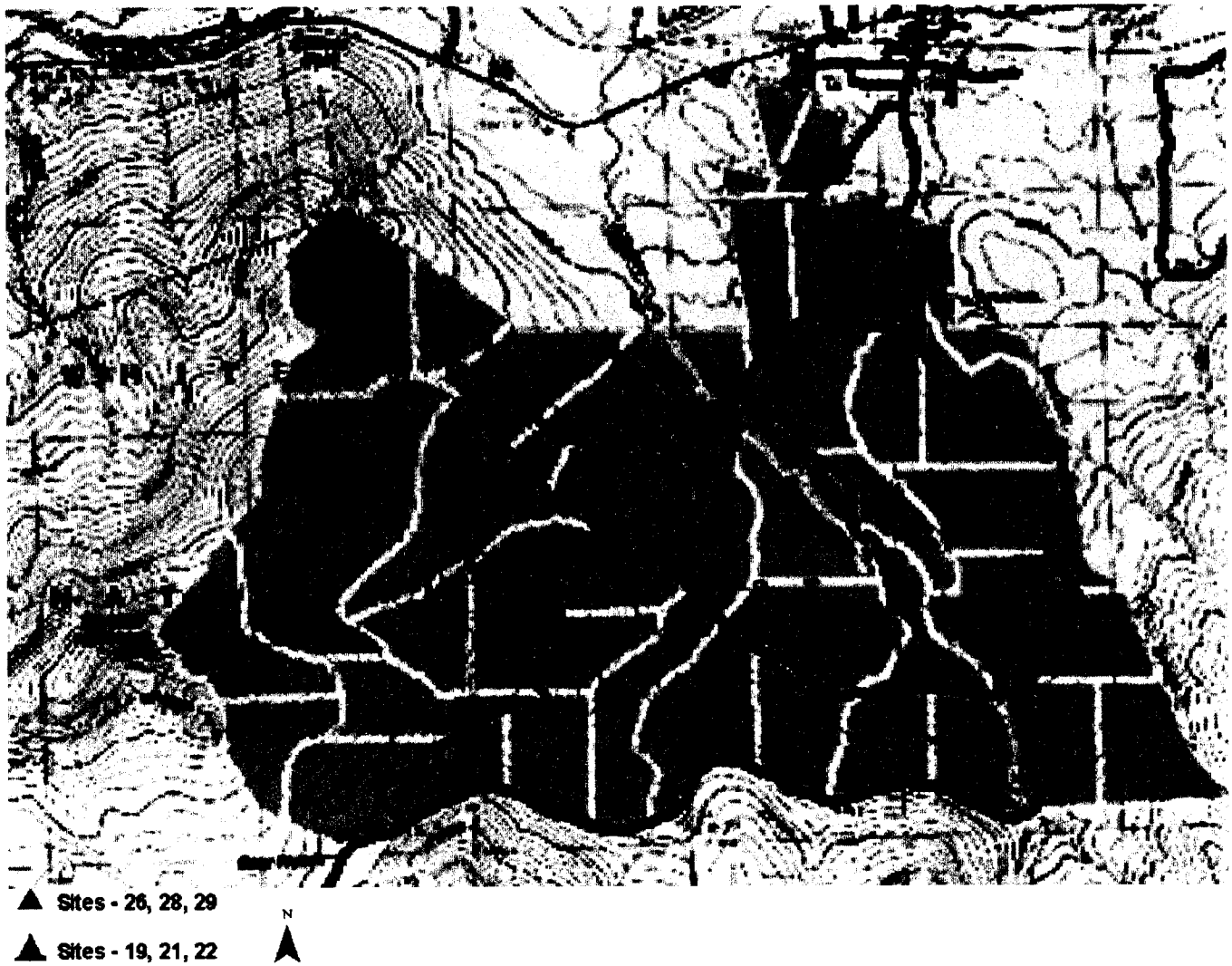


Fig. 4. USGS topographical map of Bartlett Experimental Forest showing compartments and roads (black). The research areas are located in a gently sloping region.

complex systems research center, Morse Hall) for Bartlett NH, Quad 65, publication date 1987, U.S. Geological Survey, 7.5 minute Quadrangle Series. The research sites are on gently sloping terrain as compared to the steep terrain elsewhere in BEF and surrounding White Mountain National Forest as shown by the contour lines on this map. The roads in BEF and close proximity to the town of Bartlett made these research sites easily accessible on foot.

Forest stands in BEF are even-aged and common names of individual trees have been catalogued and are listed in Table 1 (BEF USDA pamphlet).

Table 1. Tree species (% BA) found in Bartlett Experimental Forest.

Species	% of basal area (BA)
Beech	29
Red Maple	22
Yellow Birch	15
Eastern Hemlock	12
Sugar Maple	5
Paper Birch	9
White Ash	6
Red Spruce	1
Others	1

(Others include: aspen, pin cherry, striped maple, red oak, black ash, black cherry, basswood, hophornbeam, balsam fir, white pine, and red pine)

Materials and Methods

Surveys of the plant community at the research sites were conducted in Oct. 1997; Oct. 1998; May, June, Aug., and Oct. 2001; and May, June, and Oct. 2002. The forest community at the research sites is described graphically (Fig.'s 7 and 8) as all standing trees by size (> 8 cm) diameter at breast height (DBH) and by species within a tenth of a hectare circular plot centered on the previously felled research boles. The red maple research boles # 26, 28, & 29 (Area 1) and

boles # 19, 21, & 22 (Area 2) are in the center of these illustrations. The location of these research sites is indicated on a BEF map insert (Figs. 7 and 8). The basal area, $BA = \pi (0.5\ DBH)^2$, was calculated for each tree in the forest canopy within each research area. The shrub and herbaceous layers of the forest were also surveyed within the tenth of a hectare plots.

Results

Photographs of the forest floors of research Areas 1 and 2 are shown in Figs. 5 and 6, respectively. Research sites # 19, 21, & 22 (Area 2) had a thicker forest shrub and herbaceous layer than research site's # 26, 28, 29 (Area 1) and contained more trees, shrubs, and herbaceous plants, and thus less sunlight reached the forest floor at this site.

Fig. 5. The forest community of sites # 26, 28, & 29 (Area 1).

Fig. 6. The forest community of sites # 19, 21, & 22 (Area 2).

The location, species, and DBH of all trees found in research Areas 1 and 2 are shown in Figs. 7 and 8, respectively. The DBH in centimeters of the tree species is illustrated by size (less than 12 cm, 12-23 cm, 23-33 cm, > 33cm) and species. The red maple research tree stumps are numbered and located on the maps.

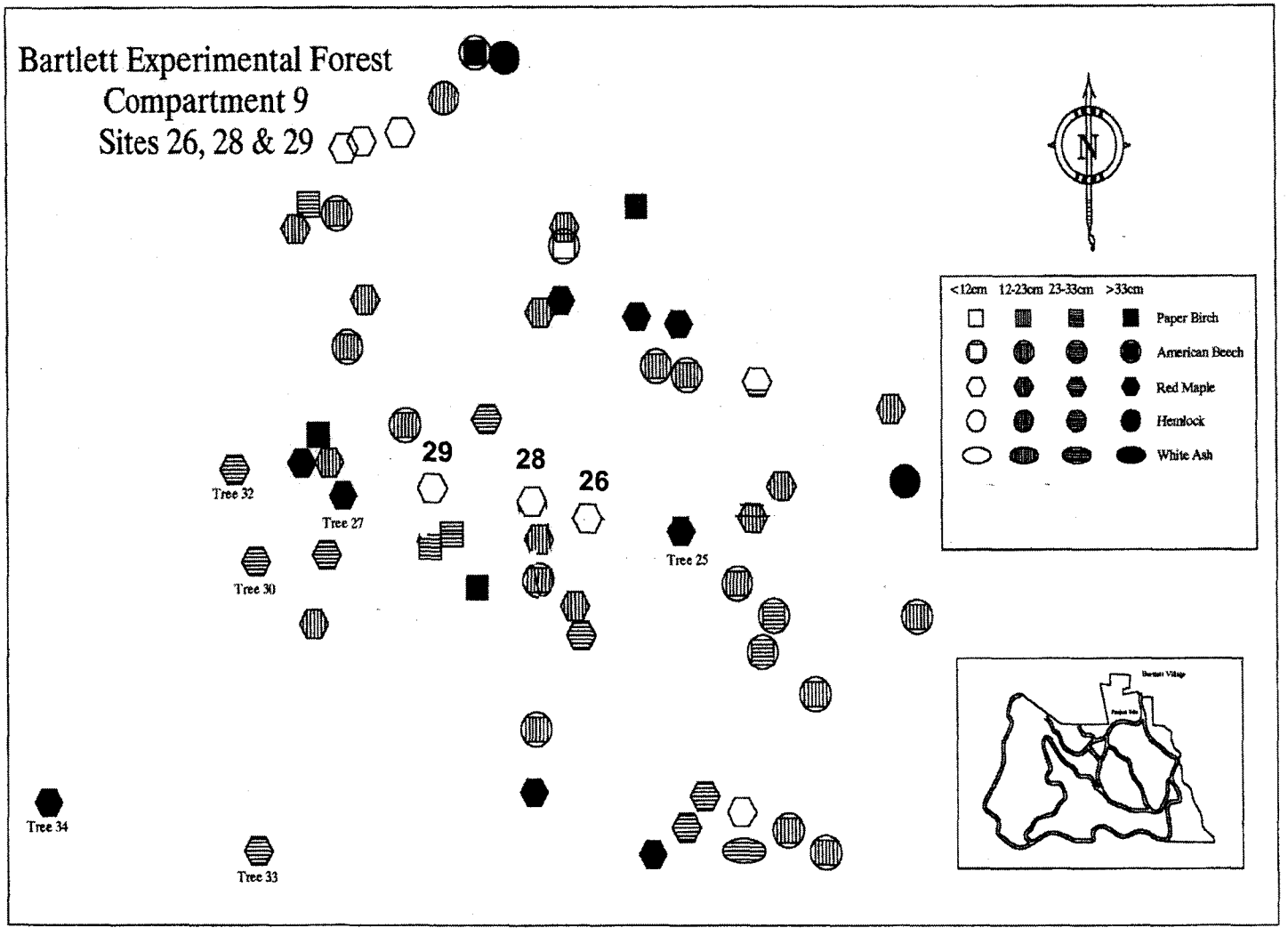


Fig. 7. Map of trees for sites # 26, 28, & 29 (Area 1). Legend indicates DBH (cm).

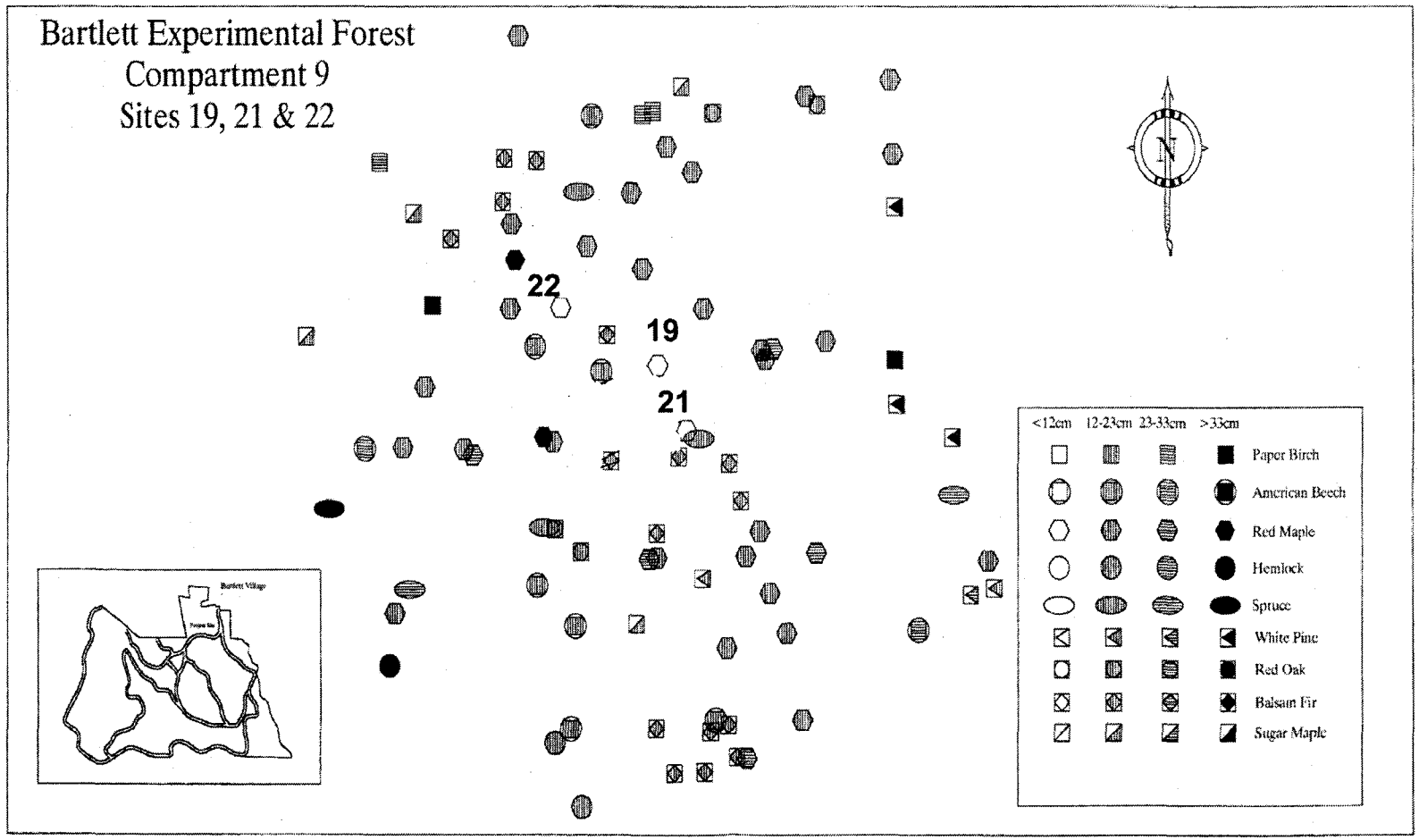


Fig. 8. Map of trees for sites # 19, 21, & 22 (Area 2). Legend indicates DBH (cm).

Measurements of DBH (cm) for each tree per research area are listed in Appendix 1 and 2. The total basal area (m^2/0.1 ha) and the number of trees per species are shown in Table 2.

Table 2. Basal area (m^2/0.1 ha) and the number of trees by species for each research area.

	Area 1		Area 2	
	BA	# of trees	BA	# of trees
Hardwoods				
Acer rubrum	2.2	33	1.4	38
Betula papyrifera	0.6	6	0.4	3
Fagus grandifolia	0.4	16	0.7	13
Fraxinus americana	< 0.1	1		
Quercus rubra			0.1	4
subtotal	3.2	56	2.6	58
Conifers				
Tsuga canadensis	0.5	3	0.3	3
Abies balsamea			0.5	27
Picea rubens			0.6	8
Acer saccharum			0.2	6
Pinus strobus			0.6	6
subtotal	0.5	3	2.2	50
Total	3.7	59	4.8	108

The data show that the total basal area of conifers is four times greater in Area 2 than in Area 1, which is not surprising since only one species of conifer is represented in Area 1. The basal area and number of deciduous trees are basically similar in the two research areas.

The diameter of trees can be an indicator of age, although not for the case of red spruce or American beech because of suppressed juvenile rings (personal conversation with Kevin T. Smith). The number of trees by size (DBH) of all

trees by research area is charted in Fig. 9. In Area 2, the majority of trees 83% (90 out of 108) are 15 – 30 cm in diameter. The data indicates that Area 2 is more of an even aged forest than Area 1.

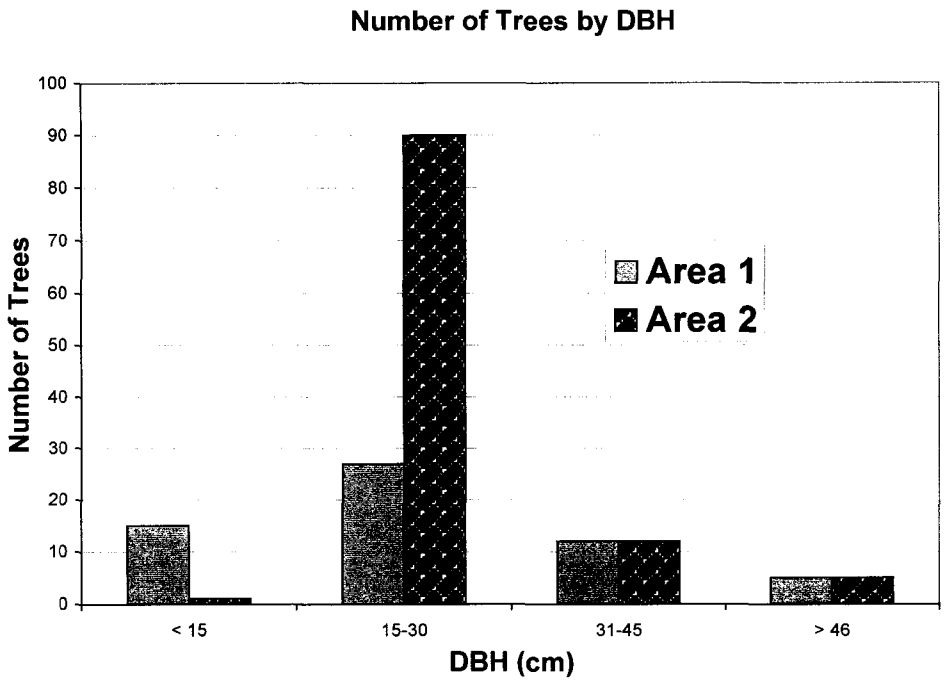
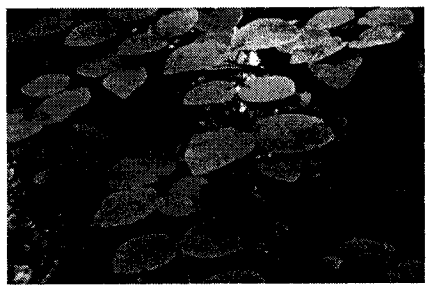


Fig. 9. The number of trees by size in centimeters (DBH) of the two research areas (Area 1 contains sites # 26, 28, & 29; Area 2 contains sites # 19, 21, & 22).

A survey of the tree saplings, shrubs and herbs at research site is listed in Appendix 3. The total vascular plant richness in the understory was 35 species. Both research areas had 16 plant species in common, with 13 additional species found only in Area 1 and six additional species found only in Area 2. Therefore, the understory plant community is more diverse in Area 1 than in Area 2.

Representative plants found in the understory of each of the research areas were photographed (Fig. 10).

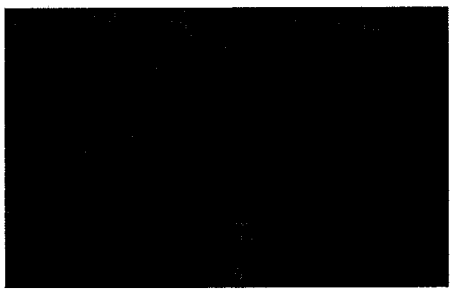
Goodyera tesselata Lodd.



Viburnum alnifolium Michx.

Acer pensylvanicum L.

Trillium undulatum Willd.



Cornus canadensis L.

Mitchella repens L.

Cypripedium acaule Aiton

Maianthemum canadense Desf.

Fig. 10. Representative plants in the understory found at the research sites.

Discussion

The DBH data of all trees in Figure 9 of research Area 2, agree with the USDA pamphlet NE-INF-78-88 describing the Bartlett Experimental Forest as an even-aged forest with 83% of all the trees 15 – 30 cm in diameter. Area 1 (sites # 26, 28, & 29) had a total of 59 mature trees (> 8 cm DBH) in 5 species and Area 2 (sites # 19, 21, & 22) had 108 mature trees (> 8 cm DBH) in nine species within the 0.1 ha plots (Table 2). Therefore, with respect to tree species, Area 2 is more diverse than Area 1. Tree saplings of *Abies balsamea* were found in Area 1, even though no mature trees of this species were found. This tree species is very tolerant of shade. Area 1 has almost twice the basal area of *Tsuga canadensis* as in Area 2, which may be limiting the growth and reproduction of *Abies* sp. even though it is shade tolerant. Area 2 is more diverse in tree species probably due to the fact that it contains almost half the basal area of *Tsuga canadensis*.

The species of understory vascular plants (tree saplings, shrubs, and herbs), on the other hand, were more diverse in Area 1 than in Area 2 (Appendix 3), even though the density of plants is greater in Area 2 than in Area 1 (Figs. 5 and 6). Both research areas had 16 plant species in common, with 14 additional species found only in Area 1 and six additional species found only in Area 2. Area 1 understory should be less diverse due to the fact that the basal area of *Tsuga canadensis* is almost twice that of Area 2. It has been shown that this tree species inhibits growth in the understory by forming very dense canopies, monopolizing resources, limiting growth, and reproduction of

The understory vascular plants (Woods 1984), and altering the pH of the soil and soil moisture (Beatty 1984) by forming the densest shade of any forest tree thereby making its own microenvironment, dry and highly acidic because of needle litter. Few plants can grow under hemlock, although *Goodyera* and *Oxalis* are two species that can and are found in Area 1. *Tsuga* sp. is the third most dominant tree species in Area 1 and apparently is not having a negative effect on the understory species.

Tsuga canadensis is considered by ecologists as a climax species in succession, while some ecologist consider *Pinus strobus* a climax species. *Pinus* sp. seedlings are moderately shade tolerant, and *Tsuga* sp. is the most shade tolerant conifer species. *Betula papyrifera* is not shade tolerant but *Fagus grandifolia* is very shade tolerant and is associated with other climax forest species such as *Acer saccharum* and *Betula alleghaniensis*. *Quercus rubra* is intolerant of shade, which may explain the absence of this species in Area 1 when combined with the presence of *Tsuga* sp., the third most dominant tree species.

These research sites did not contain any non-native invasive plant species nor did they contain any Spring ephemerals. Amongst the flowering vascular plants, 83% (15 out of 18) are Spring bloomers, with *Goodyera* and *Oxalis* being Summer flowering plants and *Aster* being a Fall flowering plant species. Both research areas contained two shrub species, *Lonicera canadensis* and *Viburnum alnifolium*, both in the family Caprifoliaceae and which bloom in May-June. Ferns found in these research areas included, *Dryopteris carthusiana* in Area 1and

Pteridium aquilinum in Area 2. Two cryptograms, *Lycopodium obscurum* and *Huperzia lucidula*, were found in both research areas. These shrubs, ferns, and club mosses are commonly found in the understory of eastern deciduous forests.

CHAPTER II

FUNGAL CULTURE EVALUATION TECHNIQUES

Introduction

The key to successful fungal isolations from various substrates is the ability to isolate only the fungi of interest. In the case of this research, effective techniques and culture media for isolating basidiomyceteous fungi from basidiocarp, bole wood, and soil were confirmed by DNA sequencing.

Culture Media

Selective media for isolation and growth of basidiomycetes have been described (e.g., Rogers et al. 1989, Harrington and Wingfield 1995, Bednarz et al. 1997, Thompson 1998, Fischer and Wagner 1999, Coetzee et al. 2001, Baumgartner and Rizzo 2001, Huss et al. 2002). Most of these media contained fungicides and antibiotics that limited or prevented the growth of non-basidiomycetous fungi and bacteria.

Polyphenol Oxidase Testing

White rot fungi can break down lignin, whereas brown rot fungi cannot. Many species of wood decay are white rotters and are able to degrade lignin and carbohydrates (cellulose and hemicellulose). All wood decay fungi produce extracellular enzymes. The media used to detect the presence of oxidase, by

discoloration are gallic (GAA) and tannic (TAA) acid media, developed by Davidson et al. (1938).

Morphological Studies

Fries grouped all fungi based on macroscopic morphological characters (Fries 1821-1832). Later, with the invention of the microscope, basidiomycetes were separated from other fungi. Patouillard (1900) divided the basidiomycetes into two classes, heterobasidiomycetes and homobasidiomycetes, on the basis of basidium morphology and the mode of basidiospore germination. Talbot (1965) further divided basidiomycetes using basidial septation as their defining character. There are published keys to identify specific groups of basidiomycete fungi in culture (e.g., Nobles 1948 and 1965, Stalpers 1978, Nakasone 1990). Today, with molecular sequence data, mycologists can separate species where morphological characters are in conflict (e.g., Hibbett and Vilgalys 1993, Hibbett and Donoghue 1995, Bruns et al. 1998, Moncalvo et al. 2000 and 2002, Binder and Hibbett 2002).

Somatic Incompatibility

Most basidiomycete fungi are heterothallic with multiallelic mating type genes to prevent self-mating (self-sterile) as opposed to homothallic fungi that can self-mate (Casselton and Olesnicky 1998). A nonself interaction would be evident as a clearing (a zone of no growth), barrage (dense mycelium), or demarcation line (dark pigment) between the two isolates grown on the same petri dish. A self (non-reaction) is demonstrated by intermingling mycelium, which results in not being able to distinguish between the two culture isolates.

Somatic incompatibility (SI) testing, mating studies, or vegetative compatibility groups (VCG, as coined by McCallum et al. 2004) illustrate this self or nonself mechanism.

The overall mechanisms of SI have been studied: Petersen (1995 a and b) discussed mating studies and the species concept for systematists; Worrall (1997) examined basidiomycetes; Boddy (2000) discussed the combative interactions between wood-decaying basidiomycetes; and Boidin (1986) cautions mycologists in making conclusions based on mating test results.

Somatic incompatibility testing has been used by many researchers: Hallenberg (1984) compared Corticiaceae species from Europe and North America; Coates and Rayner (1985) describe the 'Bow-Tie' reaction between homokaryons of *Stereum hirsutum* (Willd.:Fr.) Gray; Wilson (1990 and 1991) compared dikaryotic-monokaryotic and dikaryotic pairings of *Echinodontium tinctorium* (Ellis and Everh.) Ellis and Everh; Petersen and Bermudes (1992) separated *Panellus stypticus* (Bull.) Karst. interbreeding populations from USSR, New Zealand, Japan and eastern North America; Hansen et al. (1993a and b) studied the genetic correlation in *Heterobasidion annosum* (Fr.) Bref.; Rizzo and May (1994) did haploid-diploid matings of *Armillaria ostoyae* Romagn.; Rajchenberg and Greslebin (1995) compared the taxonomy of Argentina polypores; Petersen (1995a) looked at the long distance mating phenomena of Agarics; Banik and Burdsall (1999) paired 81 isolates of *Laetiporus cincinnatus* and *L. sulphureus;* Burrill et al. (1999) compared *Armillaria* species and *Megacollybia platyphylla* (Per.) Kotl. & Pouzar; McCabe et al. (1999) studied the

nature of heterokaryosis of *Rhizoctonia solani* Kuhn; Kauserud and Schumacher (2003) looked at geographic populations of *Trichaptum abietinum* (Dicks.:Fr.) Ryv.; Nilsson and Hallenberg (2003) investigated the *Hypochnicium punctulatum* complex; and Otieno et al. (2003) characterized *Armillaria* isolates from tea.

There is disagreement on which media and methods to use with SI testing: 3% MA in the dark (Coates and Rayner 1981); MEA (Adaskaveg and Gilbertson 1987, Holmer et al. 1994, Hallenberg 1984, Rayner and Boddy 1988, Rizzo and May 1994, Burrill et al. 1999); N6:5 medium in the dark (Fries 1987); 'barrage agar' (May 1988); Hagem's agar (Hansen et al. 1993a and b); PDA (Murphy and Miller 1993); water agar (Tsuneda et al. 1997); PDA with gallic acid (Banik and Burdsall 1999); CE/CYM agar (Calvo-Bado et al. 2000); and V8 (McCallum et al. 2004) have all been used.

Molecular methods have been compared to SI testing. Banik and Burdsall (2000) used allozyme analysis to evaluate compatibility of pairings of single spore isolates of *Laetiporus sulphureus*; Holmer et al. (1994) found that the somatic incompatibility testing agreed with the sequence of the minisatellite M13 of *Phellinus tremulae* (Bond.) Bond. & Borisov; and Stenlid (1985) identified pure cultures of *Heterobasidion annosum* by three methods: somatic incompatibility, identification of mating factors, and isoenzyme patterns.

Somatic incompatibility testing was found inferior to randomly amplified polymorphic DNA (RAPD) markers for discriminating between genotypes in populations of *Suillus granulatus* (Fr.) Kuntze (Jacobson et al. 1993). Roy et al. (1997) studying different strains of *Phlebiopsis giganteus* (Fr.) Julich saw

concordance between somatic incompatibility and RAPD analyses. Congruency was found between somatic incompatibility testing and arbitrary primed PCR (AP-PCR) during genetic studies between populations of *Fomitopsis pinicola* (Sw.:Fr.) Karst. (Hogberg et al. 1999).

Molecular Testing

Molecular methods used by other researchers have included: random amplified polymorphic DNA (RAPD); restriction fragment length polymorphism (RFLP); amplified fragment length polymorphism (AFLP); sequencing; and microsatellite testing. There is no agreement as to which molecular method is the best.

Bruns et al. (1991) summarize molecular methods and their range of discrimination power in fungal systematics. If one is studying the phylogenetic relationships from the kingdom to genera level, the nuclear rDNA (nrDNA) genes should be sequenced; from the generic to species and into the population level, the internal transcribed spacer (ITS) regions of the fungal genome should be sequenced.

In a flow chart of molecular methods, Dowling et al. (1996) reported that RFLP with staining of the agarose restriction enzyme gels has a low to moderate sensitivity, a low cost, high efficiency, and assays at greater than 100 base pair (bp) fragment size, while microsatellite testing has a high sensitivity, a moderate cost, high efficiency, and assays of 100-600 bp fragment size.

Bridge (2002) also compared the resolution of different molecular marker methods. The RAPD method investigates the variable, generally nuclear and

sometimes repetitive DNA resolved for individual, sub-specific groups. Satellite primers detect the variable, generally nuclear, repetitive DNA resolved at the individual, sub-specific groups. The AFLP method reveals a subset of total genomic DNA, resolved at the individual, sub-specific group, of some closely related species. The ITS RFLP method demonstrates nrDNA variable spacers resolved at the closely related species, and some sub-specific groups. Sequencing the ITS region of the genome confirms nrDNA variable spacers resolved at some sub-specific groups, of closely related species.

AFLP differs from RFLP in that it uses polymerase chain reaction (PCR) amplification instead of Southern hybridization for detection of restriction fragments. PCR-RFLP detects DNA sequence variation by amplifying a target genomic region, digested by restriction enzymes, then separated by electrophoresis. Sakakibara et al. (2002) could not distinguish between fungal species of *Lactarius* and others with *Cortinarius* using PCR-RFLP alone. They concluded that sequencing was required to identify the fungal species. Some fungal species may be represented by more than one profile using PCR-RFLP, whereas another profile may represent several species (Glen et al. 2001). A survey of the diversity of soil fungi by PCR-RFLP of the ITS region, yielded only one basidiomycete, *Cryptococcus*, with most others being ascomycetes (Viaud et al. 2000). There was a strong correlation between RAPD and microsatellite primed-PCR data of *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & DeNot. in pistachios (Ma et al. 2001). Direct sequencing of PCR products allows opposite-strand sequence verification, and sequence data from different organisms can

easily be compared. RFLP testing does not allow one to verify the opposite-strand of the DNA nor can one easily compare these data to the data of other organisms (Hibbett 1992). Direct sequencing of PCR product is faster than cloning and it also reduces the danger of obtaining incorrect sequences due to misincorporations by *Taq* polymerase (McCabe 1990). PCR-RFLP's, intersequence simple repeats (ISSRs), and mating studies were used to compare geographic populations of *Trichaptum abietinum* (Kauserud and Schumacher 2003).

The nrDNA is a complex gene with one to several thousand copies of the tandem repeat units: intergenic spacer (IGS) - 18S - ITS1 - 5.8S - ITS2 - 28S. The 18S and 28S (S = Svedberg sedimentation coefficient) ribosomal DNA (rDNA) genes are used in studying broad phylogenetic differences in a broad range of organisms that evolve relatively slowly (Cullings and Vogler 1998) and can be used to investigate distantly related organisms (White et al. 1990). In contrast, the ITS and intergenic spacer (IGS) regions accumulate more mutations and are used to separate species within a genus and are used in the identification of fungi (White et al. 1990). The ITS regions are used in studying interspecies (between or among) and sometimes intraspecies (in or within) and evolve relatively quickly (Cullings and Vogler 1998). The IGS region is used in population studies. The 5.8S gene region is generally used only as an alignment tool. Hibbett and Vilgalys (1993) decided that the ITS-2 region was too variable for their study of phylogenetic relationships of the genus, *Lentinus.*

The ITS region is useful for molecular systematics at the species level, and within species because of a higher degree of variation than the ribosomal genes (large and small subunit). The ITS region is a multicopy site with highly conserved priming sites. The ITS non-coding regions in fungi are each 200-400 bases long (Garber et al. 1988). In fungi, the ITS regions (1 & 2) provide a good resolution to the genus level. The ITS regions have provided phylogenetically useful data at the species and generic levels (Hibbett et al. 1995, Yan et al. 1995, Harrington and Potter 1997). In angiosperms, the ITS-2 region has been shown to be more highly conserved than the ITS-1 region (Hershkovitz and Zimmer 1996, Mai and Coleman 1997). Nuclear small-subunit rDNA 18S is approximately 1.8 kilo bases (kb) long, and the nuclear large-subunit rDNA 28S is approximately 3.4 kb. Both the small and large subunit rDNA are highly conserved regions.

The conserved ribosomal DNA gene regions (18S, 5.8S, & 28S), the variable ITS regions (ITS1 & 2), and the placement of several primers used in this research and discussed in this dissertation are shown in Figure 11.

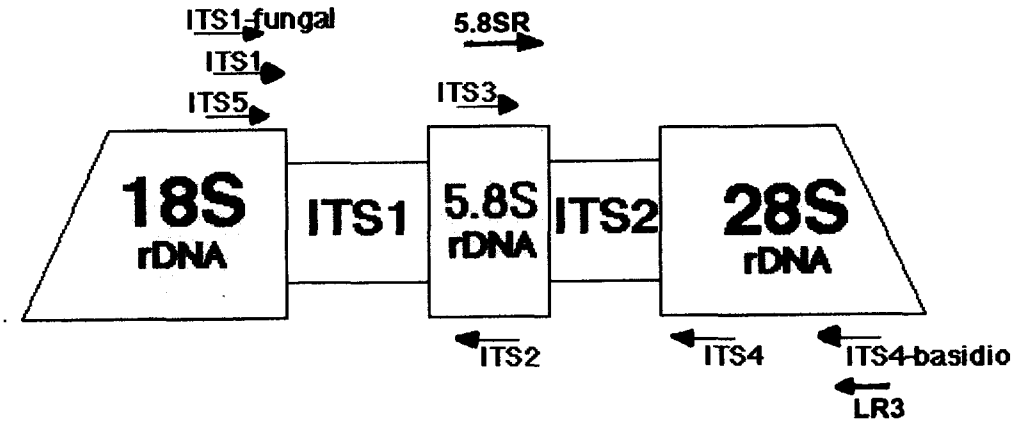


Fig. 11. Map of commonly used primers within & between the 18S and 28S ribosomal DNA genes. (http://plantbio.berkeley.edu/~bruns/)

The 5.8SR primer is located at the 34-51 base position in the 5.8S gene and the LR3 primer is located at the 654-638 base position in the large subunit rDNA gene (Vilgalys and Hester 1990) and aligns with the divergent D2 domain in that gene. The oligonucleotide primers 5.8SR and LR3 were used to amplify fungal rDNA in a study of *Cryptococcus* species. Smith and Sivasithamparam (2000) used the primer 5.8SR as one of the sequencing primers when studying species of *Ganoderma*. Fischer and Wagner (1999) used the primers 5.8SR and LR7 to amplify a fragment of nuclear DNA by PCR from herbarium material of European polypores and used RFLP analysis as an identification tool. Moncalvo et al. (2000) used 5.8SR and other primers to look at the phylogenetic relationships of agaric fungi. Moncalvo et al. (2002) has shown that the nuclear large subunit region to be most useful to infer phylogenetic relationships in basidiomycetous fungi at the genus and family levels.

ITS regions tend to be highly polymorphic and have been used extensively in fungal taxonomic and phylogenetic studies (Bridge and Arora 1998, White et

al. 1990). The ITS region of the rDNA has a high copy number, which is more sensitive for detection of fungi in wood where the total fungal DNA levels could be low during decay and when DNA degradation can occur in late term decay.

Fungal specific (ITS1F) and basidiomycete specific (ITS4B) primers have been developed (Gardes and Bruns 1993). But, (Adair et al. 2002) found that these specific primers generated non-specific amplification from three ascomycetes, and they concluded that this primer pair is not suitable to verify the presence of decay fungi in wood chips of hemlock and lodgepole pine. ITS1F and ITS4B primer pair was shown to be reliable to detect the presence of wood decay basidiomycetes in both pure culture and wood by Jasalavich et al. (2000). Jellison et al. (2002) were not able to detect *Postia placenta* (Fr.) Larsen & Lombard or *Leucogyrophana pinastri* (Fr.) Ginns & Weresub using either primer pair ITS1F / ITS1 or ITS1F / ITS4B. A PCR product was not obtained using ITS1F and ITS4B primers for several genera of ectomycorrhizal fungi and amplified some ascomycete fungi (Glen et al. 2000). Primer pair ITS1F / ITS4B did not produce adequate template, and therefore, the additional primer pair ITS5 / ITS4 was used while studying phylogenetic relationships of *Myxacium* (Seidl 2000). In a study of the taxonomy of *Rhizopogon vinicolor* species, Kretzer et al. (2003) used ITS1F and ITS4 primers to amplify both ITS regions, but when amplification of the entire region was not successful, only the ITS1 region was amplified using ITS1F and ITS2 primers.

ITS1 and ITS4 primers have been used to produce a PCR product by other researchers: Guzman-Davalos et al. (2003) to determine the infrageneric

classification of *Gymnopilus*; Hoiland and Holst-Jensen (2000) to compare *Cortinarius* species; Coetzee et al. (2001) to study the root pathogen, *Armillaria* species; Kruger et al. (2001) to use RFLP analysis to sequence data while analyzing Lycoperdales; Schulze (2000) to compare these primers and others while studying two important forest pathogens *Armillaria ostoyae* Romagn. and *Heterobasidion annosum*; Smith and Sivasithamparam (2000) to study 5 species of *Ganoderma* from Australia; Wu et al. (2000) to look at phylogenetic and biogeographic differences between Eastern Asian and Eastern North American species of *Suillus* ; Dresler-Nurmi et al. (1998) to group lignin degrading corticoid fungi; Erland (1995) to determine the abundance of *Tylospora* ectomycorrhizas in south Swedish spruce forest; and Challen et al. (2003) to study *Agaricus* species. For sequencing, ITS2, ITS3, & ITS4 were among the primers used when studying *Myxacium* (Seidl 2000). Vellinga et al. (2003) used primers ITS1-5 for sequencing in their study of the taxonomy of *Macrolepiota*.

DNA Extraction

A rapid method to extract DNA from soil was developed by Porteous and Armstrong (1991) that used several chemicals and enzymes. Adair et al. (2002), because of their concern with interference of wood extractives and decay by-products, used the Qiagen extraction kit as part of a method to extract fungal DNA from wood products. Zambino (2002) used a dry grinding method to extract DNA from spores of rusts and other plant pathogenic, obligate parasitic fungi. Zolan and Pukkila (1986) ground *Coprinus cinereus* (Schaeff.:Fr.) Gray tissue in a mortar and pestle to extract DNA.

Challen et al. (2003) used 1mL of 3% w/v Chelex in 1 mM Tris-HCL (pH 8) and ca. 200 mg. of glass beads in micro-centrifuge to extract DNA from *Agaricus* species. Chelex failed to generate high amounts of quality DNA when compared to double phenol-chloroform and CTAB extractions (Mikhailova and Johannesson 1998). Chelex-100 resin was used by Turpin et al. (1992) to study the effect of *Streptomyces* on the survival of *Salmonella* in soil, and by Moehlenhoff et al. (2001) to extract DNA and remove PCR inhibitors from fungi isolated from painted art objects.

Other researchers have used the Ready-To-Go TM PCR Beads kit. Nilsson and Hallenberg (2003) used this technique in a study of *Hyochnicium punctulatum*, as did Otieno et al. (2003) in a study of *Armillaria*.

The DNeasy® Plant Mini extraction kit has been used by: Doherty, et al. (2003) to reveal genetic variation in the symbiotic fungus of leaf-cutting ants; Moeller and Peltola (2001) in a failed attempt to obtain high molecular weight DNA from pigmented spores of *Stachybotrys chartatum* (Ehren.:Fr.) Hughes; Viaud et al. (2000) to examine the diversity of soil fungi; and Adair, et al. (2002) to detect decay fungi on wood chips.

QIAquick™ Spin has been used to purify the PCR product by other researchers in the study of *Armillaria* species (Sierra et al. 1999), *Cortinarius* (Seidl 2000), arbuscular mycorrhizal fungi (Douhan and Rizzo 2003), *Hyochnicium punctulatum* (Nilsson and Hallenberg 2003), and *Leccinum* (den Bakker et al. 2004).

Materials and Methods

Culture Media

Appendix 5 lists all manufacturers of supplies and equipment used in this research. Several different culture media were used in this research for different purposes. Some required the addition of fungicides and antibiotics in order to increase the chances of isolating basidiomycetous fungi and inhibit or slow down the growth of unwanted fungi and bacteria. The most restrictive media used in this research included: LGBDA (sometimes labeled as "L" in data excel charts or figures), LGBA (sometimes labeled as "T" or "Thorn" in data excel charts or figures), and MYBDA (sometimes label as "M" in data excel charts or figures); all contained fungicides and antibiotics. All three media formulations are detailed in Appendices 6-8 respectfully. Additional culture media used will be discussed in this and further chapters.

A microbiology laminar flow hood was used to transfer substrates (soil, bole wood, wood baits, & basidiocarp) or to sub-culture fungal growth onto culture medium. To ensure sterility of the bench top surfaces during isolations, a cleaning solution (20% commercial sodium hypochlorite bleach, 20% of 95% ethanol, and 60% distilled water, v/v) was used.

All culture plates of the following substrates: bole wood (CWD), soil, soil pit wood baits, soil jar wood baits, and basidiocarp fruiting bodies were incubated at room temperature and observed for fungal growth every week for four weeks. Distinct colonies were subcultured and isolated onto malt-yeast agar (MYA) medium containing 10 g malt extract, 2 g of yeast extract, and 20 g of agar in a

liter of distilled water (Thompson 1998). All pure fungal isolates of possible basidiomycete fungi, from all substrates, were stored using techniques described by Burdsall and Dorworth (1994) on ME medium (Nakasone 1990) for future analysis. Inoculum plugs (1 cm) of actively growing pure culture isolates on ME medium were removed and placed in cryovials covered with sterile distilled water and stored in a refrigerator. This method is preferred over yearly transfer of fungal isolates to agar slants because the isolates can be stored for at least seven years, thereby reducing genetic and morphological instability.

Polyphenol Oxidase Testing

Production of polyphenol oxidases was used to determine whether the pure culture isolates were white or brown rotting fungi. An inoculum plug (1 cm) was removed from an actively growing culture and placed mycelium side down in the center of the culture media: GAA and TAA (Appendix 9). The Bavendamm (Bavendamm 1928) positive reaction on these media contains phenolic acids that indicate the production of extracellular oxidase by the fungi. A negative reaction is associated with brown rot fungi. A positive reaction is more often associated with white rot fungi. The type of reaction on these media (described in Table 3 and shown in Fig. 12), the growth rate, and diameter in centimeters were recorded at one and two weeks for all fungal isolates (accompanying CD).

Table 3. Reactions on GAA and TAA media.

Negative	No brown discoloration of the agar
1+	Diffusion zone light to dark brown, formed under inoculum at center of fungal mat and visible from underside only
2+	Diffusion zone light to dark brown, formed under most of fungal mat but not extending to margin. Visible from underside only
3+	Diffusion zone light to dark brown, extending a short distance beyond the margin of the fungal mat and visible from the upper side
4+	Diffusion zone dark brown, opaque, extending considerably beyond margin of fungal mat
5+	Diffusion zone very intense, dark brown, opaque, forming a wide corona around inoculum. Usually with no growth on the medium

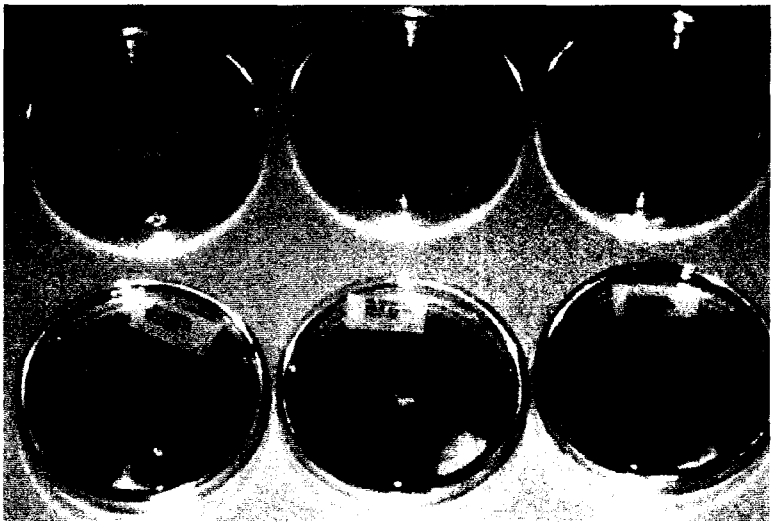


Fig. 12. These petri dishes illustrate the reactions (negative to 5+) on GAA medium. The top three petri dishes (neg to 2+) are photographed from the underside of the fungal mat. The bottom three petri dishes are photographed from the upper side of the fungal mat.

The GAA/TAA reactions of all fungal isolates were classified into 10 groups based on Davidson et al. (1938) and two additional groups (Table 4).

Table 4. GAA and TAA classification groups. Groups 1 – 10. Two additional groups are included.

Negative reaction on both GAA and TAA:
- Group 1 growth is equal on both media
- Group 2 growth is greater on GAA than on TAA
- Group 3 growth on GAA, no growth or trace on TAA

Positive reaction on both GAA and TAA:
- Group 4 no growth or trace on both media
- Group 5 no growth or trace on GAA, <25 mm growth on TAA
- Group 6 no growth or trace on GAA, 25-50 mm growth on TAA
- Group 7 growth is equal on both media
- Group 8 fair growth on GAA, good growth on TAA
- Group 9 good growth on GAA, no growth or trace on TAA, more reaction on GAA, TAA could have a negative reaction

Negative reaction on GAA, Positive reaction on TAA:
- Group 10 good growth on both media

In addition to the above 10 groups; two additional groups were defined, when the results did not fall into the above groups:
Non : no growth and no reaction on both GAA and TAA
? : none of the above

Morphological Studies

Color and texture of fungal growth and growth rate measured at one and two weeks were recorded. Microscopic observations of the hyphae and other features of each pure fungal culture isolates were done using a compound light microscope with Phloxine B and Lactophenol Cotton Blue stains (Appendix 10). Positive proof that an organism is a basidiomycete was the presence of clamp connections along a hyphal strand (Fig. 13).

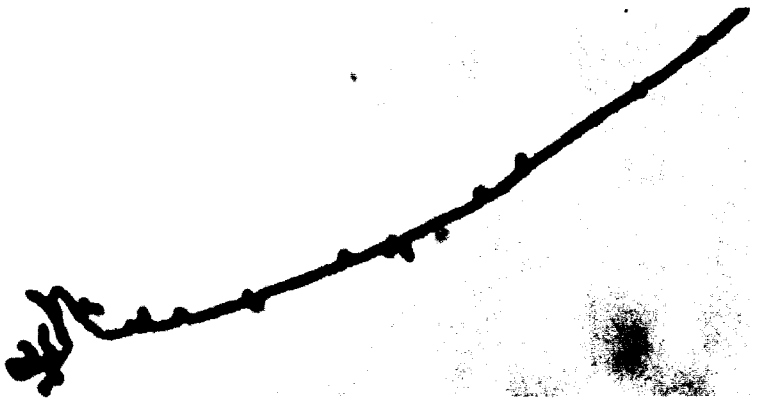


Fig. 13. Clamp connections along the hyphae as seen under the light microscope using phase contrast.

Also recorded were the color and texture of fungal isolates (macroscopic), and the growth rate measured as diameter in centimeters at one and two weeks on MEA medium (10 g malt extract and 20 g of agar in a liter of distilled water). Non-basidiomycete fungi were identified using the taxonomic keys: Wang and Zabel (1990), Watanabe (1994), and Barnet and Hunter (1972), and were not kept for further studies.

Slide cultures (Larone 1995) were made of some culture isolates that required further observations to identify them microscopically. A block of MYA (2 cm x 2 cm) was placed onto a sterile microscope slide, at the four corners of the agar, using a sterile dissecting tool, small pieces of actively growing fungal culture was pressed into the MYA agar, then a sterile cover slip was placed on top of the agar block. This slide was then placed onto a bent glass pipette (to raise the slide off the bottom of the petri dish) into a glass petri dish that contained 5 mL of sterile water and covered with the cover of the petri dish and incubated at room temperature. Slide cultures were viewed under the dissecting microscope to see if hyphal growth from the agar block had occurred onto the

cover slip. When sufficient growth had occurred, the cover slip was removed and placed onto another slide containing a stain. Then this slide was examined under the compound microscope.

Somatic Incompatibility

Somatic incompatibility testing was run on culture isolates that had similar oxidase testing reactions and micro- and macroscopic cultural characteristics. Using the bulb end of a sterilized glass pipette, inoculum plugs 1 cm in diameter from separate actively growing fungal isolates were placed 1 cm apart in the middle of the petri dish of 2 % MEA medium (personal communication with Dr. Nakasone, FPL). Plates were incubated at room temperature on the bench top, examined every week for four weeks, and the results were recorded. It was very important to observe these plates every week because some fungi can quickly grow over the other in the plate giving the appearance that they are compatible. Compatibility is demonstrated in Figure 14. Incompatibility is demonstrated in Figure 15. The dark zone and barrage of aerial hyphae between the culture isolate plugs demonstrates that these two fungal isolates are incompatible.

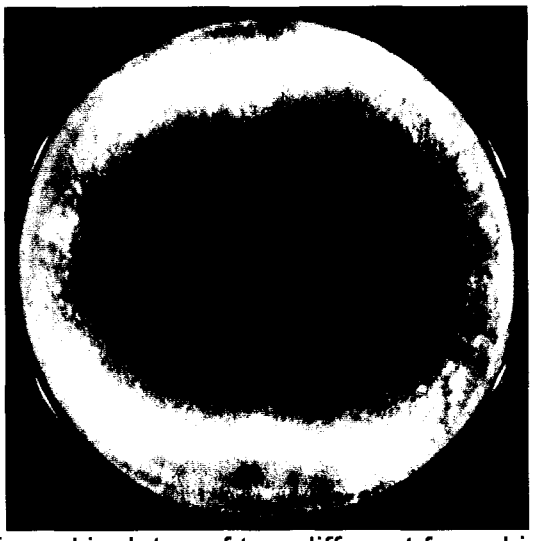


Fig. 14. Compatible fungal isolates of two different fungal isolates on ME medium.

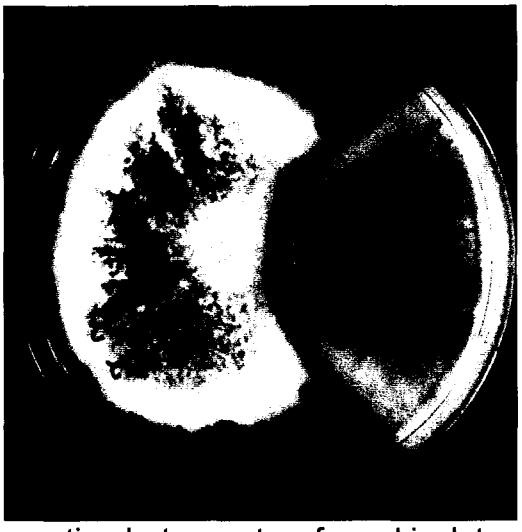


Fig. 15. Incompatible reaction between two fungal isolates showing the dark pigment and barrage between the inoculum plugs on ME medium.

Molecular Testing

Molecular techniques were used to determine similarities between fungal culture isolates from the soil and wood and to place a name (genus and species) on the culture isolates, when possible.

DNA Extraction. Aerial hyphae were removed from the MYA culture plate by gently scraping with a sterilized (dry sterilizer) and cooled dissecting needle and removing approximately 1-2 cm of actively growing mycelium at the edge of the colony and placing this mycelium into a microcentrifuge tube containing 200 µl sterile molecular water. A sterilized micropestle was used to grind and break up the hyphae. Micropipetters with sterilized disposable tips were used for all measurements.

Several methods to extract DNA from the hyphae were used. At first, the ground hyphae were used directly in the PCR (Mullis and Faloona 1987), and followed by two other methods, Chelex-100 (5% soln.) and Ready-To-Go™ PCR Beads. The best PCR product was obtained by using ground hyphae (as described in the previous paragraph) and the DNeasy® Plant Mini Kit. The DNeasy® extraction protocol is explained in detail in Appendix 11. The manufacturers instructions were followed with minor changes that included: skipping step # 1 (the grinding of hyphae with liquid nitrogen); using 40 µl of the ground hyphae in step # 2; and not centrifuging the lysate in step # 4.

Polymerase Chain Reaction (PCR). At first, the 5.8SR and LR3 primers were used, but the BLAST results from GenBank using these primers had numerous different (genera) fungal names, all with an E value of 0.0, i.e., all a

perfect match. It was concluded that most of the PCR product was in the 28S conserved region. The ITS1 and ITS4 primers (White, et al. 1990) were used for PCR.

PCR amplifies specific regions of genes. Using the ITS1 and ITS4 primers (White et al. 1990), double-stranded DNA was amplified by PCR from the end part of the nuclear 18S small-subunit ribosomal DNA (nuc-SSU rDNA), the nuclear 5.8S rDNA, both ITS regions, and the beginning part of the nuclear 28S large-subunit rDNA (nuc-LSU rDNA). The ITS regions in the fungal genome are phylogenetic variable regions. Using ITS1 and ITS4 as the PCR primers meant that some of the conserved gene regions of 18S rDNA and 28S rDNA, and the entire 5.8S rDNA gene would be included in the PCR product and both the ITS regions. Therefore, these primers would include both conserved and variable segments of the fungal genome.

Oligodeoxyribonucleotides primers were purchased from Integrated DNA Technologies, Inc. (IDT) Coralville, IA. The following primers were used in PCR and/or sequencing; they are listed in the standard 5' to 3' direction.

Vilgalys and Hester (1990) primers:
5.8SR (TCG ATG AAG AAC GCA GC)
LR3 (GGT CCG TGT TTC AAG AC)

White et al. (1990) ITS1-4 primers:
ITS1 (TCC GTA GGT GAA CCT GCG G)
ITS2 (GCT GCG TTC TTC ATC GAT GC)
ITS3 (GCA TCG ATG AAG AAC GCA GC)
ITS4 (TCC TCC GCT TAT TGA TAT GC)

Platinum® *Taq* DNA polymerase and an Eppendorf Mastercycler gradient PCR machine were used to amplify the DNA. According to the manufacturer,

this polymerase has an error rate of $1x10^{-4}$ to $2x10^{-5}$. The dNTP mixture was purchased from Sigma-Aldrich. Positive and negative controls (without DNA), to detect contamination in the reagents, were run with the unknown samples during all PCR amplifications. The positive control was a known sample that would amplify with these primers, and the negative water control contained all reagents without any DNA template. The reactions were done in PCR tube strips. The PCR protocol used in this research is outlined in detail in Appendix 12.

Total reaction volume was 50 µL and contained the following components: 10 µM of each primer, 1.5 units *Taq* polymerase, 50 µM of dNTP's, 5 µL of buffer minus Mg, 2 µL of MgCl, and 5 µL of extracted DNA template from the fungal culture isolates. Both the buffer-Mg and MgCl were supplied with the *Taq* polymerase.

The conditions of amplification were as follows: the lid was heated to 100°C, then an initial denaturation of 2 min. at 94°C, then 36 cycles of the following temperature profile: 30 sec. at 94°C denaturation, 30 sec. at 55°C annealing, and 1 min at 72°C elongation; and the run was terminated by a 7 min. extension at 72°C and held at 4°C. The annealing temperature was deduced by using the Wallace rule (Innis and Gelfand, 1990), that 4°C for every GC pair and 2°C for every AT pair are totaled for each primer. The PCR reactions were performed using an Eppendorf Mastercycler gradient machine.

Electrophoresis. The PCR products (DNA template) were electrophoresed using a 14 well, 1.5 mm comb to make the wells. A 1% agarose

gel was made using 1X TAE buffer, which according to the Owl Easycast™ owner's manual, is used when separating DNA fragments with the size of 0.5-10 kb. Invitrogen Low DNA Mass Ladder and Ready-Load™ 1 Kb plus DNA ladder were run on each gel. The electrophoresis protocol used in this research is outlined in detail in Appendix 13.

To check the quality of DNA of the PCR product, 4 µL of each PCR product was mixed with 1 µL Type II gel-loading buffer. The Owl Easycast™ Model B1 horizontal electrophoresis system makes a 9x11 cm gel. The gels were run at 90 Volts/cm for 45 min, stained with ethidium bromide, and destained in water. The gels were photographed using a NucleoTech's GelExpert 3.5, illuminated with UV light (360 nm). Figure 16 illustrates a gel after a PCR run and includes the Low DNA Mass Ladder (lanes 1 &13), Ready -Load™ 1Kb Plus Ladder (lane 14), negative control (lane 6), and a positive control (lane 12).

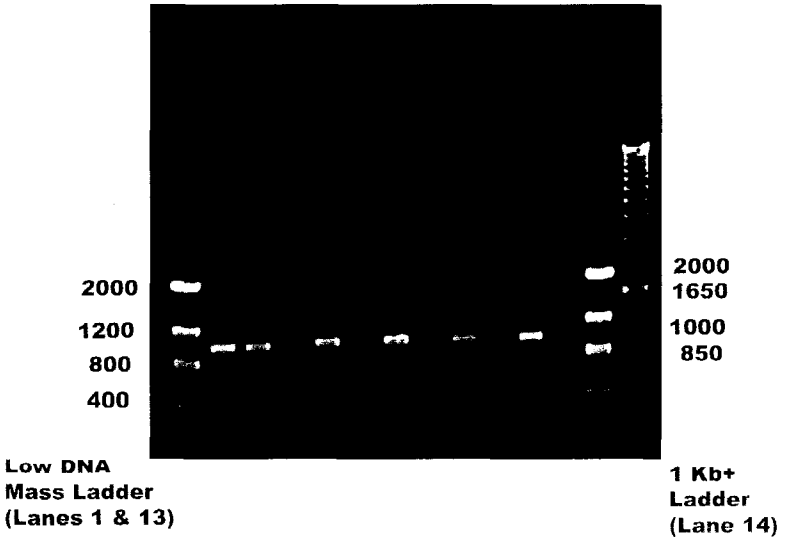


Fig. 16. Electrophoresis gel after a PCR run. Unknowns are in lanes 2-5, 7-11, negative control in lane 6, positive control in lane 12, Low DNA Mass Ladder in lanes 1 & 13, and 1Kb+ Ladder in lane 14. Unknown PCR products are ~ 1000 bp of rDNA.

The size of the PCR amplified product was more or less uniform for most taxa (just under 1000-bp of rDNA), as measured by the 1KB+ ladder. The Low Mass Ladder was used to determine the amount of PCR product. The genomic DNA template PCR products were purified using Qiagen QIAquick PCR Purification Kit and a microcentrifuge, in accordance with the manufacturer's recommendations with one addition. In step # 8, after the addition of Buffer EB to the center of the membrane, the samples stood for 1 min. prior to centrifugation. This additional drying of the membrane allowed the alcohol to dry off and would not cause problems during gel electrophoresis (personal conversation with the manufacturer). The DNA purification protocol used in this research is outlined in detail in Appendix 14.

Another agarose gel was run with the purified DNA template using only the Low DNA Mass Ladder. The gels were photographed using a NucleoTech's GelExpert 3.5 illuminated with UV light (360 nm). To determine the amount of DNA template, the samples were compared to the Low DNA Mass Ladder under UV light.

Sequencing. Some culture isolates were sequenced in both directions using the primers ITS1-4 (Fig. 17) in order to get sequence of the forward and reverse strand.

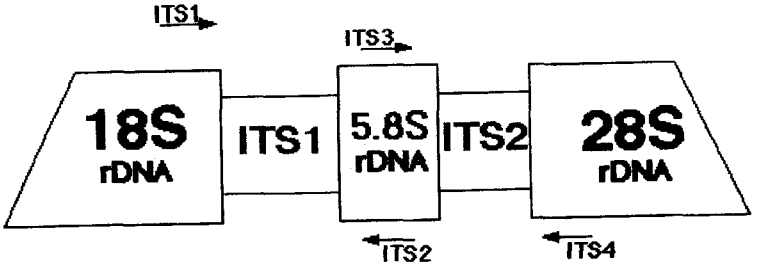


Fig. 17. ITS primers map (http://plantbio.berkeley.edu/~bruns/).

Appendix 15, outlines in detail, the methods used in this research to prepare the samples for sequencing: the PCR run with one primer and ethanol precipitation. The samples were sent to the University of New Hampshire (UNH) sequencing facility that uses an ABI 373A DNA automated sequencer with ABI prism dye terminator cycle sequencing ready reaction kit. This is a modified Sanger method of dideoxy-mediated chain termination sequencing and is a fluorescence-based method using AmpliTaq DNA polymerase.

BLAST in GenBank. All sequences were analyzed using CHROMAS software version 2.23, which was edited with EdSeq (DNAstar) to remove the extreme 5' and 3' ends of the sequences and to clean up any sequencing errors. The cleaned/edited EdSeq files were saved and each primer sequence was BLASTed in GenBank using the BLASTN program (Altschul et al. 1997). Then, using SeqMan (DNAstar) all the sequences of each primer on a specific fungal isolate were aligned and searched for the sequence of the end primers on both the forward and reverse strands to further clean the sequence data. A SeqMan file was saved containing the aligned sequences of all primers of each fungal

isolate, and a contig file of all primers of each fungal isolate was saved as an EdSeq file. The contig of each fungal isolate was then BLASTed in GenBank.

CHAPTER III

SOIL CLASSIFICATION AND SOIL FUNGAL ISOLATES

Introduction

To develop a better understanding of some of the roles that fungi play in the forest ecosystem, it is crucial to have a picture of the complex surroundings in which they exist, particularly the soil. The soil community is determined by soil properties, vegetation type, microclimate, and interactions between organisms. Soil properties include: pH, moisture, texture, nutrient content, air space, and temperature. Soil descriptions, nutrient content of the soils, and coarse woody debris nutrient content and state of decay are components important to a study of forested ecosystems, as these factors directly influence the diversity of organisms, including fungi.

Researchers are working to develop data about the soil environment in the forest ecosystem as a pathway to understanding the dynamics of the ecosystem. Entry and Backman (1995) concluded that cellulose:lignin:N ratios more accurately predicted the decomposition rate of organic matter in a terrestrial ecosystem than C:N or lignin:N ratios. In order to understand how the total amounts of N, Ca, K, Mg, and P in a forest ecosystem change over time, and which minerals change significantly, chemical analysis of soil samples have been done. For example, Federer et al. (1989) compared removal of nutrients from

forest ecosystems of timber harvesting and soil leaching. Johnson et al. (1997) demonstrated that studying the changes in soil chemistry after logging does result in an understanding of the long-term effects of harvesting on nutrient cycling of the forest. In this work, the nutrient content of the soils was analyzed, which led to an understanding of the pools of exchangeable cations. Lang and Forman (1978) studied the detrital dynamics of the Hutcheson Memorial Forest in New Jersey, a mature oak forest. They attempted to further understand the elemental input and turnover time in the forest. In their work, coarse woody debris and soil samples were analyzed for nutrient and organic matter content.

In the northeastern U.S. there are a number of processes that are creating a trend toward acidification of soils in the forest ecosystem. Nitrogen deposition in the northern hemisphere typically indirectly affects woodland organisms but, in general, nitrogen deposition trends towards acidification of the soil, thereby affecting the soil biota (Wall et al., 2001). One measurable effect of an increase in pH is that there are higher populations of earthworms, resulting in a decrease of organic matter in the soils (Kourtev et al., 1998). Tsobel (1990) showed that the pH of the upper horizons of the soil is increased after forest clear-cutting. Small changes in the pH of spodosol soils at the Hubbard Brook Experimental Forest have been shown to have a large effect on sulfate adsorption in the mineral soil after forest clear cutting (Nodvin et al. 1986). In addition, Berthelsen and Steinnes (1995) studied the accumulation patterns of heavy metals in the soil profiles affected by forest clear-cutting. The disturbance of factors balancing a forest ecosystem often has an indirect effect on species within the system.

Losses in biodiversity may alter biogeochemical pathways and ecosystem resilience (Vitousek and Matson, 1984).

The soil fungi are involved in many functions of the ecosystem including:

1) regulation of biogeochemical cycles;

2) translocation and holding of nutrients for plants;

3) soil structure and aggregation; and

4) bioremediation of pollutants.

Anderson (1995) and Hammond et al. (1995) predicted that a greater number of species occur in the soil than above ground. There is no complete list of all species found in soils.

Time course collections of soil and litter for the culture of fungi have been done (e.g., Behera and Dash 1979, Christensen 1969, Gochenaur 1978, Kjoller and Struwe 1987, Smeltzer et al. 1986). Christensen (1969) collected samples of forest soils from mid-June to early August and November in Wisconsin and found that there were variations in densities for dominant taxa over the seasons. Gochenaur (1978) collected soil samples three times a month for 15 months in New York and found seasonal changes in propagule densities. Behera and Dash (1979) collected soil samples bimonthly for a year in Indian crop fields and found that the number of fungal colonies and the number of species were highest during the winter and the numbers decreased with increase in the depth of the soil. Smeltzer et al. (1986) collected soil samples two times a month from late May to early September in a mixed hardwood forest in Vermont to understand soil microorganism populations with soil compaction. They did not see monthly

variation of organisms and concluded that this was due to the fact that they collected only during summer conditions. Kjoller and Struwe (1987) collected litter in Denmark every month for two years and found indications of high cellulolytic activity during the winter with maximum activity in February. These studies suggest that variation in the composition of the fungal flora in the soil occur throughout the year, and therefore, time course collections are necessary to qualify this variation.

In the work reported here, the focus is on the dynamics specifically of basidiomycetes across soil horizons extending from the forest floor to the lower mineral soils. Methods for baiting fungi have been employed by other researchers (e.g., Tsuneda and Thorn 1995, Archer et al. 1995, Cookson and Pham 1995, Freitag and Morrell 1990, Jasalavich et al. 2000, Johnston and Aust 1994).

Materials and Methods

In the present research, soil and bole wood were collected and cultured during two seasons (Spring and Fall). Red maple and white pine wood baits were used to increase the chances of isolating wood decay fungi from the soil. All cultures, subcultures, and French square jar red maple and white pine wood bait experiments were incubated at room temperature.

Collecting and Processing Soil Samples

To collect soil samples, soil pits were dug at each of the six research sites next to previously felled red maple logs (boles). Soil samples were collected in June 1997, June 1999, and October 1999. For improving the chances of

isolating possible basidiomycetous fungi, an additional collection of one organic and one mineral soil horizon occurred in August 1999 at site # 28. Soil processing methods were fine-tuned and the most fruitful method was used during the October 1999 collection. During each collection date, soil samples (one organic and two different mineral soil horizons) were taken from each soil pit using aseptic techniques (Thompson 1998), placed into sterile plastic containers, and stored in a cooler for transport back to the laboratory for further processing. The vertical face of each soil pit was scraped horizontally with a sterilized trowel to expose each soil horizon. Samples of soil were removed by using a sterilized trowel, excavating horizontally into each soil horizon. Soil pit wood baits were then inserted, during the June 1997 collection, into the excavated horizons, and this process is described under soil pit wood bait culture method. During other collections, soil pits were dug in adjacent areas near the bole so as not to disturb the soil pit baits.

Direct Soil Culture Method

The June 1997 soils were processed using a modified sieve method of Thorn et al. (1996) as described by Thompson (1998). This method is referred to as the "sieve" method. In addition to this method, a few un-washed grains of each soil were streaked across the culture plates; and referred to as the "direct" method. Culture media used with these soils collected in June 1997 were LGBDA and MYBDA (Thompson 1998). For each soil horizon, ten replicate plates were used for each method and each type of culture medium.

Soils collected in June 1999 were processed using the Thorn, et al. (1996) sieving method with one minor change: the soil solution was placed on an orbital shaker at room temperature not at 4 °C. For each soil horizon, ten replicate plates of each medium: LGBDA, MYBDA, and 2% water agar (WA) were analyzed. Appendix 16 describes the processing method used with these soils. However, this sieving method yielded many non-basidiomycete fungal colonies on the water agar medium, and the LGBDA and MYBDA culture media produced no fungal growth at all on most plates. With the organic soils, all MYBDA plates except one, yielded no fungal growth; all LGBDA medium culture plates except eight, yielded no fungal growth; the culture plates with fungal growth yielded several to many species of fast growing zygomycetous fungi and yeasts. With the mineral soils, 74% of all plates (LGBDA and MYBDA) produced no growth; other culture plates had a few species of zygomycete fungi and yeasts.

To address this problem, soil samples were collected in August 1999 behind bole # 28 of the organic soil horizon (Oe) and one mineral soil horizon (Bhs). With this collection of soil samples, dilution tests were run to determine what method would yield more possible basidiomycetes and fewer non-basidiomycete fungi. The media used for this experiment were MYBDA and LGBDA, three plates per method per medium per soil sample. Appendix 17 describes the processing method used with these soils. The organic soil was a 0.5% dilution and the mineral soil was a 2% and 4% dilution.

Soil samples collected in October 1999 was processed using the method that was the most fruitful during the August soil dilution testing, with a slight

modification for the organic soils. The "liquid" (suspended organic particles on the tilted sieve) portion of the washed soil was diluted more and the "grains" (settled mineral particles) were diluted less. Appendix 18 describes the processing method used with these soils. Two and a half grams of organic soil and five grams of mineral soil were washed and diluted. One mL of a 1% dilution of "liquid" washed organic soil and a half mL of a 0.25% dilution of "grains" were streaked onto the culture medium using a turntable and sterile glass "hockey" stick. One mL of a 2% dilution of "liquid" washed mineral soil and half mL of 2% dilution of "grains" were streaked onto the culture medium. The culture media used for the soils collected in October were LGBDA, LGBA (Thorn et al. 1996), and MYBDA, 3 plates each per soil sample.

All culture plates were observed daily for fungal growth during the first week and then weekly for a total of four weeks. All fungal growth was sub-cultured onto MYA medium for microscopic observations and determination as to whether they were putative basidiomycetous fungi. All possible basidiomycete fungi were kept for further studies.

Soil Classification and Chemical Analysis

Using ArcMap software and unpublished data from a pilot project by the US Forest Service (USFS) and Natural Resource Conservation Service (NRCS), the soils for Bartlett Experimental Forest were classified. Using the Munsell soil chart, the organic and mineral soils at each research site were classified by color. The descriptions and ecological properties of all soil horizons at each research site are illustrated using Adobe Illustrator. The description of all soil horizons at

each research site included: depth (cm), matrix, iron concentrations, texture, structure, boundary, and an estimation of stone and roots.

Soil temperatures of the soil horizons were taken during all collections. The UNH Soils Analytical Services Laboratory, Durham New Hampshire, determined the mineral content, percent organic matter, and pH, on the June 1997 soil samples and additional June 1999 soil horizons. Soil pH and other chemical analyses were done on the soils (that were cultured from) from the June 1997 collection. This included pH, percent organic matter (LOI-550), and parts per million of calcium, phosphorus, magnesium, and potassium (Mehlich 3). These tests were performed using standard soil testing methods of the North East USA (Agricultural Experimental Station, Delaware, bulletin # 493, 1995).

Soil Jar Wood Bait Culture Method

In a previous study (Thompson 1998), 250 mL French square jars containing red maple wood baits (5 x 2.5 x 1.5 cm) were used in an attempt to attract decay fungi from the soil samples into the wood baits. However, the red maple wood baits were apparently too large for successful infection by basidiomycete fungi in the soil samples in the given time frame (personal communication with Suki Croan Wisconsin Forest Products Lab, Jan. 1999). In this study, much smaller, 5 mm square, previously sterilized red maple and white pine wood baits were used (Fig. 18). Using aseptic techniques, approximately 5 g of one soil horizon per research site was placed into a jar containing a sterile moist paper towel. Then the wood baits (4 of each wood type/jar) were placed on top of the soil, red maple at one end and white pine at opposite end of the jar.

Approximately 5 g of the same soil horizon were placed on top of the wood baits, just enough to cover the wood. The sterile lids were placed back onto the jars making sure that air could enter the jars (not screwed on tight). The jars were incubated at room temperature on their flat sides (Figure 19). Soil samples collected in June 1999, and October 1999, were used to set up these jar wood bait cultures. Jars were observed regularly to ensure that the paper towels remained moist. Sterile distilled water (5 mL) was added to the jars, when no condensation (respiration) could be seen on the upper glass of the jar or if the soils and paper towels appeared to be drying out.

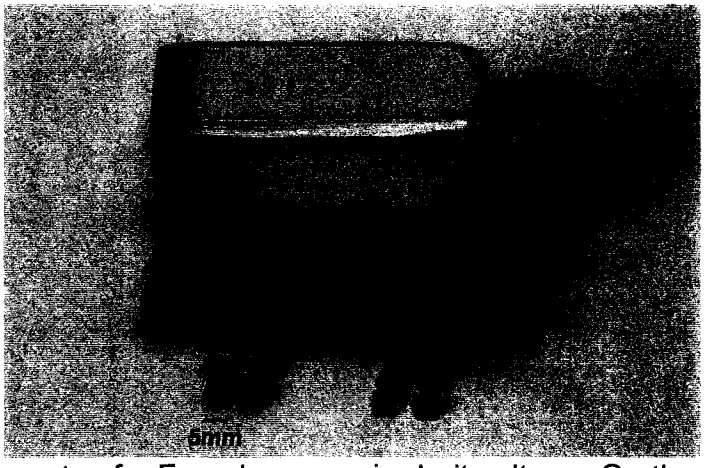


Fig. 18. Components of a French square jar bait culture. On the left are the red maple wood baits, on the right are the white pine wood baits.

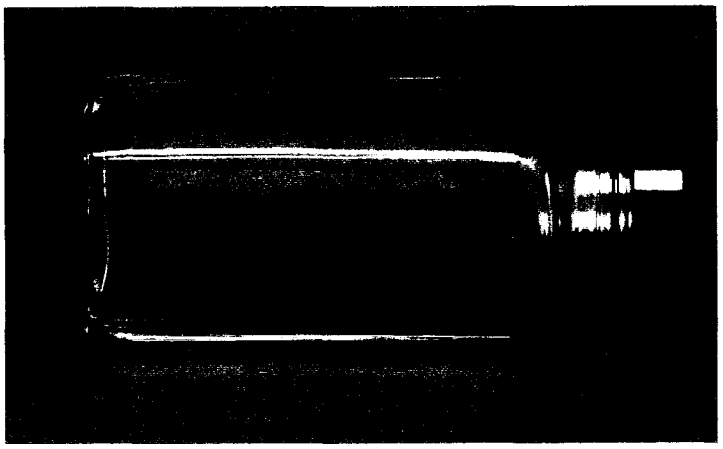


Fig. 19. French jar wood bait culture including sterile water and single soil horizon from one research site.

On two occasions (given below) one red maple and white pine wood bait were removed from the jars using aseptic techniques. All sides of the wood were rubbed on sterile paper towels to remove excess soil and fungal growth. Then, all sides of the wood were cut off exposing the inner wood. The inner wood was then split in two, with each piece being placed onto separate MYBDA culture plates. The culture plates were observed every week for four weeks. Suspect basidiomyceteous fungi were sub-cultured onto MYA medium and kept for further testing.

The June soil jar wood baits were incubated from June 3, 1999, and sampled on October 2, 2000, (16 + months) and on July 2, 2001, (25 + months). The October soil jar wood baits were incubated from October 18, 1999, and sampled on February 14, 2001, (16 months) and on July 12, 2001, (21 months).

In February 2001, the paper towels in the October 1999 jars, containing the organic soils from several of the research sites, were totally decayed. They were processed at 21 months instead of waiting longer. When the jars were

opened to remove the wood baits, many of the wood baits were completely decayed and not recoverable from the jars.

Soil Pit Wood Bait Culture Method

Sterile soil pit red maple wood baits (5 x 2.5 x 1.5 cm) with a length of cotton string tied to each bait (Fig. 20) were inserted into each soil horizon (organic and two mineral), that were going to be cultured during the June, 1997 collection. These baits were placed at the six research sites and left undisturbed for three years and four months. Flagging tape was attached to the end of the string that was placed over the bole for locating them. The soil pits were back-filled with sterile sand around the soil pit wood baits to prevent exposing the wood to a different soil horizon. The remainder of each soil pit was back-filled with the excavated soil (Fig. 21).

Fig. 20. Soil pit red maple wood bait with attached string and flagging prior to insertion into the soil horizons.

Fig. 21. Back fill of sterile sand around the soil pit baits (site # 29).

The soil pit wood baits were retrieved during October 2000 and placed into separate sterile plastic containers to be transported to the laboratory. At the laboratory, using aseptic techniques, the baits were cleaned with sterile paper towels. These baits were aseptically opened to expose the inner wood. Inner wood chips (ca. 3 x 3 mm) were removed aseptically using forceps and placed onto three types of selective media (5 plates for each medium), LGBDA (Thompson 1998), LGBA (Thorn, et al. 1996), and MYBDA (Thompson 1998). The culture plates were observed every week for four weeks. Putative basidiomyceteous fungi were sub-cultured onto MYA medium for further testing.

The morphological and molecular methods used to analyze the fungal isolates are described in Chapter 2.

Results

Temperature of Soil Horizons

Temperatures (°C) of each soil horizon are listed in Appendix 4, by collection date and research site. Temperatures were taken of all soil horizons

during the June 1997, collection. Only three soil horizons (organic and two mineral) were collected and cultured from each research site (the asterisk indicates the soil horizons that were not cultured for fungi during the June 1997, collection). As expected, the temperatures of the organic soils during the October collection were dramatically lower than during the June collections. In fact, the temperatures of these organic soils were lower than the mineral soils, with the onset of winter.

Soil Classification

The soil classification map of Bartlett Experimental Forest includes the roads and the surrounding area (Fig. 22). All research sites are located in an area with soil type 143B: Monadnock fine sandy loam, with a 3-8 percent slope that is very stony. Soils in the Monadnock series have very deep, well-drained soils on glaciated uplands. These soils are classified as coarse-loamy over sandy or sandy-skeletal, isotic, frigid, Typic Haplorthods.

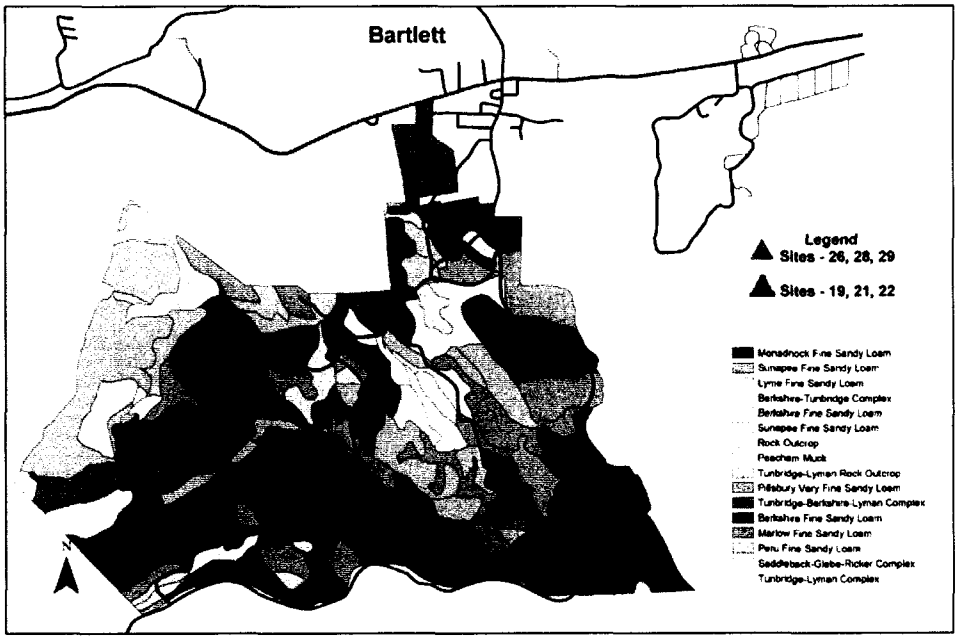


Fig. 22. Soil classification map of Bartlett Experimental Forest. All research sites are in the 143B Monadnock fine sandy loam. This is unpublished data from a pilot project of the USFS and NRCS (permission from Steve Fay).

The old soil classification system in comparison to the new classification system for organic soils are as follows:

Old	New	
L	Oi	slightly decomposed, fibric, fiber content > 40%
F	Oe	fragmented, hemic, fiber content 17-40%
H	Oa	humus, highly decomposed, fiber content < 17%

The organic soils at these research sites were either **Oe** or **Oeb**, b meaning buried. The mineral soils at the research sites included: **E** - eluvial, grayish color due to the loss (leaching) of Fe, Al and clay into the lower mineral horizons, leaving a concentration of sand and silt particles; **B** - illuvial, accumulation of nutrients and organic matter; **Bs** - spodic with accumulation of Fe and Al oxides; **Bhs** - dispersible organic matter (podzolization), and accumulation of Fe & Al

oxides; **Bw** - developed color or structure, weathered but not dark enough to be a spodic; **BC** - parent material (C), soil forming.

During the June 1997, soil collection, the soil horizons for each research site were described and ecological properties recorded (Tables 5-10). Each soil pit was dug to the BC soil horizon and the depth was measured in centimeters from the boundary of the organic and first mineral soil horizon. Using the Munsell soil color chart (1994, Macbeth Corp., New Windsor NY) the matrix of each soil horizon was determined, and includes the hue YR (yellow red), and value/chroma.

Hue: the measure of the chromatic composition of light that reaches the eye.
Value: the degree of lightness or darkness of a color in relation to a neutral gray scale.
Chroma: the relative purity or strength of the spectral color.
Matrix: the dominant color or background color on which mottles (redox features) are compared. Iron concentration is the amount of mottles (few, common or many) and the matrix color of those mottles.
Texture: determines the relative proportions of sand, silt, and clay in the soil, using the hand USDA texture field method and soil ribbon test.
Structure: the arrangement of primary soil particles into compound particles or aggregates (platy, prismatic, angular blocky, subangular blocky, or granular).
Boundary: the topography (smooth, wavy, irregular, or broken), which refers to the irregularities of the surface that divides the horizons and the distinctness (abrupt, clear, gradual, or diffuse), which refers to the thickness of the zone within which the boundary is located.
Stone: the percentage amount of gravel (2 mm – 3 inches), cobble (3 –12 inches), and stone (12 – 24 inches) found in that soil horizon.
Roots: the amount of roots found in the soil horizon (few 0-2%, common 2-20%, many 20% +).

Adobe Illustrator software was used to diagrammatically illustrate the description and ecological properties of each soil horizon at each site, and includes the depth in centimeters, the boundaries between the soil horizons, and the amount of roots and stones found in each horizon (Figs. 23-28).

Table 5. Site # 19 soil description and ecological properties.

Site # 19	Horizon				
	Oe	E	Oeb	Bhsb	BCb
Depth	8 - 0 cm	0 - 1 cm	1 - 11 cm	11 - 21 cm	21 + cm
Matrix	10 YR 2/1	10 YR 6/1	10 YR 2/1	5 YR 3/4	10 YR 5/6
Fe Conc.				mod. 2.5 YR 4/6	few 10 YR 5/8
Texture		fine sandy loam		few sandy loam	coarse sandy loam
Structure		mod. granular		weak granular	weak subangular blocky
Boundary	clear wavy	clear broken	clear broken	clear irregular	
Stone	0, 0, 0	5, 0, 0	0, 0, 0	20, 0, 0	10, 10, 0
Roots	many	few	few	many	none

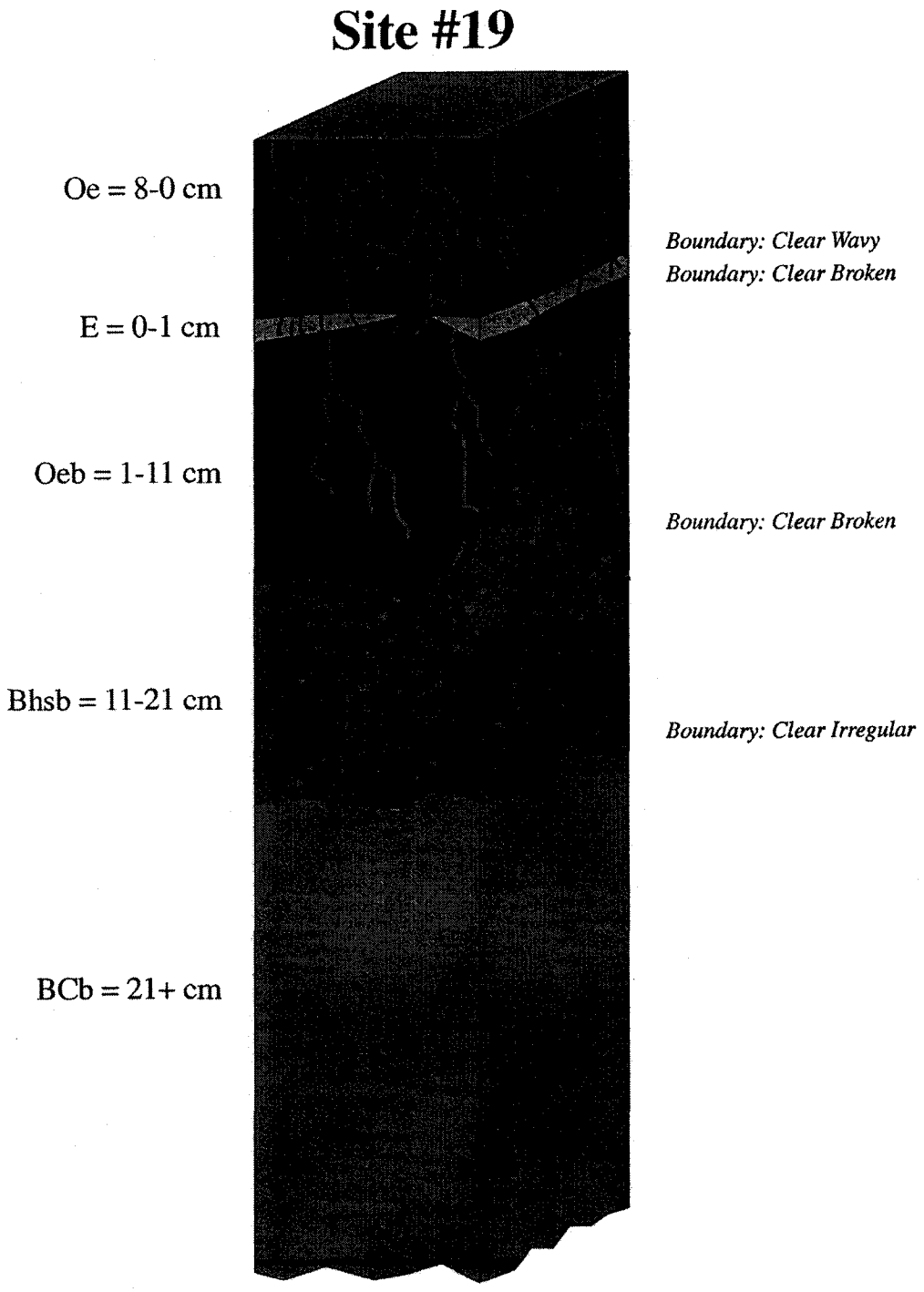


Fig. 23. Soil description and ecological properties of site # 19.

Table 6. Site # 21 soil description and ecological properties.

Site # 21	Horizon			
	Oe	E	Bhs	BC
Depth	7 - 0 cm	0 - 3 cm	3 - 11 cm	11 + cm
Matrix	10 YR 2/1	10 YR 6/1	2.5 YR 2.5/4	10 YR 5/6
Fe Conc.				few 5 YR 3/4
Texture		fine sandy loam	fine sandy loam	sandy loam
Structure		mod. granular	weak granular	weak subangular blocky
Boundary	clear smooth	clear broken	clear wavy	
Stone	0, 0, 0	10, 0, 0	5, 0, 0	10, 10, 0
Roots	many	common	few	none

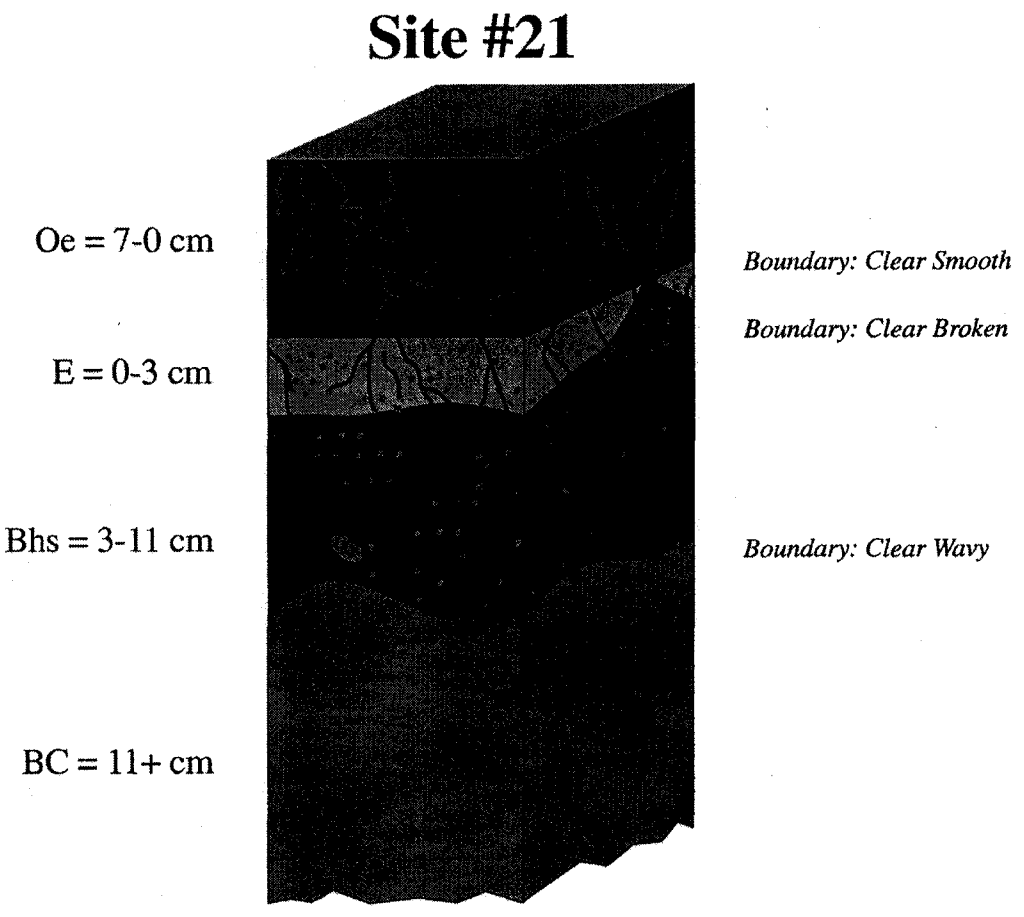


Fig. 24. Soil description and ecological properties of site # 21.

Table 7. Site # 22 soil description and ecological properties.

Site # 22	Horizon		
	Oe	Bhs	BC
Depth	5 - 0 cm	5 - 15 cm	15 + cm
Matrix	10 YR 2/1	5 YR 3/4	10 YR 5/6
Fe Conc.			few 10 YR 3/6
Texture		sandy loam	coarse sandy loam
Structure		weak granular	weak subangular blocky
Boundary	clear wavy	clear wavy	
Stone	0, 0, 0	10, 0, 0	20, 10, 0
Roots	many	common	none

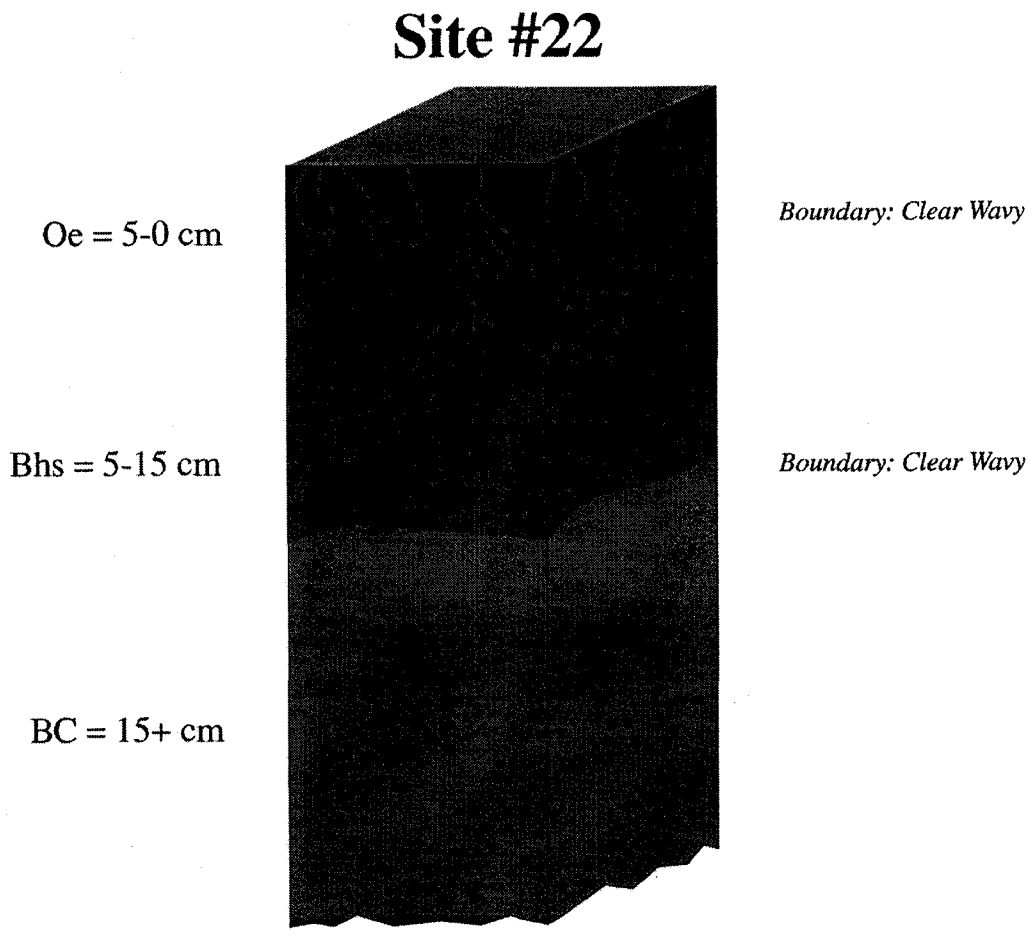


Fig. 25. Soil description and ecological properties of site # 22.

Table 8. Site # 26 soil description and ecological properties.

Site # 26	Horizon			
	Oe	E	Bw	BC
Depth	5 - 0 cm	0 - 10 cm	10 - 16 cm	16 + cm
Matrix	10 YR 2/1	10 YR 7/1	7.5 YR 4/6	10 YR 4/6
Fe Conc.		few 5 YR 5/3	few 5 YR 4/4	
Texture		fine sandy loam	sandy loam	coarse sandy loam
Structure		mod. granular	mod. granular	mod. granular
Boundary	clear broken	clear broken	gradual wavy	
Stone	5, 0, 0	10, 0, 0	5, 0, 0	20, 20, 0
Roots	many	common	common	common

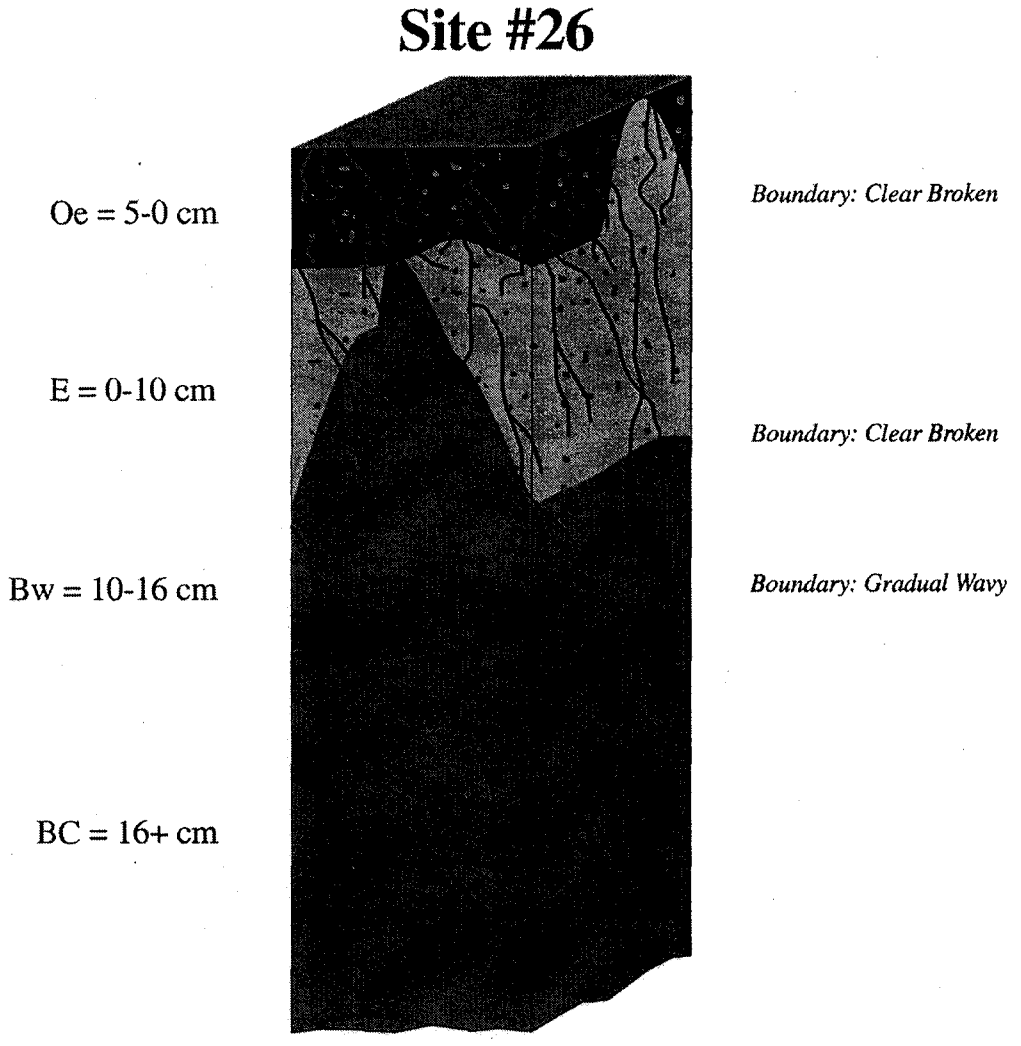


Fig. 26. Soil description and ecological properties of site # 26.

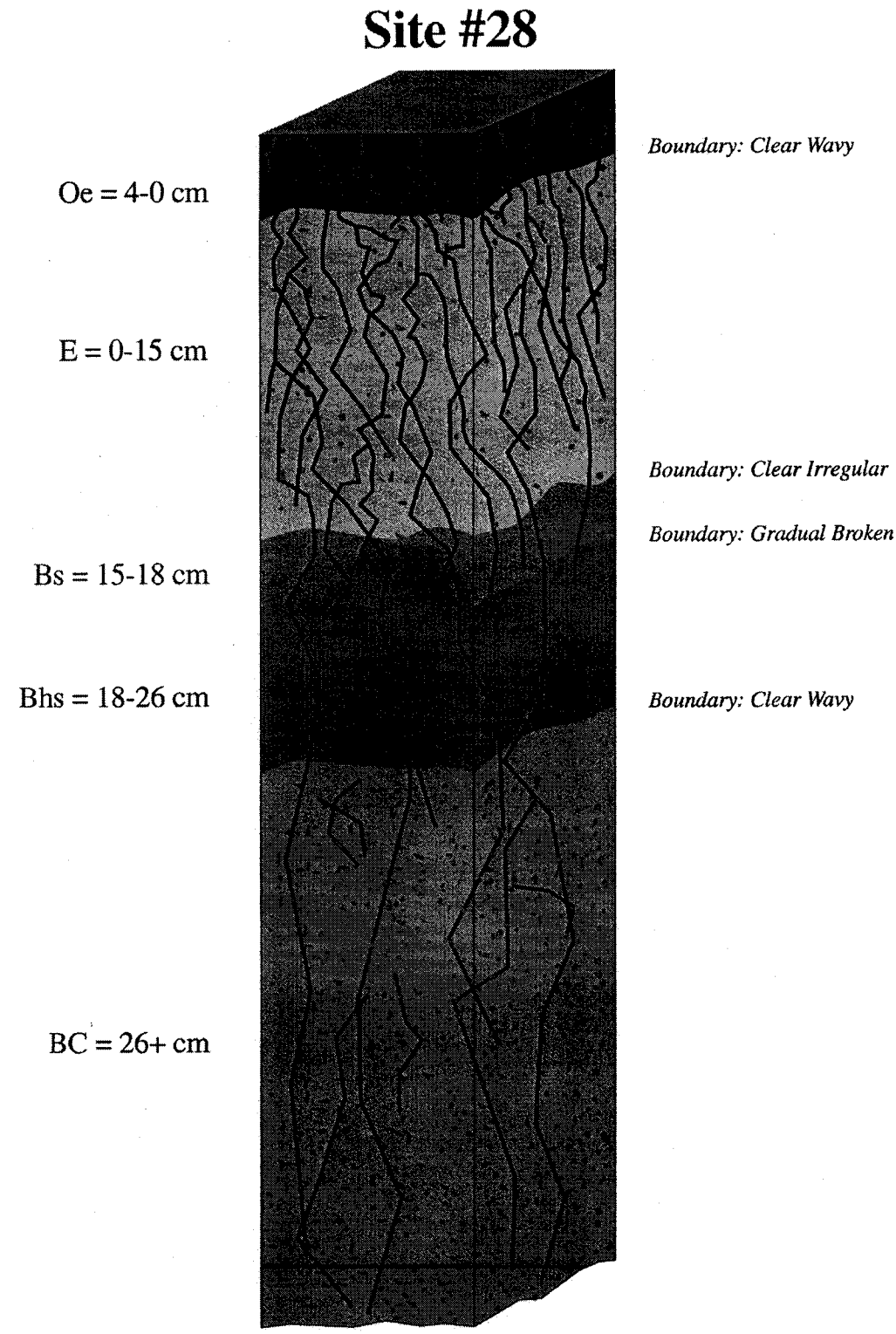


Fig. 27. Soil description and ecological properties of site # 28.

Table 9. Site # 28 soil description and ecological properties.

Site # 28	Horizon				
	Oe	E	Bs	Bhs	BC
Depth	4 - 0 cm	0 - 15 cm	15 - 18 cm	18 - 26 cm	26 + cm
Matrix	10 YR 2/1	10 YR 7/1	7.5 YR 5/3	5 YR 4/6	10 YR 6/6
Fe Conc.		few 5 YR 4/6		few 5 YR 3/4	few 10 YR 5/8
Texture		fine sandy loam	fine sandy loam	sandy loam	coarse sandy loam
Structure		weak granular	mod. granular	mod. granular	mod. granular
Boundary	clear wavy	clear irregular	gradual broken	clear wavy	
Stone	0, 0, 0	5, 0, 0	5, 0, 0	5, 0, 0	20, 0, 0
Roots	many	many	few	few	few

Table 10. Site # 29 soil description and ecological properties.

Site # 29	Horizon				
	Oe	E1	E2	Bw	BC
Depth	5 - 0 cm	0 - 3 cm	3 - 5 cm	5 - 23 cm	23 + cm
Matrix	10 YR 2/1	10 YR 7/1	7.5 YR 5/2	7.5 YR 4/6	10 YR 6/6
Fe Conc.				few 5 YR 4/6	few 10 YR 5/8
Texture		fine sandy loam	fine sandy loam	sandy loam	coarse sandy loam
Structure		weak granular	mod. granular	mod. granular	mod. granular
Boundary	clear wavy	clear wavy	clear wavy	gradual irregular	
Stone	10, 0, 0	5, 0, 0	5, 0, 0	20, 0, 0	20, 30, 0
Roots	many, charcoal	many	many	common	few

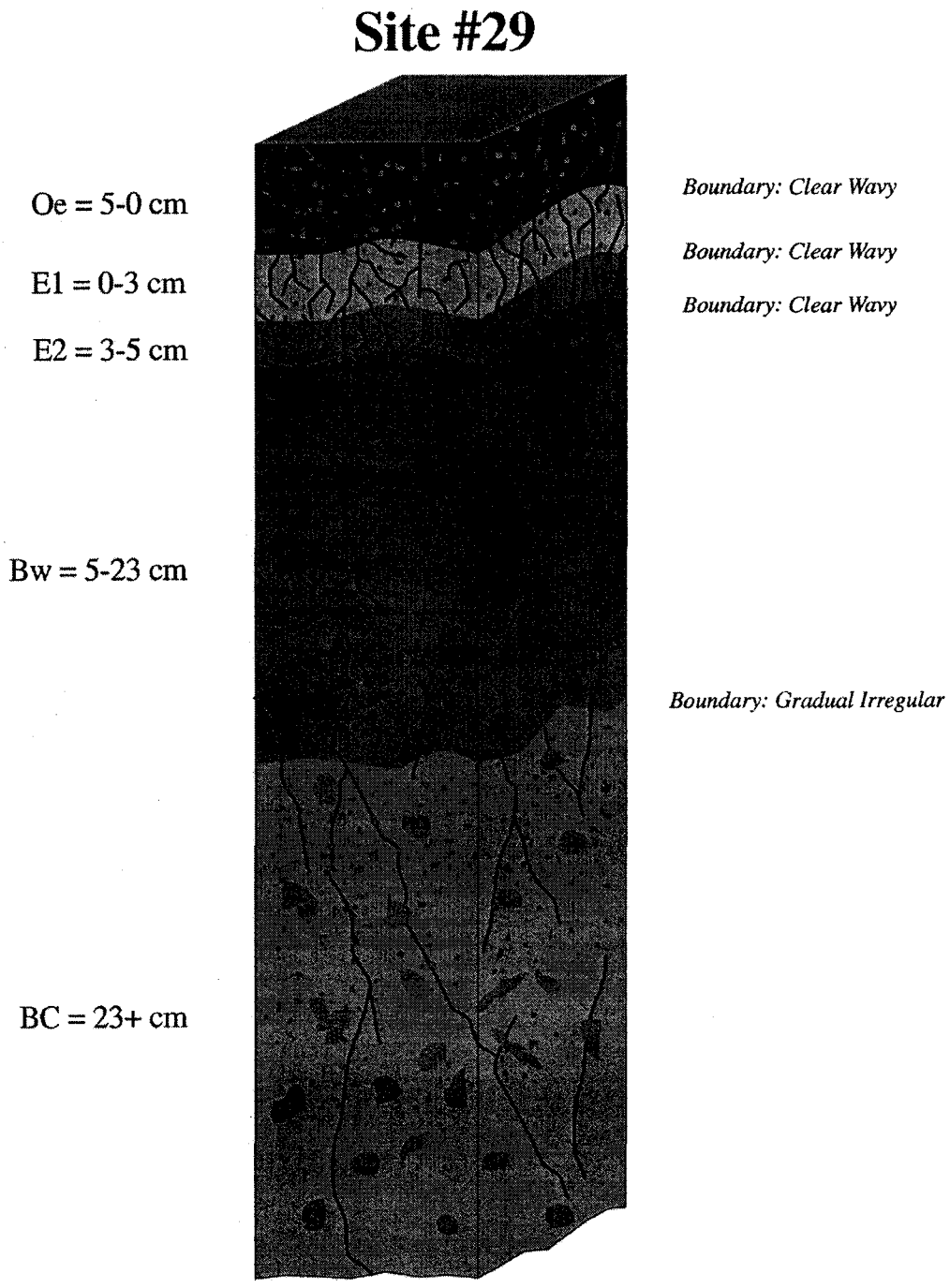


Fig. 28. Soil description and ecological properties of site # 29.

Site's # 26, 28, and 29 had deeper soils than site's # 19, 21, and 22. Site # 22 is the only research site that did not have an E horizon and was the shallowest soil. The other research sites had typical spodosol soils.

Soil pH and Chemical Analysis

Appendix 19 lists the pH and chemical analyses of the soil horizons that were cultured from the June 1997, collection. Bar graphs of pH levels, parts per million (ppm) of magnesium (Mg), calcium (Ca), potassium (K), phosphorus (P), and % organic matter (OM) are shown in Figures 29-34, respectfully.

Statistical analysis, ANOVA, was used to compare the chemical analysis between the organic (Oe) and the mineral soil horizons (E and BC or BCb) for pH, Mg, Ca, K, P, and % OM of the research sites. There was a significant difference between the organic soils (Oe) and the mineral soil (BC or BCb) horizons for magnesium, calcium, potassium, and % organic matter. There was a significant difference between the organic soils (Oe) and the mineral soil (E) horizons for percent organic matter. The p values were less than 0.05.

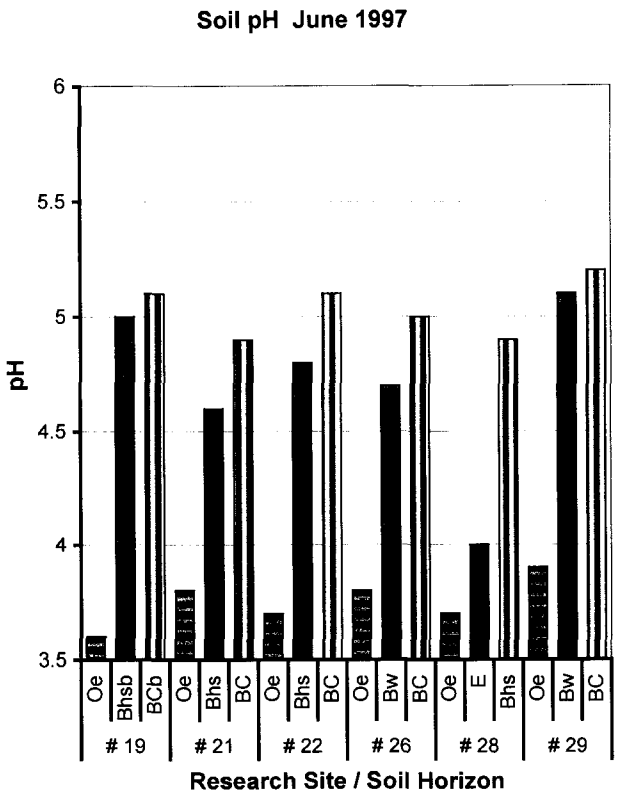


Fig. 29. June 1997 soil pH for all research sites and soil horizons.

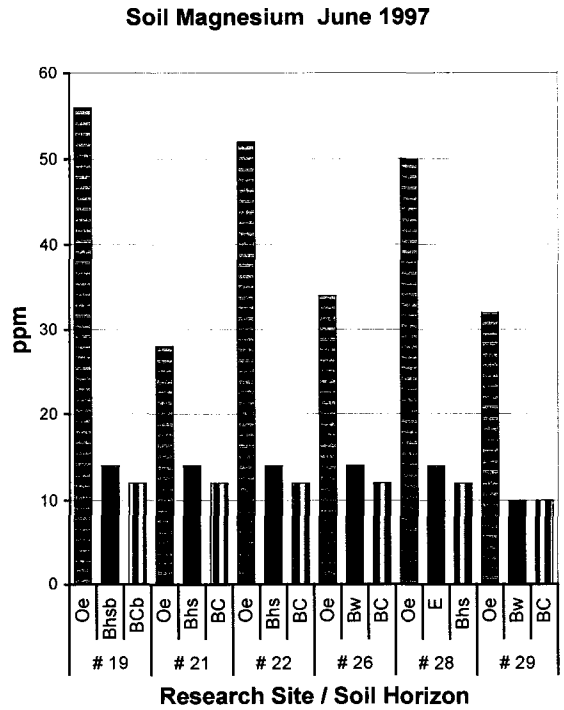


Fig. 30. June 1997 soil magnesium (ppm) for all research sites and soil horizons.

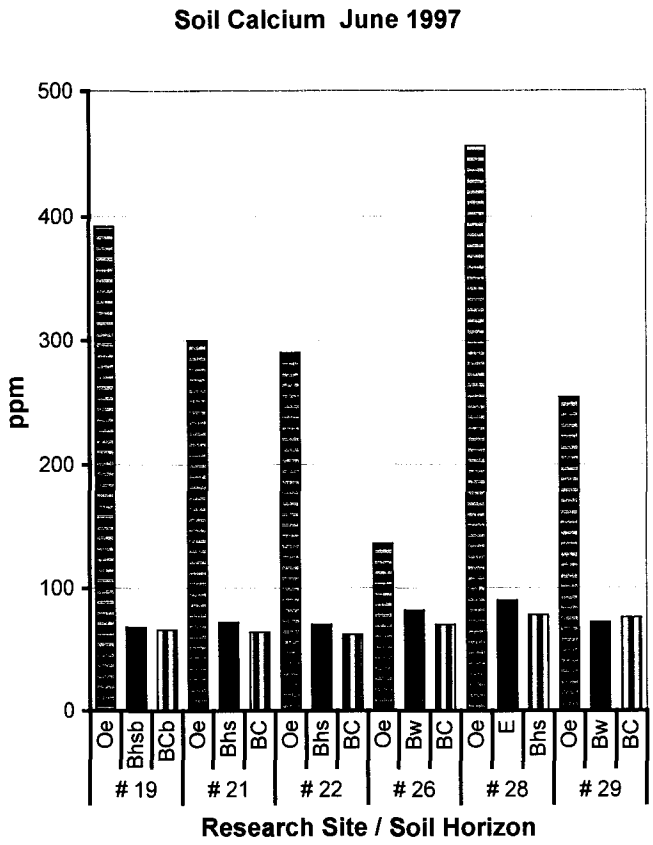


Fig. 31. June 1997 soil calcium (ppm) for all research sites and soil horizons.

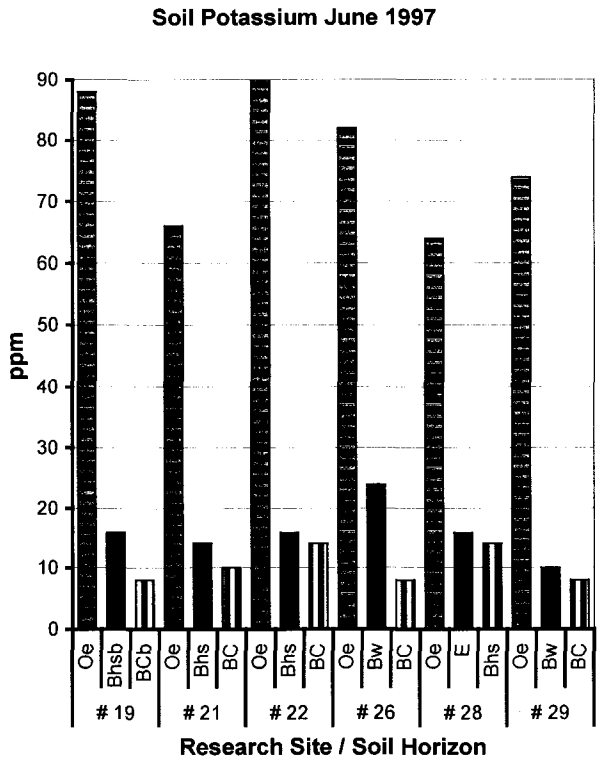


Fig. 32. June 1997 soil potassium (ppm) for all research sites and soil horizons.

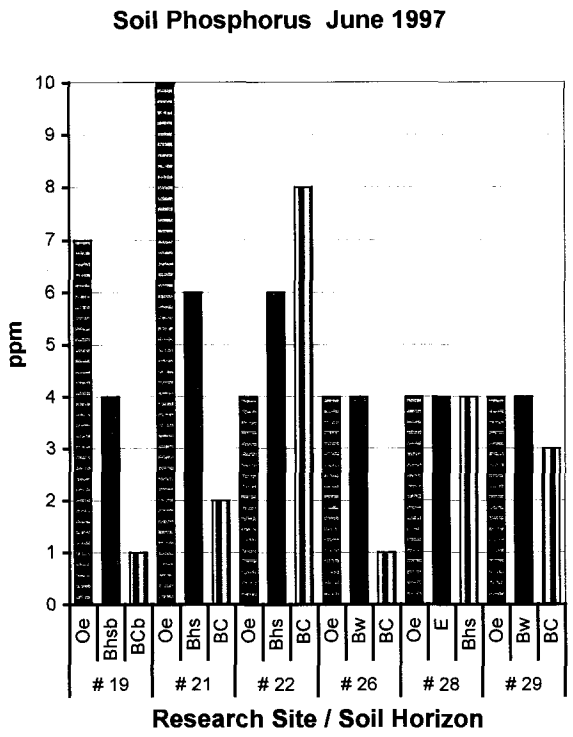


Fig. 33. June 1997 soil phosphorus (ppm) for all research sites and soil horizons.

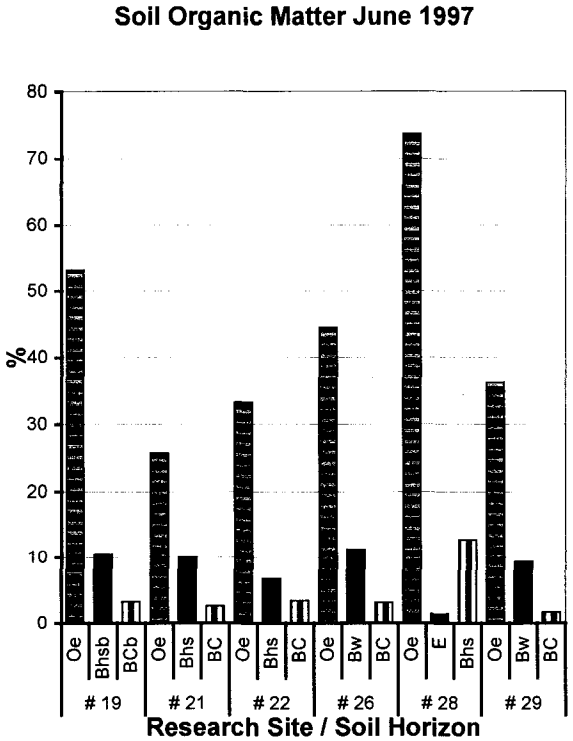


Fig. 34. June 1997 soil organic matter (%) for all research sites and soil horizons.

The pH was the lowest in the organic soils and highest in the lower mineral soil horizons, which is what would be expected. The magnesium was highest in the organic soils and almost the same in the two mineral soil horizons, with a slightly lower amount in the lower mineral soil horizon. The calcium was highest in the organic soil horizons and almost the same in both of the mineral soil horizons. The potassium was highest in the organic soil horizons and the lowest in the lower mineral soil horizons. The phosphorus was the highest in the organic or upper mineral soil horizon, except for site # 22. At site # 22 the lowest phosphorus was in the organic and highest in the lower mineral soil horizon, the complete reverse of the other five research sites. The organic matter was the highest in the organic soil horizon and lowest in the lower soil horizons, except for site # 28. In site # 28, the upper mineral soil horizon had less organic matter than the lower mineral soil horizon, which is to be expected for an E horizon.

Fungal Cultural Characteristics

A key to the summarized cultural characteristics for microscopic and macroscopic data of fungal isolates is given in Table 11.

Table 11. Key to summarized cultural characteristics.

Microscopic: 0 = non-clamps
1 = clamp connection
2 = multiple clamps
3 = oidia
4 = basidium and spores

Growth Rate at 2 weeks: 1 = 3.0 cm and less
2 = + 3.0 cm
3 = full plate (FP)

Color: 0 = no color, almost no color
1 = off white, white
2 = with yellow, brown or other non-white color

Texture: 0 = no aerial hyphae, cloudy, scattered, waxy, or sodden
1 = felty
2 = velvety
3 = downy or hairy
4 = floccose
5 = cottony or cobwebby
6 = plumose, radiating out, or feathery
7 = pulverulent or dry
8 = woolly
9 = silky

Fungal Isolates Directly from Soils

Table 12 summarizes the cultural data for all the fungal isolates directly from soil of the six research sites. The full data results on these isolates can be found on the accompanying CD.

Table 12. Summary cultural data of fungal isolates directly from soil by collection date and research site.

Collection Date	Fungal Isolate [U]	Soil Horizon	GAA/TAA[V]	M[W]	GR[X]	Color[Y]	Texture[Z]
	Site # 19						
Jun-99	19-1-3 L 1	Oe	7	3	3	2	1
	Site # 21						
Jun-97	21-1-3 L	Oe	5	0	3	1	7

Collection Date	Fungal Isolate [U]	Soil Horizon	GAA/TAA[V]	M[W]	GR[X]	Color[Y]	Texture[Z]
Jun-97	21-1-8 LS 2	Oe	3	0	3	1	1
Jun-97	21-2-2 LD 5B	Bhs	?	0	3	1	1
Jun-97	21-2-3 LS 1	Bhs	4	3	3	1	2, 3
Jun-97	21-2-7 LD 2	Bhs	?	0	3	1	1, 5, 9
Jun-97	21-3-10 LD 1	BC	9	0	3	1	1
Jun-99	21-1-4 L 2	Oe	?	0	2	1	1, 2
Jun-99	21-2-1 M 1	E	3	0	2	0	1, 2
Jun-99	21-2-3 M 3	E	non		2	0	3
Oct-99	21-1 OSG L3 2	Oe	10	0	3	2	1, 3
Oct-99	21-1 OSG T1 2	Oe	?	0	1	2	6
Oct-99	21-1 OSL L1 1	Oe	1	0	2	1	3
Oct-99	21-3 MSL M1 2 A	BC	3	0	2	1	0, 3
Oct-99	21-3 MSL M1 2 B	BC	3	0	2	1	0, 1
	Site # 22						
Jun-97	22-1-9 MS 1	Oe	non	0	2	1	0
Jun-99	22-3-4 M	BC	?	4	1	1	3
Oct-99	22-1 OSG M2 1	Oe	7	0	1	2	6
Oct-99	22-1 OSG M2 3	Oe	7	0	1	2	2
Oct-99	22-1 OSG T3 1	Oe	7	0	1	2	1, 2
Oct-99	22-1 OSL L2 1	Oe	7	0	1	2	6
Oct-99	22-1 OSL T2 1	Oe	7	0	1	2	0
Oct-99	22-2 MSG T2 1	Bhs	non	0	1	2	6
	Site # 26						
Jun-97	JB 26-3-1 16 wks M	BC	3	0	3	1	6
Jun-99	26-3-4 M 1	Bw	3	0	2	1	0
Jun-99	26-3-5 M 1	Bw	?	0	3	1	1, 3
Oct-99	26-1 OSL T3 2	Oe	5	3	2	2	1, 3
Oct-99	26-2 MSG M2 1	Bw	4	1	2	1	1, 6
Oct-99	26-2 MSG M2 1 A	Bw	7	0	2	1	1
Oct-99	26-2 MSG M2 1 B	Bw	7	1	2	1	1, 3
Oct-99	26-2 MSG M2 3 A	Bw	3	0	1	1	0, 1
Oct-99	26-2 MSG M2 3 B	Bw	3	0	1	1	0, 1
Oct-99	26-2 MSG M2 4	Bw	4	1	2	1	1, 3
Oct-99	26-2 MSL M2 1	Bw	3	0	1	0	0
Oct-99	26-2 MSL M3 1	Bw	3	0	3	1	1, 5
Oct-99	26-2 MSL M2 2	Bw	3		2	1	0, 3
Oct-99	26-3 MSG M3 1 A	BC	3	0	1	0	0, 1
Oct-99	26-3 MSG M3 1 B	BC	3	0	1	0	0, 1
	Site # 28						
Jun-97	28-2-2 MD 1	E	4	0	1	1	0, 3

Collection Date	Fungal Isolate [U]	Soil Horizon	GAA/TAA[V]	M[W]	GR[X]	Color[Y]	Texture[Z]
Jun-99	28-1-4 L A & B	Oe	non			2	0
Jun-99	28-2-1 WA 1	E	3	0	2	1	1
Jun-99	28-3-3 WA 2	Bhs	10	0	1	2	3, 9
Aug-99	OS G 0.5 L (10-8)	Oe	?	0	3	2	3, 8
Aug-99	OS G 0.5 L (9-27)	Oe	3		2		
Aug-99	OS G1 L	Oe	non		2	0	1, 3
Aug-99	OS L 0.5 L (10-3)	Oe	3		1	1	3
Aug-99	OS L 0.5 (9-27)	Oe	3		2		
Aug-99	MS 3.5 UG L A	Bhs	3	0	1	1	1, 3
Aug-99	MS 3.5 UG L B	Bhs	5	0	3	1	0, 1, 5
Aug-99	MS 3.5 UG L	Bhs	1		1		
Aug-99	MS 3.5 UL L	Bhs	?		2		
Aug-99	MS 3.5 2L 1 L	Bhs	3		2		
Aug-99	MS 3.5 2G 0.5 L	Bhs	3		2		
Aug-99	MS 3.5 2G 0.5 L	Bhs	3	0	2	0	0, 1, 3
Aug-99	MS 3.5 2G 1 M	Bhs	non	0	2	1	0, 3, 5
Aug-99	MS 3.5 4G 0.5 L	Bhs	3		2		
Aug-99	MS 3.5 4G 1 M	Bhs	7	0	3	1	1, 2, 3
Aug-99	MS 5.0 UG L (9-2)	Bhs	7	0	3	1	3, 4, 9
Aug-99	MS 5.0 UG L (9-27)	Bhs	3		2		
Aug-99	MS 5.0 UL L (9-27)	Bhs	3		2		
Aug-99	MS 5.0 UL L A	Bhs	3		2	0	1
Aug-99	MS 5.0 UL L B	Bhs	?	0	2	2	1
Aug-99	MS 5.0 2G 0.5 L	Bhs	3		2	0	0, 1, 3
Aug-99	MS 5.0 2L 0.5 M	Bhs	4	0	1	1	3, 7
Aug-99	MS 5.0 4G 1.0 L	Bhs	3		2		
Aug-99	MS 5.0 4G 1.0 L red	Bhs	non		2	2	3, 4
Aug-99	MS 5.0 4L 0.5 L 1	Bhs	5	0	1	2	
Aug-99	MS 5.0 4L 0.5 L	Bhs	3	0	1	0	0, 1, 3
Oct-99	28-1 OSG L2 2	Oe	5	0	2	2	5
Oct-99	28-1 OSL L2 2	Oe	5	3	2	1	1, 3
Oct-99	28-1 OSL L2 2 A	Oe	5	3	2	1	3
Oct-99	28-1 OSL L2 2 B	Oe	5	3	2	1	3, 6
Oct-99	28-1 OSL L2 3	Oe	5	1	2	1	1, 3
Oct-99	28-1 OSL T1 1	Oe	5	0	2	2	1
Oct-99	28-1 OSL T2 1	Oe	5	0	2	2	2, 9
Oct-99	28-1 OSL T2 2	Oe	10	0	1	2	5
Oct-99	28-2 MSG M2 3	E	2	0	2	0	0, 1, 3

Collection Date	Fungal Isolate[U]	Soil Horizon	GAA/TAA[V]	M[W]	GR[X]	Color[Y]	Texture[Z]
	Site # 29						
Jun-97	29-1-8 LD 2	Oe	?	3	3	1	4
Jun-97	29-1-9 MS 5B 1	Oe	6	0	3	2	4
Jun-97	29-1-9 MS 5B 2	Oe	6	0	3	2	4
Jun-99	29-1 1ml L 3	Oe	non	0	2	0	1, 9
Jun-99	29-1-1 L 1	Oe	non	0	2	0	1
Jun-99	29-1-1 L 6	Oe	non	0	2	0	1
Jun-99	29-1-3 L 1	Oe	non	0	2	0	0, 1
Jun-99	29-1-4 WA 1	Oe	7	0	3	1	1, 9
Jun-99	29-1-4 WA 2	Oe	7	0	3	0	1, 9
Jun-99	29-1-5 L 2	Oe	?	0	2	0	1
Jun-99	29-1-5 WA 1	Oe	?	3	3	1	1, 2
Oct-99	29-1 OSG M3 1	Oe	3	1	2	1	3
Oct-99	29-1 OSG T1 4	Oe	non	0	2	1	3
Oct-99	29-1 OSG T3 1	Oe	?	0	2	1	3, 5
Oct-99	29-1 OSG L1 1	Oe	non	0	2	0	0
Oct-99	29-1 OSL M1 2	Oe	non	0	2	1	3
Oct-99	29-1 OSL M1 4	Oe	?	0	1	2	1
Oct-99	29-1 OSL L2 4	Oe	1	0	2	0	3
Oct-99	29-1 OSL T2 1 A	Oe	4	0	1	2	6
Oct-99	29-1 OSL T2 1 B	Oe	4	0	1	2	3
Oct-99	29-2 MSG L2 2	E2	4	0	1	2	5, 8
Oct-99	29-2 MSL M3 4	E2	4	0	2	1	0, 3, 9
Oct-99	29-3 MSG M1 2	Bw	non	0	2	0	0, 3

U: Fungal Isolate: first number = the site number, second number = the soil horizon (1 organic, 2 & 3 mineral), third number = the culture plate number. Media type (L= LGBDA, T = LGBA, M = MYBDA, WA = water agar). S = sieve, D = direct, MSG = mineral soil grain, OSG = organic soil grain, OSL = organic soil liquid, OSG = organic soil liquid. Last number = isolate number.
V: Oxidase reaction (Table 4, p. 41)
W: Microscopic characteristics (Table 11, p. 80)
X: Growth rate at 2 weeks (Table 11, p. 80)
Y: Morphological color (Table 11, p. 80)
Z: Morphological texture (Table 11, p. 80)

With the direct soil culture method of the June 1997, 1999, and October 1999, collections, for all research sites: 62.5% were white rotters (GAA/TAA groups 4-9) and 37.5% were brown rotters (GAA/TAA groups 1-3). Of the white rotters, 70% were from the organic soil horizons, 71% were isolated on

LGBDA/LGBA medium and 19% on MYBDA medium. Of the white rotters, 30% were from the mineral soil horizons, 33% were isolated on LGBDA/LGBA medium and 66.7% on MYBDA medium. Of the brown rotters, 22% were from the organic soil horizons, 75% were isolated on LGBDA/LGBA and 25% on MYBDA. Of the brown rotters, 72% were from the mineral soil horizon, none were isolated on LGBDA/LGBA, and 93% on MYBDA.

June Jar Wood Bait Fungal Isolates

The cultural data of the June jar wood bait fungal isolates are summarized in Table 13. The full data results of these isolates can be found on the accompanying CD. All wood baits were cultured onto MYBDA medium. The soil was collected in June 1999 and the wood baits were isolated from on October 2, 2000, and July 2, 2001.

Table 13. Summary cultural data of fungal isolates from June jar wood baits (soil).

Fungal Isolate [U]	Soil Horizon	GAA/TAA [V]	M [W]	GR [X]	Color [Y]	Texture [Z]
Wood baits placed onto MYBDA medium on 10-2-00						
19-3 WP 1	BCb	3	0	3	1	1
19-3 WP 2	BCb	3	0	3	1	1, 3
22-3 WP 1	BC	non	1	2	0	9
28-1 WP	Oe	non	0	3	1	5
28-3 RM A	Bhs	3	1	3	1	6
28-3 RM B	Bhs	3	0	3	1	6
28-3 WP 2A	Bhs	3	0	3	1	6
28-3 WP 2B	Bhs	3	0	2	1	6, 8
Wood baits placed onto MYBDA medium on 7-2-01						
26-2 WP 2 4	E	6	1	2	2	3, 8
26-2 WP 2 3	E	6	1	2	2	3, 8
26-2 WP 1 1	E	6	1	2	2	3, 8
28-3 WP 2 2	Bhs	?	1	3	1	6
28-3 WP 2 1	Bhs	?	1	3	1	6
28-3 RM 1 1	Bhs	?	1	3	1	6

U: Fungal Isolate: first number = the site number, second number = the soil horizon (1 organic, 2 & 3 mineral), WP = white pine, RM = red maple, last number and letter = the isolate number
V: Oxidase reaction (Table 4, p. 41)
W: Microscopic characteristics (Table 11, p. 80)
X: Growth rate at 2 weeks (Table 11, p. 80)
Y: Morphological color (Table 11, p. 80)
Z: Morphological texture (Table 11, p. 80)

With the June jar wood bait culture method for all research sites, 33% were white rotters (GAA/TAA groups 4-9) and 67% were brown rotters (GAA/TAA groups 1-3). Both white and brown rotters were isolated only from the mineral soil horizons.

June jar wood bait cultures from sites # 21 and # 29 only produced *Trichoderma* sp., *Mucor* sp., other fast growing zygomycete fungi, and *Cephalosporium* sp. These non-basidiomycete fungi were found on most culture plates of all sites. Some culture plates yielded *Mortierella* sp. Basidiomycete

fungi were isolated from the second mineral soil horizon (Bhs) of site # 28, the first mineral soil horizon (E) of site # 26, the second mineral soil horizon (BC) of site # 22, and the second mineral soil horizon (BCb) of site # 19. These basidiomcyete fungi were isolated from red maple wood baits from site # 28 and on the white pine wood baits from sites # 19, # 22, # 26, and # 28.

October Jar Wood Bait Fungal Isolates

The cultural data of the October jar wood bait fungal isolates are summarized in Table 14. The full data results of these isolates can be found on the accompanying CD. The soil was collected in October 1999 and the wood baits were isolated from on February 14, 2001, and July 12, 2001.

Table 14. Summary cultural data of fungal isolates from October jar wood baits (soil).

Fungal Isolate [U]	Soil Horizon	GAA/TAA [V]	M [W]	GR [X]	Color [Y]	Texture [Z]
Wood baits placed onto MYBDA medium on 2-14-01						
28-1 WP 2 2	Oe	6	1	2	1	5, 8
28-2 WP 2 2	E	6	1	2	1	5, 8
28-2 WP 2 3	E	6	1	2	1	5, 8
28-2 WP 2 1	E	6	1	2	1	5, 8
28-2 WP 1 6	E	6	1	2	1	5, 8
28-2 WP 1 5	E	6	1	2	1	5, 8
28-2 WP 1 4	E	6	1	2	1	5, 8
29-1 RM 1 3	Oe	4	1	2	1	6
29-1 RM 2 1	Oe	4	1	2	1	6
29-1 WP 1	Oe	7	0	2	2	0, 3, 7, 8
29-1 WP 2	Oe	7	0	2	2	0, 3, 7, 8
Wood baits placed onto MYBDA medium on 7-12-01						
21-1 WP 2	Oe	4	1, 3	1	1	1
28-2 WP 8	E	6	1	3	2	3, 8
28-2 WP 7	E	6	1	3	2	3, 8
28-2 RM 1 1	E	6	1	3	2	3, 8
28-3 WP 2 2	BC	6	1	2	2	8

U: Fungal Isolate: first number = the site number, second number = the soil horizon (1 organic, 2 & 3 mineral), WP = white pine, RM = red maple, last number and letter = the isolate number
V: Oxidase reaction (Table 4, p. 41)
W: Microscopic characteristics (Table 11, p. 80)
X: Growth rate at 2 weeks (Table 11, p. 80)
Y: Morphological color (Table 11, p. 80)
Z: Morphological texture (Table 11, p. 80)

With the October jar wood bait culture method of all research sites, 100% were white rotters (GAA/TAA groups 4-9). Of these white rotters, 38% were isolated from the organic soil horizons and 63% from the mineral soil horizons.

October jar wood bait cultures from sites # 26, # 22, and # 19 only produced *Trichoderma* sp., *Mucor* sp., other fast growing zygomycete fungi, and *Cephalosporium* sp. These non-basidiomycete fungi were found on most culture plates. Some culture plates yielded *Penicillium* sp., yeast, *Mortierella* sp., or

bacteria. Basidiomycete fungi were isolated from the organic soil horizon (Oe) from sites # 21, # 28, and # 29, the first mineral soil horizon (E) of site # 28, and the second mineral soil horizon (BC) of site # 28. These basidiomycete fungi were isolated from the red maple wood baits from sites # 28 and # 29, on the white pine wood baits from sites # 21, # 28, and # 29.

Soil Pit Wood Bait Fungal Isolates

The soil pit red maple wood baits were inserted into the soil horizons at the research sites during the June, 1997 collection. They were removed three years and four months later in October 2000.

Figure 35 illustrates the raw sterile red maple wood used as a soil pit bait. Figures 36-41 illustrate the soil pit baits from the research soil horizons at each research site (photographed after culturing from them).

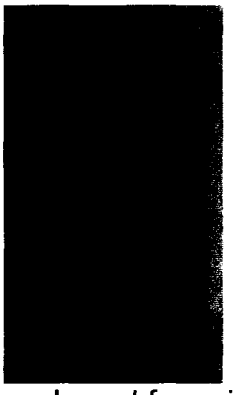
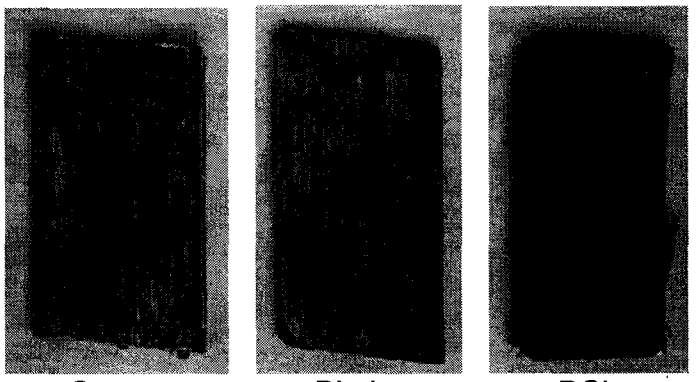
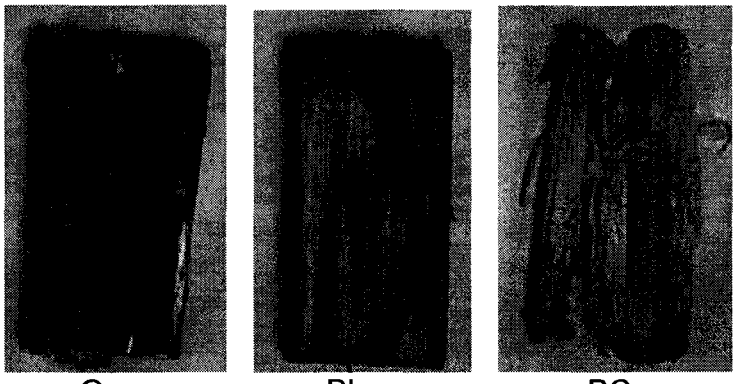


Fig. 35. Raw/sterile red maple wood used for soil pit baits, prior to insertion into three of the soil horizons of each research site.



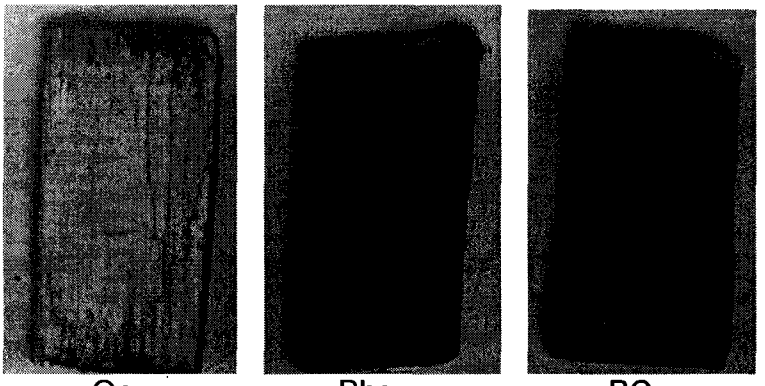
Oe Organic | Bhsb Mineral | BCb Mineral

Fig. 36. Soil pit baits from site # 19 by soil horizon.



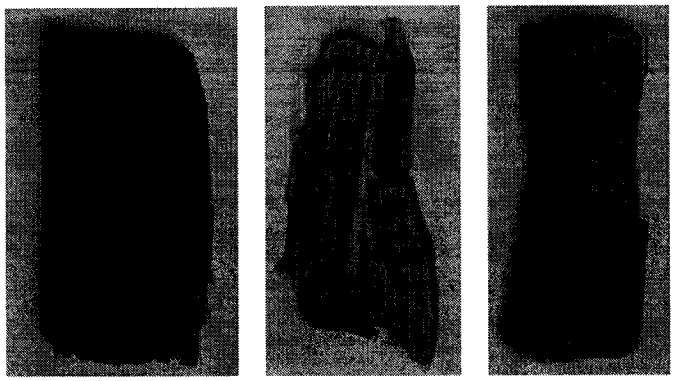
Oe Organic | Bhs Mineral | BC Mineral

Fig. 37. Soil pit baits from site # 21 by soil horizon.



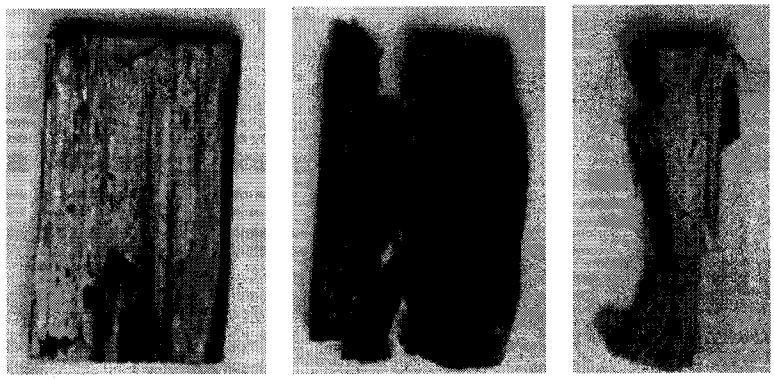
Oe Organic | Bhs Mineral | BC Mineral

Fig. 38. Soil pit baits from site # 22 by soil horizon.



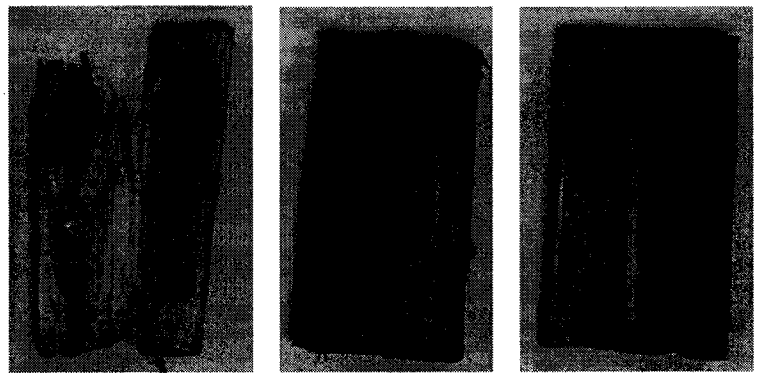
Oe Organic | Bw Mineral | BC Mineral

Fig. 39. Soil pit baits from site # 26 by soil horizon.



Oe Organic | E Mineral | Bhs Mineral

Fig. 40. Soil pit baits from site # 28 by soil horizon.



Oe Organic | Bw Mineral | BC Mineral

Fig. 41. Soil pit baits from site # 29 by soil horizon.

Table 15 is the summarized cultural data of the soil pit wood bait fungal isolates. Full data results of these isolates can be found on the accompanying

CD. The baits were placed onto MYBDA, LGBDA, and Thorn (LGBA) culture media.

Table 15. Summary cultural data of fungal isolates from soil pit baits removed from the soil horizons on October 2000.

Fungal Isolate [U]	Soil Horizon	GAA/TAA [V]	M [W]	GR [X]	Color [Y]	Texture [Z]
19-1 MYBDA 1B	Oe	5	1	3	1	1, 3
19-1 MYBDA 3B	Oe	5	1	3	1	1, 3
19-1 Thorn 2B	Oe	5	1	3	1	1, 3
19-1 LGBDA 1B	Oe	5	1	3	1	1, 3
19-2 LGBDA 2B	Bhsb	4	1	3	1	0, 3
19-3 MYBDA 1B	BCb	4	1	3	1	0, 3
19-3 MYBDA 1A	BCb	4	0	3	1	0, 3
21-1 MYBDA 1A	Oe	5	1	3	1	1, 3
21-1 MYBDA 2A	Oe	5	1	3	1	1, 3
21-1 MYBDA 3B	Oe	5	1	3	1	1, 3
21-3 MYBDA 1A	BC	4	1	3	1	0, 3
21-3 MYBDA 2A	BC	3	1	3	1	7
22-1 MYBDA 1B	Oe	5	1	3	1	1, 3
22-1 LGBDA 2A	Oe	5	1	3	1	1, 3
22-1 LGBDA 3A	Oe	5	1	3	1	1, 3
22-1 Thorn 1A	Oe	5	1	3	1	1, 3
22-1 MYBDA 3A	Oe	5	1	3	1	1, 3
26-3 MYBDA 1A	BC	?	1	3	0	6
28-1 MYBDA 1A	Oe	5	1	3	1	1, 4
28-1 LGBDA 1A	Oe	6	1	3	1	1, 4
28-1 LGBDA 2A	Oe	6	1	3	1	1, 4
28-1 Thorn 1A	Oe	5	1	3	1	1, 4
28-2 MYBDA 1A	E	6	1	3	1	1, 4
28-2 Thorn 2B	E	5	1	3	1	1, 4
28-2 Thorn 1A	E	4	1	3	1	1, 4
28-2 Thorn 2A	E	6	0	2	1	1, 4
28-2 LGBDA 2B	E	5	1	2	1	1, 4
29-1 MYBDA 2B	Oe	4	1	2	1	1, 4
29-1 MYBDA 1A	Oe	4	1	2	1	1, 4

Fungal Isolate [U]	Soil Horizon	GAA/TAA [V]	M [W]	GR [X]	Color [Y]	Texture [Z]
29-1 MYBDA 3B	Oe	5	0	2	1	1, 4
29-3 MYBDA 2A	BC	?	1	3	0	6
29-3 MYBDA 1A	BC	?	1	3	0	6
29-3 MYBDA 3A	BC	?	1	3	0	6

U: Fungal Isolate: first number I= the site number, second number = the soil horizon (1 organic, 2 & 3 mineral), last number and letter = the isolate number
V: Oxidase reaction (Table 4, p. 41)
W: Microscopic characteristics (Table 11, p. 80)
X: Growth rate at 2 weeks (Table 11, p. 80)
Y: Morphological color (Table 11, p. 80)
Z: Morphological texture (Table 11, p. 80)

With the soil pit wood bait culture method for all research sites: 97% were white rotters (GAA/TAA groups 4-9) and 3% were brown rotters (GAA/TAA groups 1-3). Of the white rotters, 68% were from the organic soil horizons, 42% were isolated on LGBDA/LGBA medium and 58% on MYBDA. Of the white rotters, 32% were from the mineral soil horizons, 56% were isolated on LGBDA/LGBA medium and 44% on MYBDA medium. Of the brown rotters, 100% were from the mineral soil horizons and were isolated on MYBDA medium.

Trichoderma sp., *Mucor* sp., and *Cephalosporium* sp. were found on many of the soil pit bait culture plates. Some plates produced no growth. Basidiomycetes were isolated from site # 29 organic soil horizon (Oe) and second mineral soil horizon (BC), from site # 28 in organic soil horizon (Oe) and first mineral soil horizon (E), from site # 26 second mineral soil horizon (BC), from site # 22 in organic soil horizon (Oe), from site # 21 in organic soil horizon (Oe) and second mineral soil horizon (BC), from site # 19 from organic soil horizon (Oe) and both mineral soil horizons (Bhsb and BCb). MYBDA medium yielded 21, LGBDA yielded 7, and LGBA yielded 6 putative basidiomycetous fungi, respectively.

Sequencing

A selected number of soil fungal isolates were sequenced, primarily from one research site, # 28, although a few fungal isolates from other research sites also were sequenced. Tables 16-18 list the GenBank BLAST identities for these isolates. They are listed by research site, culture method (direct soil, June jar bait [6JB], October jar bait [10JB] and soil pit baits [SPB]), collection date, soil horizon, primers used in sequencing, and GenBank accession number.

Table 16. GenBank BLAST results of soil fungal isolates from sites # 21, 22, 26.

	Fungal Isolate [z]	Collecion Date	Horizon	Primers	BLAST results	Accession #
Soil	21-3-10 LD 1	June 97	BC	ITS 1-3	*Thanatephorus cucumeris*	AF455463
Soil	22-3-4 M	June 99	BC	ITS 1&2	*Ganoderma* sp.	AJ749970
Soil	26-3-4 M 1	June 1999	Bw	ITS1-3	uncultured fungus	AF461657
Soil	26-1 OSL T3 2	Oct. 1999	Oe	ITS1-3	*Hypholoma sublateritium*	AY534117
Soil	26-2 MSG M2 1 B	Oct. 1999	Bw	ITS1 & 3	*Mycena aff. murina*	AF335444
Soil	26-2 MSG M2 3 B	Oct. 1999	Bw	ITS1-3	uncultured fungus	AF461657
Soil	26-2 MSG M2 4	Oct. 1999	Bw	ITS1 & 3	*Mycena aff. murina*	AF335444
Soil	26-2 MSL M3 1	Oct. 1999	Bw	ITS1-3	uncultured fungus	AF461657
Soil	26-2 MSL M2 2	Oct. 1999	Bw	ITS1-3	uncultured fungus	AF461657
Soil	26-3 MSG M3 1 A	Oct. 1999	Bw	ITS1-3	uncultured fungus	AF461657
6JB	26-2 WP 2 4	June 1999	E	ITS1-3	*Hypholoma sublateritium*	AY534117
6JB	26-2 WP 1 1	June 1999	E	ITS1-3	*Hypholoma sublateritium*	AY534117
SPB	26-3 MYBDA 1A	Oct. 2000	BC	ITS1 & 3	*Fomes fomentarius*	AY354213

Z: Fungal Isolate: see Tables 12-15

Figure 42 illustrates the basidiomycete fungal isolates identified by sequencing per soil horizon for research site # 26.

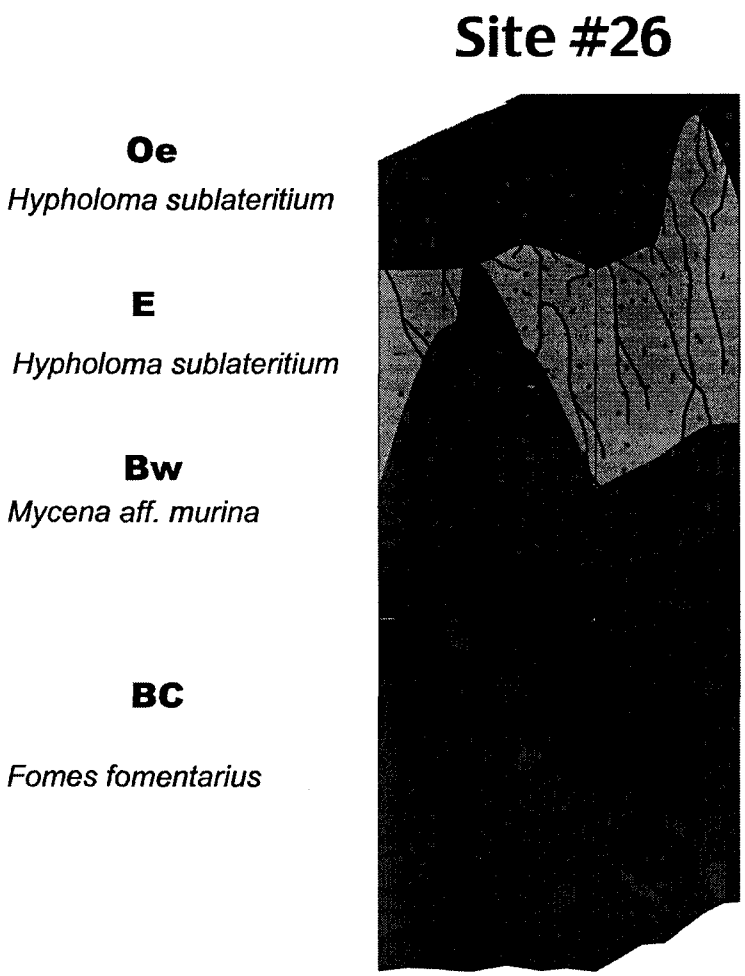


Fig. 42. Identified basidiomycete fungi by soil horizon, site # 26.

Table 17. GenBank BLAST results of soil fungal isolates from site # 28.

	Fungal Isolate [z]	Collecion Date	Horizon	Primers	BLAST results	Accession #
Soil	28-2-2 MD 1	June 1997	E	ITS1-4	*Ganoderma* sp.	AJ749970
Soil	28-1-4 L A	June 1999	Oe	ITS1-4	*Inonotus glomeratus*	AF247968
Soil	28-2-1 WA 1	June 1999	E	ITS1-4	*Irpex lacteus*	AB079265
Soil	28-3-3 WA 2	June 1999	Bhs	ITS1-4	Mycorrhizal fungal sp.	AY228346
Soil	OS G 0.5 L (10-8)	Aug. 1999	Oe	ITS1 & 2	*Armillaria gallica*	AY190248
Soil	OS G1 L	Aug. 1999	Oe	ITS1-3	*Myrothecium inundatum*	AJ302005
Soil	OS L 0.5 L (10-8)	Aug. 1999	Oe	ITS1, 2, 4	*Verticillium bulbillosum*	AJ292410
Soil	MS 3.5 UG L B	Aug. 1999	Bhs	ITS1-4	*Polyporus brumalis*	AY494980
Soil	MS 3.5 UL L	Aug. 1999	Bhs	5.8SR, ITS1	ascomycete	/
Soil	MS 3.5 2G 0.5 L	Aug. 1999	Bhs	ITS1 & 2	*Myrothecium inundatum*	AJ302005
Soil	MS 3.5 2G 1 M	Aug. 1999	Bhs	ITS1 & 2	*Myrothecium inundatum*	AJ302005
Soil	MS 3.5 4G 1 M	Aug. 1999	Bhs	ITS1-3	*Echinodontium taxodii*	AF455458
Soil	MS 5.0 UG L (9-2)	Aug. 1999	Bhs	ITS1 & 2	*Fusarium tricinctum*	AY188923
Soil	MS 5.0 UG L (9-27)	Aug. 1999	Bhs	ITS1 & 2	*Myrothecium inundatum*	AJ302005
Soil	MS 5.0 UL L A	Aug. 1999	Bhs	ITS1 & 2	*Verticillium bulbillosum*	AJ292410
Soil	MS 5.0 UL L B	Aug. 1999	Bhs	5.8SR, ITS 1	*Stereum* species	AY207328
Soil	MS 5.0 2G 0.5 L	Aug. 1999	Bhs	ITS1 & 2	*Myrothecium inundatum*	AJ302005
Soil	MS 5.0 2L 0.5 M	Aug. 1999	Bhs	ITS1-3	*Skeletocutis nivea*	AJ006679
Soil	MS 5.0 4L 0.5 L 1	Aug. 1999	Bhs	ITS1 & 2	*Cladosporium elatum*	AF393699
Soil	MS 5.0 4L 0.5 L	Aug. 1999	Bhs	ITS1-3	*Verticillium bulbillosum*	AJ292410
Soil	28-1 OSL L2 3	Oct. 1999	Oe	5.8SR, ITS 1 & 2	*Lecythophora* sp.	AY354246
Soil	28-1 OSL L2 2	Oct. 1999	Oe	LR3, ITS1-4	*Hypholoma sublateritium*	AY534117
Soil	28-1 OSG L2 2	Oct. 1999	Oe	ITS1-3	*Hypoxylon fragiforme*	AJ390403
Soil	28-1 OSL L2 2B	Oct. 1999	Oe	5.8SR, ITS1-4	*Hypholoma sublateritium*	AY534117
Soil	28-1 OSL T2 2	Oct. 1999	Oe	ITS1-4	*Armillaria ostoyae*	AJ250055
Soil	28-1 OSL L2 2A	Oct. 1999	Oe	5.8SR, ITS1-4	*Hypholoma sublateritium*	AY534117
Soil	28-1 OSL T1 1	Oct. 1999	Oe	5.8SR, ITS1-4	*Hypholoma sublateritium*	AY534117
Soil	28-2 MSG M2 3	Oct. 1999	Oe	ITS1-3	uncultured fungus	AF461617
6JB	28-1 WP	June 1999	Oe	ITS1-3	uncultured fungus	AF504844
6JB	28-3 RM A	June 1999	Bhs	5.8SR, ITS1-3	*Phanerochaete rimosa*	AY219349
6JB	28-3 RM B	June 1999	Bhs	ITS1-3	*Phanerochaete rimosa*	AY219349
6JB	28-3 RM 1 1	June 1999	Bhs	5.8SR, ITS1-3	*Phanerochaete rimosa*	AY219349
6JB	28-3 WP 2B	June 1999	Bhs	5.8SR, ITS1	uncultured fungus	AF461617
10JB	28-1 WP 2 2	Oct. 1999	Oe	LR3, 5.8SR, iTS1-4	*Hypholoma sublateritium*	AY534117
10JB	28-2 WP 2 1	Oct. 1999	E	ITS1-4	*Hypholoma sublateritium*	AY534117
10JB	28-2 WP 1 6	Oct. 1999	E	ITS1-4	*Hypholoma sublateritium*	AY534117
10JB	28-2 WP 8	Oct. 1999	E	ITS1-4	*Hypholoma sublateritium*	AY534117
10JB	28-2 RM 1 1	Oct. 1999	E	ITS1-4	*Hypholoma sublateritium*	AY534117
10JB	28-3 WP 2 2	Oct. 1999	BC	ITS1-4	*Hypholoma sublateritium*	AY534117
SPB	28-1 MYBDA 1A	Oct. 2000	Oe	ITS1-4	*Hypholoma sublateritium*	AY534117
SPB	28-2 MYBDA 1A	Oct. 2000	E	ITS1-4	*Hypholoma sublateritium*	AY534117
SPB	28-2 Thorn 1A	Oct. 2000	E	ITS 1,3,4	*Hypholoma sublateritium*	AY534117

	Fungal Isolate [Z]	Collecion Date	Horizon	Primers	BLAST results	Accession #
SPB	28-2 Thorn 2A	Oct. 2000	E	ITS1-4	*Hypholoma sublateritium*	AY534117

Z: Fungal Isolate: see Tables 12-15

Figure 43 illustrates the basidiomycete fungal isolates identified by sequencing per soil horizon for research site # 28.

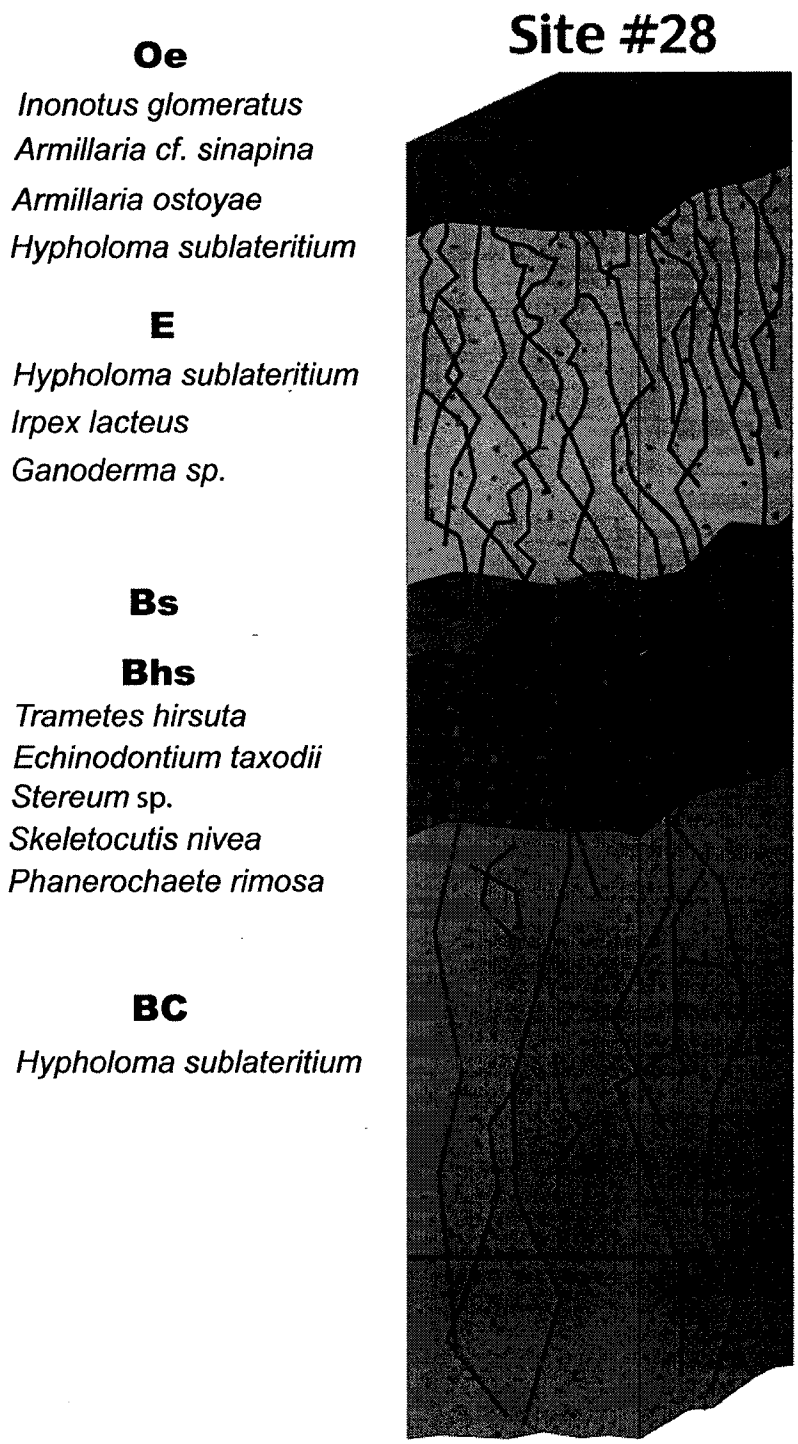


Fig. 43. Identified basidiomycete fungi by soil horizon, site # 28.

Table 18. GenBank BLAST results of soil fungal isolates from site # 29.

	Fungal Isolate [Z]	Collection Date	Horizon	Primers	Blast results	Accession #
Soil	29-1-8 LD 2	June 1997	Oe	ITS1-3	*Thanatephorus cucmeris*	AF455461
Soil	29-1-9 MS 5B 1	June 1997	Oe	ITS1 & 3	*Phellinus ignarius*	AF455434
Soil	29-1-9 MS 5B 2	June 1997	Oe	ITS1-3	*Trametes versicolor*	AY309017
Soil	29-1-1 L 6	June 1999	Oe	ITS1 & 3	*Coprinus fissolanatus*	AF345812
Soil	29-1-4 WA 2	June 1999	Oe	ITS1-3	*Verticillium bulbillosum*	AJ292410
Soil	29-1-5 WA 1	June 1999	Oe	ITS1-3	*Thanatephorus cucmeris*	AF455463
Soil	29-1 OSG M3 1	Oct. 1999	Oe	ITS1 & 3	*Myrothecium inundatum*	AJ302005
Soil	29-1 OSL T2 1 A	Oct. 1999	Oe	ITS1-3	*Armillaria* sp.	AY554331
Soil	29-2 MSG L2 2	Oct. 1999	E2	ITS1-3	*Armillaria* sp.	AY554331
Soil	29-2 MSL M3 4	Oct. 1999	E2	ITS1-3	*Armillaria sp.*	AY554331
Soil	29-3 MSG M1 2	Oct. 1999	Bw	ITS1-3	*Reniforma strues*	AF444573
10JB	29-1 RM 1 3	Oct. 1999	Oe	ITS1 & 3	un ID Basidiomycete	AF241332
SPB	29-1 MYBDA 1A	Oct. 2000	Oe	ITS1 & 3	*Megacollybia platyphylla*	AF498289
SPB	29-3 MYBDA 2A	Oct. 2000	BC	ITS1 & 3	*Phanerochaete rimosa*	AY219349

Z: Fungal Isolate: see Tables 12-15

Figure 44 illustrates the basidiomycete fungal isolates identified by sequencing per soil horizon for research site # 29.

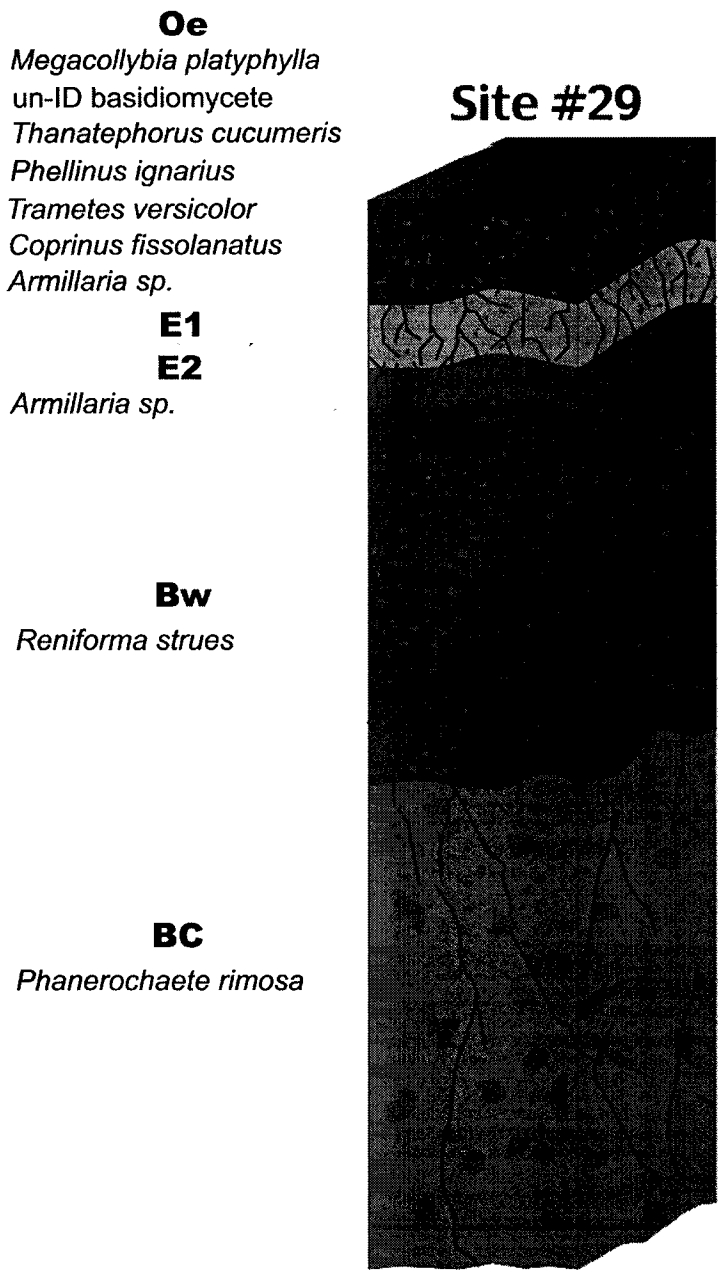


Fig. 44. Identified basidiomycete fungi by soil horizon, site # 29.

Soil basidiomycete isolates identified by sequencing are listed below by collection date, order, and family per research site (Table 19). Order and family names selected using Hawksworth et al (1995).

Table 19. Soil basidiomycete isolates by research site, method, order, family and soil horizon.

Site # 26			
Method	Order	Family	Soil Horizon
Direct			
Hypholoma sublateritium	Agaricales	Strophariaceae	Oe
Mycena aff. murina	Agaricales	Tricholomataceae	Bw
June Jar Bait			
Hypholoma sublateritium	Agaricales	Strophariaceae	E
Soil Pit Bait			
Fomes fomentarius	Poriales	Coriolaceae	BC

Site # 28			
Method	Order	Family	Soil Horizon
Direct			
Inonotus glomeratus	Hymenochaetales	Hymenochaetaceae	Oe
Armillaria cf. *sinapina*	Agaricales	Tricholomataceae	Oe
Armillaria ostoyae	Agaricales	Tricholomataceae	Oe
Hypholoma sublateritium	Agaricales	Strophariaceae	Oe
Ganoderma sp.	Ganodermatales	Gandermataceae	E
Irpex lacteus	Stereales	Steccherinaceae	E
Skeletocutis nivea	Poriales	Coriolaceae	Bhs
Echinodontium taxodii	Stereales	Echinodontiaceae	Bhs
Stereum species	Stereales	Stereaceae	Bhs
June Jar Bait			
Phanerochaete rimosa	Stereales	Meruliaceae	Bhs
October Jar Bait			
Hypholoma sublateritium	Agaricales	Strophariaceae	Oe, E, BC
Soil Pit Bait			
Hypholoma sublateritium	Agaricales	Strophariaceae	Oe, E

Site # 29			
Method	Order	Family	Soil Horizon
Direct Soil			
Thanatephorus cucmeris	Ceratobasidiales	Ceratobasidiaceae	Oe
Phellinus ignarius	Hymenochaetales	Hymenochaetaceae	Oe

Site # 29			
Method	Order	Family	Soil Horizon
Direct Soil			
Trametes versicolor	Poriales	Coriolaceae	Oe
Coprinus fissolanatus	Agaricales	Coprinaceae	Oe
Armillaria sp.	Agaricales	Tricholomataceae	Oe, E2
Reniforma strues	basidio. yeast		Bw
October Jar Bait			
un-ID Basidiomycete			Oe
Soil Pit Bait			
Megacollybia platyphylla	Agaricales	Tricholomataceae	Oe
Phanerochaete rimosa	Stereales	Mertuliaceae	BC

Tables 20 (basidiomycete) and 21 (non-basidiomycete) summarize the GenBank identities for the soil fungal isolates.

Table 20. GenBank sequence identification of basidiomycete soil fungal isolates.

Culture Method	Soil Horizon	Blast results	Accession #	# of Isolates
Direct Soil	Oe	*Lecythophora* sp.	AY354246	1
"	Oe	*Hypholoma sublateritium*	AY534117	5
"	Oe	*Hypoxylon fragiforme*	AJ390403	1
"	Oe	*Armillaria ostoyae*	AJ250055	1
"	Oe, BC	*Thanatephorus cucmeris*	AF455461	3
"	Oe	*Phellinus ignarius*	AF455434	1
"	Oe	*Trametes versicolor*	AY309017	1
"	Oe	*Coprinus fissolanatus*	AF345812	1
"	Oe, BC	*Verticillium bulbillosum*	AJ292410	4
"	Oe, Bhs	*Myrothecium inundatum*	AJ302005	5
"	Oe, E2	*Armillaria* sp.	AY554331	3
"	Oe	*Inonotus glomeratus*	AF247968	1
"	Oe	*Armillaria* cf. *sinapina*	AY228346	1
"	E	*Ganoderma* sp.	AJ749970	1
"	E	*Irpex lacteus*	AB079265	1
"	Bhs	mycorrhizae fungal sp.	AY219840	1
"	Bhs	*Trametes hirsuta*	AY494980	1
"	Bhs	*Echinodontium taxodii*	AF455458	1
"	Bhs	*Fusarium tricinctum*	AY188923	1
"	Bhs	*Stereum* species	AY207328	1
"	Bw	*Mycena aff. murina*	AF335444	2
"	Bw	*Reniforma strues*	AF444573	2
"	BC	*Ganoderma* sp.	AJ749970	2
Spring Jar Bait	E	*Hypholoma sublateritium*	AY534117	2
"	Bhs	*Phanerochaete rimosa*	AY219349	3
October Jar Bait	Oe	un ID Basidiomycete	AF241332	1
"	Oe, E, BC	*Hypholoma sublateritium*	AY534117	6
Soil Pit Bait	Oe, E	*Hypholoma sublateritium*	AY534117	4
"	Oe	*Megacollybia platyphylla*	AF498289	1
"	BC	*Phanerochaete rimosa*	AY219349	1
"	BC	*Fomes fomentarius*	AY354213	1

Table 21. GenBank sequence identification of non-basidiomycete or un-cultured fungus soil fungal isolates.

Culture Method	Soil Horizon	BLAST result	Accession #	# of Isolates
Direct Soil	Oe	*Hypoxylon fragiforme*	AJ390403	1
"	Oe	uncultured fungus	AF461617	1
"	Oe	*Lecythophora* sp.	AY354246	1
"	Oe	*Myrothecium inundatum*	AJ302005	2
"	Oe	*Verticillium bulbillosum*	AJ292410	2
"	Bhs	Ascomycete	/	1
"	Bhs	*Myrothecium inundatum*	AJ302005	4
"	Bhs	*Fusarium tricinctum*	AY188923	1
"	Bhs	*Cladosporium elatum*	AF393699	1
"	Bhs	*Verticillium bulbillosum*	AJ292410	2
"	Bhs	mycorrhizae fungal sp.	AY219840	1
"	Bw	uncultured fungus	AF461657	5
Spring Jar Bait	Oe	uncultured fungus	AF504844	1
"	Bhs	uncultured fungus	AF461617	1

Discussion

Direct and sieved soil methods used to process the June 1997 soils produced almost no putative basidiomyceteous fungi. Out of the 720 plates inoculated from all research sites, only 11 fungal isolates were kept, four were isolated using MYBDA and seven were isolated using LGBDA medium. Out of these 11 isolates, six were from the organic soil, four were from the upper mineral soil horizon, and one was from the lower mineral soil horizon.

Sieved soil methods used to process the June 1999 soils produced numerous no growth plates. Out of the 540 plates inoculated from all research sites, only 18 fungal isolates were kept, five using MYBDA, five using water agar medium, and eight using LGBDA medium. Out of these 18 isolates, 11 were from the organic soil and from the mineral soil, three were from the upper mineral soil horizon, and four were from the lower mineral soil horizon.

Modified sieve methods used with the October 1999 soils produced more putative basidiomyceteous fungi. Out of the 216 plates inoculated from all research sites, 44 fungal isolates were kept, 21 using MYBDA, 12 using LGBA (Thorn), and 11 using LGBDA media. Out of these 44 isolates, 26 were from the organic soil and from the mineral soil, 13 from the upper mineral soil horizon and five from the lower mineral soil horizon. Out of these 44 isolates, 22 were from "grains" and 22 were from "liquid".

Of the 14 fungal isolates kept from the June 1999 soils in the French square jar wood bait experiments, 11 came from white pine wood, and three came from red maple wood. Out of the 14 isolates, one came from the organic soil horizon, three came from the upper mineral soil horizon, and 10 came from the lower mineral soil horizon.

Several of the soil pit baits were fragile, and were held together only by the string. Many of the soil pit baits from organic soils were covered with roots, and white resupinate fruiting bodies. The soil pit bait from the mineral soil (Bhs) from site # 28 was extremely fragile without a color change in the wood. Thirty-four fungal isolates were kept from this culture method, 21 using MYBDA, seven using LGBDA, and six using LGBA (Thorn) media. Of these 34 isolates, 20 came from the organic soil horizons, six came from the upper mineral soil horizon, and eight from the lower mineral soil horizon.

Most literature/keys available are designed to identify fungal isolates from basidiocarps; unknown fungal isolates isolated from soil are impossible to identify by culture characteristics and GAA/TAA testing alone. Of the fungal isolates that

were sequenced, basidiomycete fungi were retrieved from organic and mineral soil horizons using all methods (directly from soil, jar wood baits, and soil pit wood baits).

Analysis of cultural characteristics (Tables 12-15) of all six research sites and all soil culture methods (direct, jar bait, soil pit bait), for the June 1997, 1999, and Oct. 1999 soil collections revealed that: 75% were white rotters (GAA/TAA groups 4-9) and 25% were brown rotters (GAA/TAA groups 1-3). Sixty percent of the white rotters were from the organic soil horizons and 40% were from the mineral soil horizons. Sixteen percent of the brown rotters were from the organic soil horizons and 84% were from the mineral soil horizons.

Analysis of wood bait culture methods (jar bait & soil pit bait) for all six research sites and all soil horizons, for the June 1997, 1999, and October 1999 soil collections revealed that: 87% were white rotters (GAA/TAA groups 4-9) and 13% were brown rotters (GAA/TAA groups 1-3). Of the white rotters, 53% were from the organic soil horizons and 47% were from the mineral soil horizons. Of the brown rotters, 100% were from the mineral soil horizons.

The basidiomycete isolates from the soils identified by sequencing were from six orders: Agaricales (26), Ceratobasidiales (3), Hymenochaetales (2), Poriales (4), Ganodermatales (2), and Stereales (7).

Basidiomycete fungi from the Strophariaceae and Tricholomataceae families were isolated from the organic and mineral soils of sites # 26, 28, and 29. Additional basidiomycete families isolated from organic soils include: Hymenochaetaceae, Ceratobasidiaceae, Coprinaceae, and Coriolaceae.

Additional basidiomycete families isolated from mineral soils include: Ceratobasidiaceae, Ganodermataceae, Meruliaceae, Steccherinaceae, Echinodontiaceae, Stereaceae, and Coriolaceae. Families and orders were determined using Hawksworth et al (1995). With this limited data set, it appears that mineral soils are quite diverse in species of basidiomycete fungi.

CHAPTER IV

COARSE WOODY DEBRIS AND BOLE WOOD FUNGAL ISOLATES

Introduction

CWD is the least studied component of the forest biomass. The estimate of above- and below-ground biomass in a deciduous temperate forest is 300 t/ha (Boddy and Watkinson 1995). Wood decomposes slowly and forms a reservoir of nutrients. CWD can remain in the forest for many years before it completely decomposes and a natural forest contains CWD in various stages of decay. Decaying wood may persist in soils for over 1000 years (McFee and Stone 1986). CWD is important to the environment for other organisms and provides a site for seedling establishment for mosses, plants, and lichens and serves as a substrate for fungi to utilize. It also provides a habitat for vertebrates, invertebrates, and many microorganisms. "Up to two thirds of wildlife species use dead wood structures or woody debris for some portion of their life cycle" (Brown 2000, p.5). CWD affects the forest ecosystem processes including soil retention (physical), nutrient cycling (chemical), and wildlife habitat (biological) (Harmon et al. 1986). The holistic approach to forest management, "New Forestry," recognizes the need to retain adequate amounts of CWD after harvesting to preserve the important ecological functions in a forest with the chief concern being the long-term impact of organic matter losses (Caza 1993).

Current forest management practices greatly reduce the amount of CWD normally found in a natural ecosystem (Spies et al. 1988). CWD is a major structural component of a natural forest and understanding its functions should be an important component of ecologically minded forest management practices.

Elton (1966 p.279) states, "If fallen timber and slightly decayed trees are removed (from a natural forest) the whole system is gravely impoverished of perhaps more than a fifth of its fauna." For example, hollow dead trees provide many birds nesting sites (Bull 2000, Huss and Bednarz 2000, Bunnell et al. 2002), birds prefer more decay-prone hardwood tree species (Bunnell et al. 2002), wood-boring beetle larvae live in CWD (Duane et al. 2000, Ross 2000), ants use CWD for nesting from early dead wood through advanced decay (Lindgren and MacIsaac 2000), and there is a succession of insects that occur in dead and decaying wood (Grove 2002, Grove and Bashford 2003). In fact, fungal inoculations have been made into conifer trees to promote cavity excavation by woodpeckers in western Washington in managed forests (Huss and Bednarz 2000).

High levels of nitrogen deposition in the temperate forests is threatening the function of these forests by bringing about an imbalance in soil nutrients which in turn affects the plant community composition (Aber et al. 1989). Reduced growth of the forest is due to losses in soil cations as a result of these increased levels of nitrogen (Aber 1992, Likens et al. 1996). Many functions of CWD are documented, but little is known of the fungi and nutrient cycling processes in comparison with the mineral soil (Cazares and Trappe 2000).

Arthur and Fahey (1990) compared literature and (Weaver 1975, Lang and Forman 1978, Henderson et al. 1978, Cromack 1973) conducted research regarding temperate deciduous forests. They concluded that these forests have lower amounts of dead wood biomass than coniferous forests, but higher nutrient concentrations are found in decaying deciduous species, suggesting the importance of this dead wood as a slow pool of nutrients for forest health by contributing to important nutrient cycles.

CWD plays an important function in the cycling of nutrients as a short-term sink and a long-term source of nutrients in the forest ecosystems (Harmon et al. 1986). Several researchers have studied the nutrient content in CWD. For example, calcium was shown to be released similar to dry weight loss in leaf and branch litter by decomposition at the Hubbard Brook Forest in New Hampshire (Gosz et al. 1973). Percent of nitrogen was shown to increase significantly with the decrease in branch diameter size (Lang and Forman 1978). In the early stages of decay of boles of balsam fir in New Hampshire there was an initial loss of nutrients due to the sloughing off of the bark (Lambert et al. 1980). Bark contains 12 times more phosphorus and 24 times more calcium than fresh wood (Lambert et al. 1980). Studying balsam fir forests in New Hampshire, Lang et al. (1981) did not see significant changes in nutrient content of decaying boles at various stages of decay.

In red spruce and balsam fir in New Hampshire, Foster and Lang (1982) showed that nitrogen accumulated in the bark and that concentrations increased significantly during decay, while sodium was released from decaying wood at the

same rate as mass loss. They suggested that nitrogen might be translocated from the forest floor into the wood via fungal hyphae. Phosphorus concentrations fluctuated from accumulation and loss during the decay of older boles, suggesting that nitrogen and phosphorus limits the decomposer organism's growth, and these values may represent the elemental composition of the fungal biomass in decaying boles. Calcium concentrations increased during decay suggesting its translocation into the boles as calcium oxalate from fungal mycelia. It appeared to these researchers that the immobilization of magnesium by decay organisms was significant.

Edmonds (1987) examined the influence of the microclimate and substrate chemistry on decomposition rates and nutrient dynamics in four ecosystems in western Washington. Significant increases of elemental concentrations of nitrogen, phosphorus, calcium, and sodium were found with increasing decay of CWD (Arthur and Fahey 1990). These high amounts of calcium in dead wood could affect the long-term availability in natural forests. Dead wood above and on the forest floor represented a significant amount of nitrogen and phosphorus (Keenan et al. 1993). In decaying boles of *Pinus contorta* var. *murayana* Greb. & Balf., the nitrogen content was 75% greater than in sound boles initially, with a net release of calcium, magnesium and potassium (Busse 1994).

More recent research has shown that earlier work indicating an accumulation of nutrients in decaying wood may be in question, as volume loss may not have been considered. Krankina et al. (1999) found that *Pinus sylvestris* L. released essential nutrients in the early stages of decay, while *Betula pendula*

Roth. lost its nutrients more gradually because it retained its bark longer. Their calculations were adjusted for the loss of density and volume of the CWD. Allen et al. (2000) analyzed each log studied for C, N, Ca, Mg, K, and P. As decay progressed, nitrogen concentration increased, while the concentrations of potassium and magnesium declined in the early stages of decay, but remained constant during the later stages of decay in a temperate New Zealand forest. Over time nitrogen and minerals accumulate in CWD (Cazares and Trappe 2000). Studying the phosphorus and nitrogen cycle contribution of CWD in three Rocky Mountain forests, Prescott and Laiho (2000) concluded that CWD is not a significant source of available nitrogen in these forests. Krankina et al. (1999) concluded that when the natural forest landscape is converted to rotational forest harvesting the naturally occurring nutrient cycling by CWD is disrupted by the clear cut and thereby is a loss of a nutrient source for that forest.

Wells and Boddy (1990) and Wells et al. (1990) have shown that wood-decaying fungi opportunistically uptake and translocate phosphorus long distances from their surroundings to the substrate they are colonizing. Lindahl et al. (1999) demonstrated the translocation of ^{32}P from a wood-decomposing saprotrophic fungus, *Hypholoma fasciculare*, to an ectomycorrhizal fungus, *Suillus variegatus*, and their plant hosts. Cord-forming saprotrophic fungi take up nutrients from the soil and litter and may be the major pathway for nutrient relocation (Wells et al. 2001).

CWD is an important pool of carbon in the forest ecosystem. Clear cutting of forests can change the carbon storage capacity of the forest and can change a

forest ecosystem from a CO_2 source to a CO_2 sink during regeneration (Kawaguchi and Yoda 1986). Leached organic carbon and organic nitrogen from the organic soil horizon after clear cutting was retained in the mineral soil horizons and indicates microbial immobilization (Pirainen et al. 2002). Increase in CO_2 will bring greater amounts of carbon to the soil and will increase microbial growth, which in turn will immobilize soil nutrients and make them less available to plants (Morgan 2002).

Other researchers have focused on the relationship of fungal (sporocarps) taxa found in the forest and on the stage of decay of the CWD. Studying mountain beech, Allen et al. (2000) visually estimated the percentage of each log touching the ground and studied the diversity of fungi found among logs unrelated to succession. They concluded that log size, autumn collection, and habitat heterogeneity are factors in log fungal taxa diversity. Graham and Cromack (1982) found it helpful to describe their research sites indicating the tree size (by species), illustrating the downed bole wood, and determining the decay class of the downed bole wood for then understanding decomposition and nutrient content. They recorded the length and end diameters of all downed bole wood greater than 10 cm in diameter, concluding that dead boles are an integral component of the forest ecosystem.

Decay classes have been described and used by other researchers (Appendix 20), almost all relating to evergreen softwood tree species, with the exception of Lang and Forman (1978) who studied an oak forest in New Jersey.

They only used two decay classes to describe the changes during decomposition. Others have used five decay classes to describe their boles.

Materials and Methods

Techniques used in this research include those recently developed (Thompson 1998) and those newly developed were used to isolate species of basidiomycetes in the CWD of *Acer rubrum* (red maple) and contiguous soil horizons at six previously selected sites at the Bartlett Experimental Forest (BEF) in the White Mountain National Forest, Bartlett, New Hampshire.

The USFS scientists selected and numbered *Acer rubrum* trees in compartment 9 of Bartlett Experimental Forest in 1990. Some of these trees were cut down, sectioned into boles, and left on the ground for seven years. For this research, the boles selected were from trees numbered 19, 21, and 22 in one area of the forest and 26, 28, and 29 in another area. Tree numbers are hereafter designated as research sites. The two research areas are about 100 feet from each other and can be reached by following a path in the forest. Figure 45 illustrates the stump and sections (bole) of the tree bole on the ground of each research site #'s 19, 21, & 22 (Area 2). Figure 46 illustrates the stump and sections of the bole on the ground of each research site #'s 26, 28, & 29 (Area 1). The X designates the side and the bole of each tree where the soil pits were dug to collect soil and bole wood samples for culturing. The lengths of the bole sections used in this study were: site #19 (5'6"), #21 (4'2"), #22 (7'1"), #26 (4'), #28 (7'5"), and #29 (9'1").

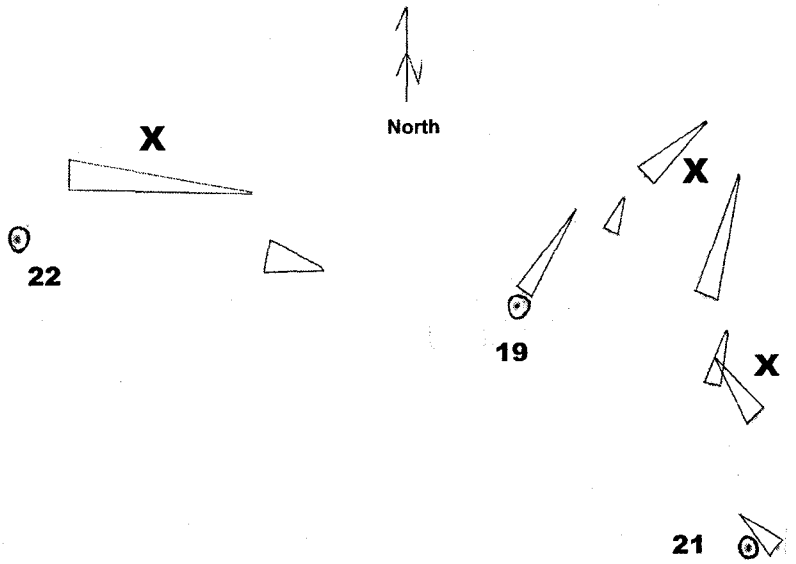


Fig. 45. Layout of the boles and stumps of research sites # 19, 21, & 22. . The "X" designates the bole that was used for the soil and bole chip collections. The "circle" represents the tree stump.

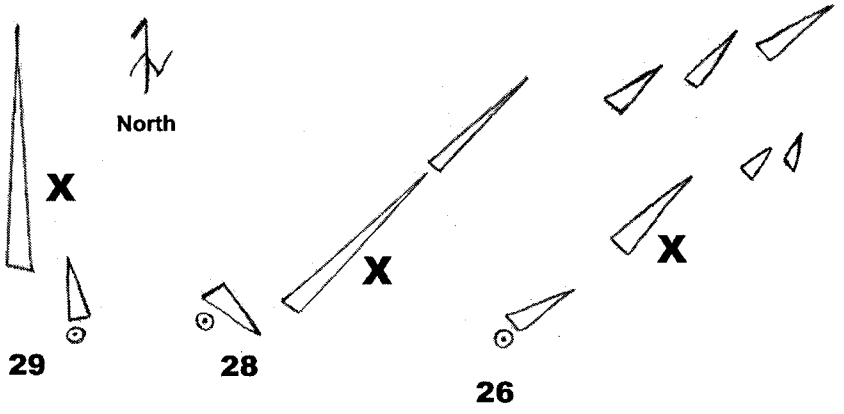


Fig. 46. Layout of the boles and stumps of research sites # 26, 28, & 29. The "X" designates the bole that was used for the soil and bole chip collections. The "circle" represents the tree stump.

Crosscut wood pieces that were hand-sawn from each decaying bole in June 1997, June 1999, and October 1999 were placed into paper bags and returned to the laboratory for further studies. Bole wood samples from site # 21 that were collected during the June 1997 collection are shown in Figure 47.

Inner wood bole chips (approximately 3 mm square) were removed aseptically, and placed onto sterile media. Bole chips from the June 1997 and June 1999 collections were placed onto 10 replicate plates each of MYBDA and LGBDA media. For the June 1997 collection, 4 bole chips were placed on each plate of medium. For the June 1999 and October 1999 collections, 2 bole chips were placed on each plate of medium. Bole chips from the October 1999 collection were placed onto 5 replicate plates each of MYBDA, LGBDA, and LGBA (T) media.

Fig. 47. Bole wood from research site # 21 (1997) showing the demarcation (dark zone lines) between different decay organisms (compartmentalization).

The decay classes of the boles were determined at the time of each collection. Hardwood boles are slow to decay so only 3 decay classes were used to describe the decomposition state of the research boles. The decay classes used in this research are listed in Table 22. A knife was used to test the structural integrity of the boles. During each bole wood collection, the rate of decomposition of the boles was classified using this system.

Table 22. Decay classes of *Acer rubrum* used in this research.

	I	II	III
bark	intact	sloughing	absent
twigs, branches	present	decaying	absent
bole shape	round	oval	none
structural integrity	sound	heartwood present	none
litter	no accumulation	accumulation	accumulation
vegetation	none	seedlings, herbs	include moss

The June 1997 and June 1999 bole wood samples were analyzed by the UNH Soils Analytical Services Laboratory for mineral content, percent organic matter, and pH. The red maple wood used for the soil pit baits was also analyzed. These tests were performed using standard soil testing methods of the North East USA (Agricultural Experimental Station, Delaware bulletin # 493, 1995) and included pH, percent organic matter (LOI-550), and parts per million of calcium, phosphorus, magnesium and potassium (Mehlich 3).

The morphological and molecular methods used to analyze the fungal isolates are described in Chapter 2.

Results

During the October 1997 visit to the research sites, it was noted that on boles # 19, 21, and 22 moss was growing at the cut area of the bole wood where samples were collected in June 1997. It was also noted that the bark had fallen off, so these boles have some characteristics of more than a decay class 1. As shown in Figure 48, the bark has fallen off bole # 28. All research boles were very hard and had sound integrity throughout all collection dates. During all bole

wood collections, all boles were in decay class 1. As of Fall 2002 all research boles were at the decay class 2 stage of decomposition.

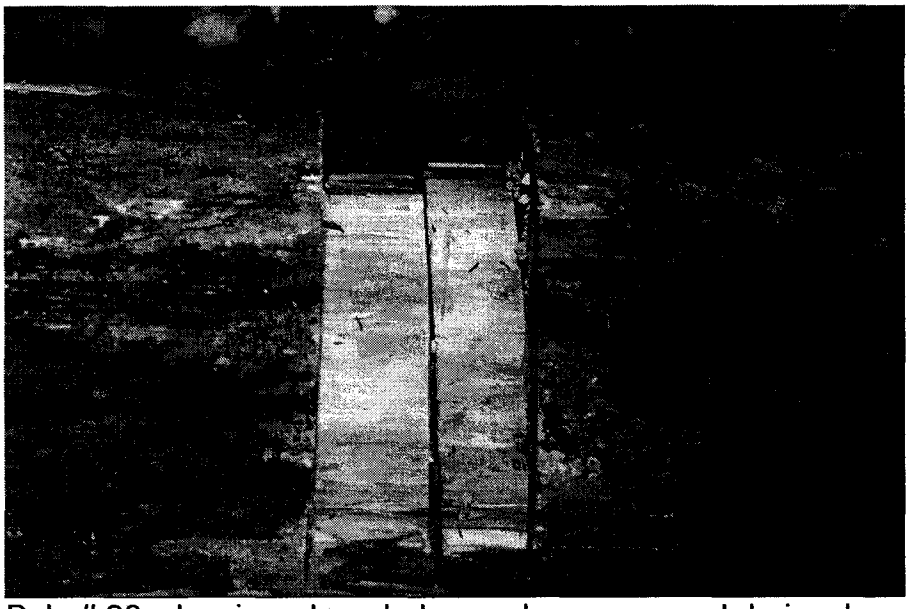


Fig. 48. Bole # 28, showing where bole wood was removed during June 1997 collection. Photographed October 1997 during sporocarp collection trip. This bole was rated as a decay class 1 during the collection; since then the bark has fallen off and it could be a decay class 1+.

The chemical analyses (Mg, Ca, K, & P) of the June 1997 and June 1999 bole wood collections and the sterile soil pit bait wood are charted in Figures 49 and 50, respectfully. The individual mineral content is charted separately in Figures 51-54, including the June 1997 and June 1999 collection years. Appendix 21 shows the results of this chemical analysis.

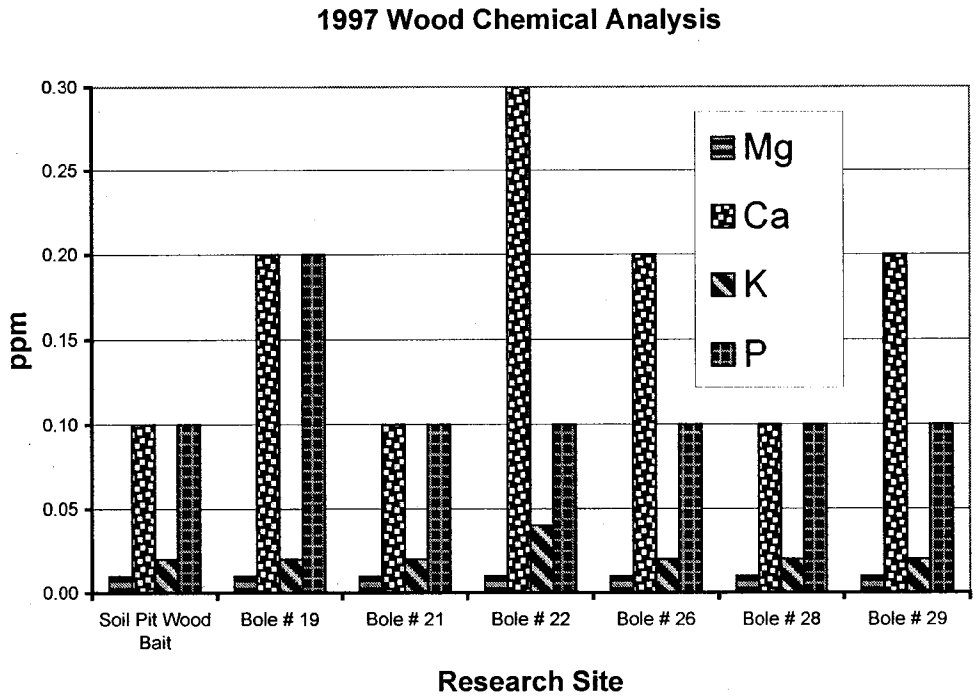


Fig. 49. Chemical analysis: Mg, Ca, K, & P (ppm) of soil pit bait (red maple) wood and June 1997 bole wood samples per research site.

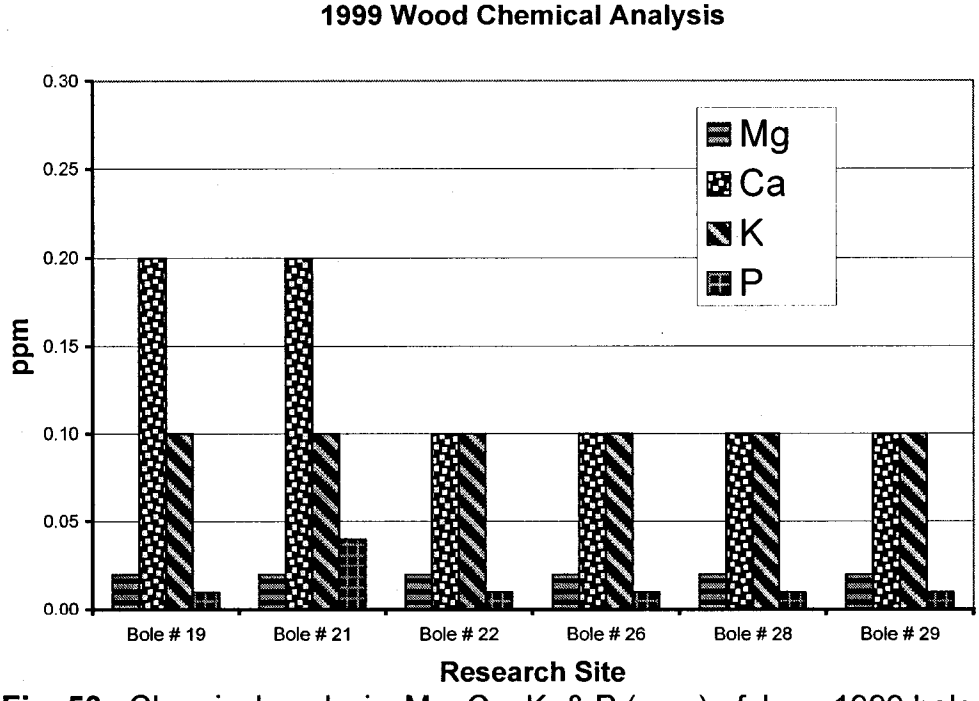


Fig. 50. Chemical analysis: Mg, Ca, K, & P (ppm) of June 1999 bole wood samples per research site.

Figures 51-54 illustrate each mineral analysis for both the June 1997 and June 1999 collection of bole wood samples (using the same scale).

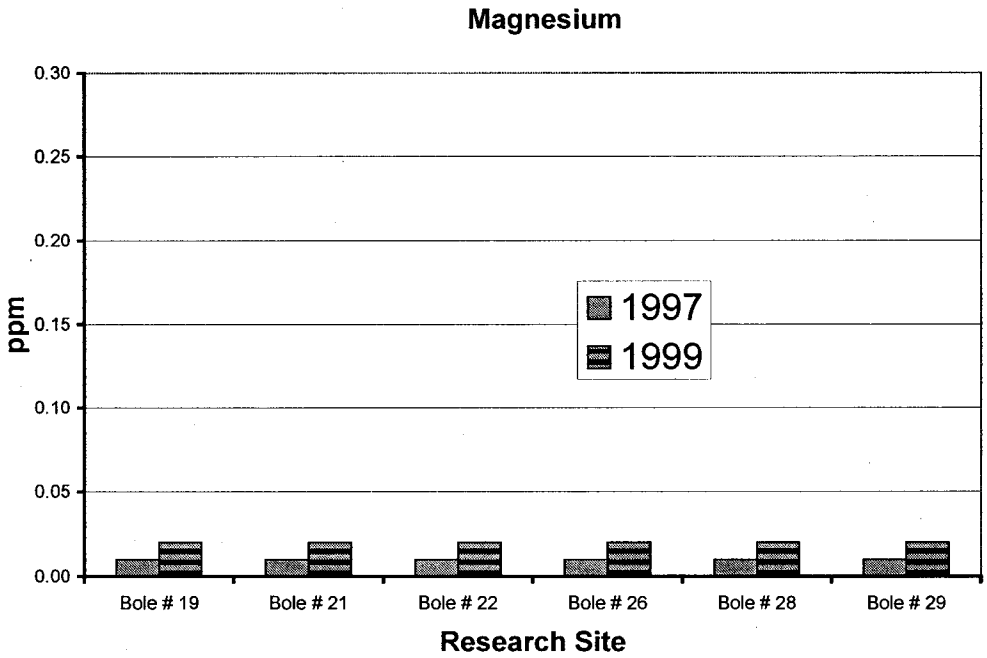


Fig. 51. Comparison of magnesium levels (ppm) in bole wood samples for all research sites and both collection dates (June 1997 & June 1999).

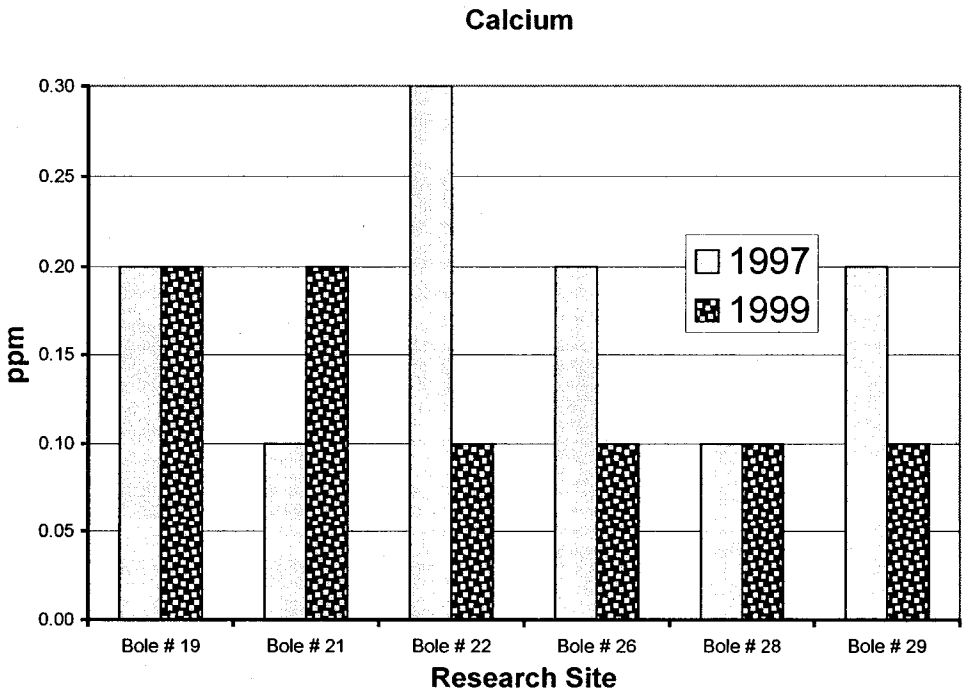


Fig. 52. Comparison of calcium levels (ppm) in bole wood samples for all research sites and both collection dates (June 1997 & June 1999).

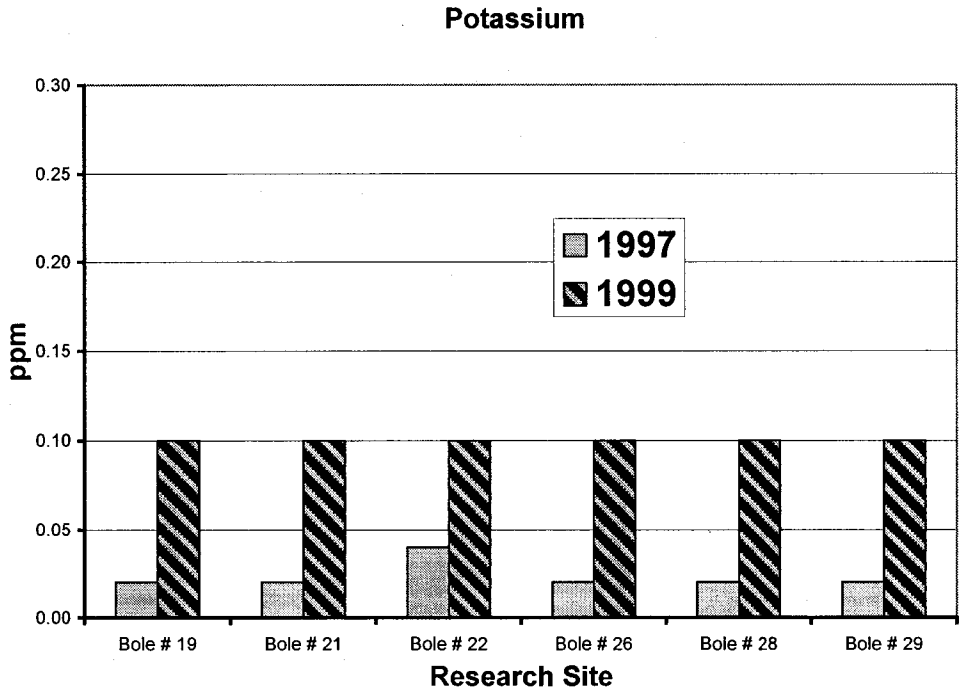


Fig. 53. Comparison of potassium levels (ppm) in bole wood samples for all research sites and both collection dates (June 1997 & June 1999).

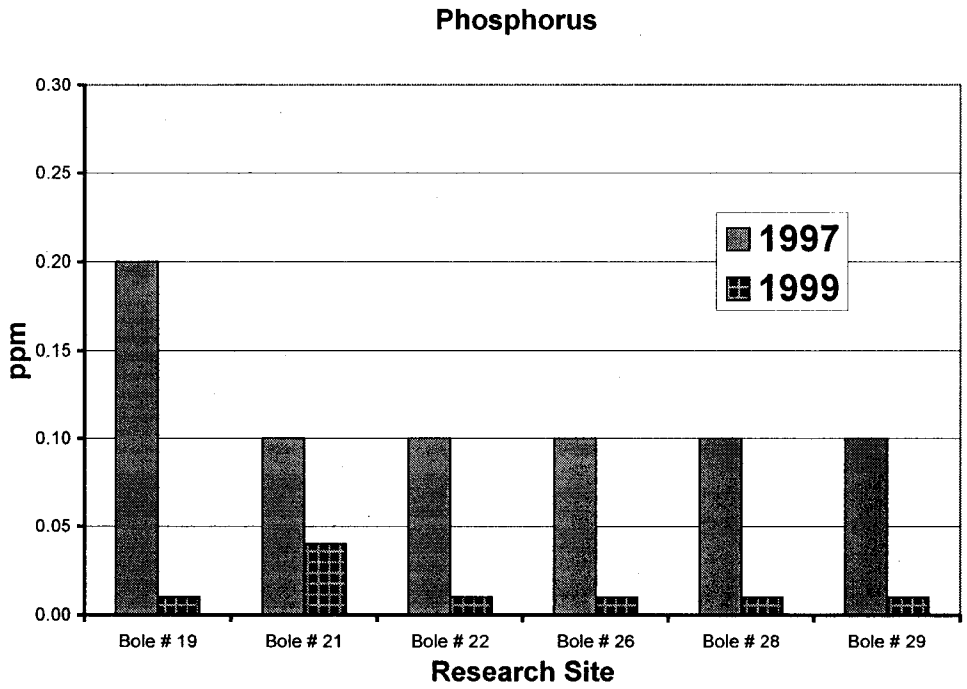


Fig. 54. Comparison of phosphorus levels (ppm) in bole wood samples for all research sites and both collection dates (June 1997 & June 1999).

The 1997 chemical analyses show that the Mg levels for all research sites, Ca levels for sites # 21 and 28, K levels for all but site # 22 and P levels for all but site # 19 are the same as fresh non-decayed red maple wood used for the soil pit baits. These data support the decay class data that all bole wood collections were at decay class 1, seven years after felling. The bark on all boles was fully intact during the June 1997 collection. The 1999 chemical analyses show that the magnesium levels of all research sites, the calcium levels of four of the research sites (excluding # 22 & 29), the potassium levels of all research sites, and the phosphorus levels of five of the research sites (excluding # 21) are the same. When comparing the 1997 to the 1999 chemical analyses of the research bole woods, all magnesium levels had increased; calcium levels in four research sites (excluding # 22 & 29) decreased; all potassium levels had increased five fold; and five of the phosphorus levels had decreased ten fold. The percent of organic matter did not change from 1997 to 1999.

Summary of the cultural data of the bole chip isolates are listed in Table 23. Full cultural data of these isolates are found on the accompanying CD.

Table 23. Summary cultural data of fungal isolates from bole chip (CWD) fungal isolates by collection date and research site.

Collection Date	Fungal Isolate [U]	GAA/TAA [V]	M [W]	GR [X]	Color [Y]	Texture [Z]
	Site # 19					
Jun-97	19-1-1 MYBDA	5	1	3	2	3
Jun-97	19-1-1 LGBDA	5	1	3	2	3
Jun-97	19-2-1 MYBDA	5	1	3	2	3
Jun-97	19-2-4 LGBDA	5	1	3	2	3
Jun-97	19-3-1 MYBDA	5	1	3	1	3
Jun-97	19-3-2 LGBDA	5	1	3	2	3
Jun-97	19-3-3 MYBDA	5	1	3	2	3
Jun-97	19-4-1 LGBDA	5	1	3	2	3

Collection Date	Fungal Isolate [U]	GAA/TAA [V]	M [W]	GR [X]	Color [Y]	Texture [Z]
Jun-97	19-4-2 MYBDA	5	1	3	2	1, 3, 4
Jun-97	19-5-1 MYBDA	5	1	3	2	3, 4
Jun-97	19-5-1 LGBDA	5	1	3	2	3
Jun-97	19-5-4 LGBDA	3	3	3	0	4
Jun-97	19-6-2 LGBDA	5	1	3	2	3
Jun-97	19-6-3 MYBDA	5	1	3	2	3
Jun-97	19-7-1 MYBDA	5	1	3	2	3
Jun-97	19-7-4 LGBDA	5	1	3	2	3
Jun-97	19-8-1 MYBDA	?	1	3	2	3
Jun-97	19-8-4 LGBDA	5	1	3	2	3
Jun-97	19-9-2 MYBDA	5	1	3	2	3
Jun-97	19-9-4 MYBDA	5	1	3	2	3
Jun-97	19-9-4 LGBDA	5	1	3	2	3
Jun-97	19-10-1 MYBDA	5	1	3	2	3
Jun-97	19-10-3 LGBDA	5	1	3	2	3
Jun-99	19-1-1 MYBDA	3	1	3	0	3
Jun-99	19-1-1 LGBDA	3	1	3	1	
Jun-99	19-1-4 LGBDA	3	1	3	0	3
Jun-99	19-2-3 MYBDA	3	1	3	0	3
Jun-99	19-3-1 MYBDA	5	1	3	0	3
Jun-99	19-3-1 LGBDA	3	1	3	0	3
Jun-99	19-3-3 MYBDA	5	1	3	0	3
Jun-99	19-4-1 MYBDA	3	1	3	0	3
Jun-99	19-4-3 MYBDA	5	1	3	0	3
Jun-99	19-5-1 LGBDA	4	1	3	0	3
Oct-99	19 M1 1	5	0	3	1	3, 5
Oct-99	19 M3 1	3	1	3	1	3
Oct-99	19 L1 1	5	1	3	1	3
Oct-99	19 L3 1	3	1	3	1	3, 7
Oct-99	19 T2 1	3	1	3	1	3, 7
	Site # 21					
Jun-97	21-1-1 LGBDA (m)	5	1	3	2	3
Jun-97	21-1-2 MYBDA	5	1	3	0	3
Jun-97	21-2-3 LGBDA	6	1	3	1	3, 8
Jun-97	21-3-1 MYBDA	5	0	2	1	3
Jun-97	21-4-1 LGBDA	5	1	3	2	5
Jun-97	21-5-1 LGBDA	5	1	3	1	3
Jun-97	21-5-3 LGBDA	5	0	3	0	3
Jun-97	21-5-3 MYBDA (m)	3	1, 4	2	2	3
Jun-97	21-5-4 LGBDA	5	1	3	2	3
Jun-97	21-5-4 MYBDA	3	1, 4	3	1	3
Jun-97	21-6-2 MYBDA	5	1	3	1	3
Jun-97	21-7-2 MYBDA	5	1	3	2	3. 8

Collection Date	Fungal Isolate [U]	GAA/TAA [V]	M [W]	GR [X]	Color [Y]	Texture [Z]
Jun-97	21-8-1 MYBDA	3	1, 4	3	1	3, 6, 7
Jun-97	21-9-1 MYBDA	3	1, 4	3	1	3, 6, 7
Jun-97	21-10-1 MYBDA	3	4	3	1	0, 5
Jun-97	21-10-3 LGBDA	5	1	3	1	3
Jun-97	21-10-4 MYBDA	3	1, 4	3	1	3, 6, 7
Jun-99	21-1-2 MYBDA	5	1	1	1	3
Jun-99	21-1-2 LGBDA	5	1	2	1	3
Jun-99	21-1-3 MYBDA	5	1	3	0	3
Jun-99	21-1-4 LGBDA	5	1	2	1	3
Jun-99	21-2-2 MYBDA	3	1	3	1	3, 7
Jun-99	21-2-3 MYBDA	3	1	3	1	3
Jun-99	21-2-3 A LGBDA	3	1	3	1	6
Jun-99	21-2-3 B LGBDA	3	1	3	1	3, 7
Jun-99	21-3-3 MYBDA	5	1	2	1	3
Jun-99	21-3-4 LGBDA	5	1	2	1	3
Jun-99	21-4-1 MYBDA	3	1	3	1	3, 6
Jun-99	21-4-4 LGBDA	3	1	3	1	3, 6
Jun-99	21-5-1 LGBDA	3	1	3	0	2, 7
Jun-99	21-5-2 MYBDA	3	1	3	1	3, 6
Jun-99	21-5-2 LGBDA	3	1	3	1	3, 7
Jun-99	21-5-3 MYBDA	4	1	2	1	6
Oct-99	21 M2 2	3	1	3	1	3, 7
Oct-99	21 M3 1	3	1	3	1	3, 7
Oct-99	21 L1 2	5	0	3	1	2, 3
Oct-99	21 L3 1	3	1	3	1	3, 7
Oct-99	21 L3 2	3	1	3	1	3, 7
Oct-99	21 T2 1	3	0	3	1	2, 3
Oct-99	21 T1 2	5	0	3	2	2, 3
	Site # 22					
Jun-97	22-1-3 MYBDA (m)	4	1	1	0	
Jun-97	22-2-1 MYBDA	5	1	3	0	5
Jun-97	22-3-1 MYBDA	4	1	1	0	
Jun-97	22-3-2 MYBDA	5	1	3	1	3
Jun-97	22-3-3 MYBDA	4	1	1	0	
Jun-97	22-5-1 MYBDA	4	1	1	0	
Jun-97	22-6-1 MYBDA	5	1	3	2	9
Jun-97	22-6-2 MYBDA	5	1	3	1	4. 5. 9
Jun-97	22-6-4 MYBDA	3	1, 4	3	1	7, 9
Jun-97	22-7-1 MYBDA	3	1, 4	3	1	7, 9
Jun-97	22-8-2 MYBDA	3	1, 4	3	1	7, 9
Jun-97	22-9-2 MYBDA	3	1, 4	3	1	7, 9
Jun-97	22-9-3 MYBDA	3	1, 4	3	1	7, 9
Jun-97	22-9-4 MYBDA	3	1, 4	3		6, 7

Collection Date	Fungal Isolate [U]	GAA/TAA [V]	M [W]	GR [X]	Color [Y]	Texture [Z]
Jun-97	22-10-3 MYBDA	3	1	3	1	7, 9
Jun-99	22-1-2 MYBDA	3	1	3	1	7, 9
Jun-99	22-2-4 MYBDA	3	1	3	1	7, 9
Jun-99	22-4-3 MYBDA	3	4	1	2	
Jun-99	22-5-1 MYBDA	3	4	1	2	
Jun-99	22-5-3 MYBDA	?	1	3	1	9
	Site # 26					
Jun-97	26-1-1 MYBDA	5	1	3	0	3
Jun-97	26-1-3 LGBDA	5	1	3	1	8
Jun-97	26-2-3 MYBDA	5	1	3	0	3
Jun-97	26-2-4 LGBDA	5	1	2	1	3
Jun-97	26-3-1 LGBDA	5	1	2	1	3
Jun-97	26-3-2 MYBDA	5	1	3	0	3
Jun-97	26-4-1 MYBDA	5	1	3	0	3
Jun-97	26-4-3 LGBDA	?9	1	3	0	3
Jun-97	26-4-3 MYBDA	5	1	3	0	3
Jun-97	26-5-1 MYBDA	5	1	3	0	3
Jun-97	26-5-3 LGBDA	5	1	3	0	3
Jun-97	26-6-2 MYBDA	5	1	3	1	3
Jun-97	26-6-1 A MYBDA	5	1	3	0	3
Jun-97	26-7-1 LGBDA	5	1	3	0	3
Jun-97	26-7-4 MYBDA	5	1	2	1	3
Jun-97	26-8-2 LGBDA	5	1	3	0	5, 9
Jun-97	26-9-1 MYBDA	5	1	3	0	3
Jun-97	26-9-3 MYBDA	5	1	3	0	3
Jun-97	26-10-2 A MYBDA	5	1	3	0	3
Jun-97	26-10-2 B MYBDA	5	1	3	0	3
Jun-97	26-10-2 LGBDA	5	1	2	1	3
Jun-99	26-1-3 MYBDA	3	1	3	0	5, 9
Jun-99	26-1-4 LGBDA	3	1	3	0	5, 9
Jun-99	26-2-1 LGBDA	3	1	3	0	5, 9
Jun-99	26-2-2 MYBDA	3	1	3	0	5, 9
Jun-99	26-2-4 LGBDA	3	1	3	0	5, 9
Jun-99	26-3-1 LGBDA	3	1	3	0	5, 9
Jun-99	26-3-2 LGBDA	3	1	3	0	5, 9
Jun-99	26-3-4 LGBDA	3	1	3	0	5, 9
Jun-99	26-3-4 MYBDA	3	1	3	0	5, 9
Jun-99	26-4-2 LGBDA	3	1	3	0	5, 9
Jun-99	26-4-3 MYBDA	3	1	3	0	5, 9
Jun-99	26-4-3 LGBDA	3	1	3	2	0
Jun-99	26-5-1 LGBDA	3	1	3	0	5, 9
Jun-99	26-5-3 MYBDA	3	1	3	0	5, 9
Oct-99	26 M1 1	3	1	3	1	6

Collection Date	Fungal Isolate [U]	GAA/TAA [V]	M [W]	GR [X]	Color [Y]	Texture [Z]
Oct-99	26 M2 2A	4	1	2	1	3, 6
Oct-99	26 M2 2B	5	1	3	1	0
Oct-99	26 M2 3	3	1	3	1	5, 7
Oct-99	26 M3 1A	5	1	2	1	3, 6
Oct-99	26 M3 1B	5	1	3	1	3
Oct-99	26 M3 2A	5	1	2	2	3
Oct-99	26 M3 2B	?5	0	3	1	6
Oct-99	26 L1 1A	5	0	2	1	3, 6
Oct-99	26 L1 1B	?5	0	3	2	6
Oct-99	26 T2 2	7	1	3	2	3
Oct-99	26 T3 1	3	0	3	2	3, 5
Oct-99	26 T3 2	5	0	3	2	3, 7
	Site # 28					
Jun-97	28-1-1 LGBDA	5	1	3	2	8
Jun-97	28-1-3 MYBDA	5	1	2	1	4, 7
Jun-97	28-1-3 B MYBDA (m)	5	1	2	1	4, 7
Jun-97	28-3-1 LGBDA	5	1	3	2	8
Jun-97	28-3-3 LGBDA	5	1	3	2	8
Jun-97	28-3-4 LGBDA	5	1	3	2	8
Jun-97	28-4-1 MYBDA	8	2	3	1	3, 5
Jun-97	28-4-2 A MYBDA	5	1	2	1	4, 6, 7
Jun-97	28-4-3 MYBDA	4	1	2	1	4, 6, 7
Jun-97	28-5-2 MYBDA	5	1	2	1	4, 6, 7
Jun-97	28-5-4 LGBDA	5	1	2	1	4, 6, 7
Jun-97	28-6-1 LGBDA	4	1	2	1	4, 6, 7
Jun-97	28-6-3 B MYBDA	5	1	2	1	4, 6, 7
Jun-97	28-7-1 B LGBDA	5	1	2		
Jun-97	28-7-2 LGBDA	5	1	2	0	4
Jun-97	28-8-1 A MYBDA	4	1	2	0	4
Jun-97	28-8-2 B MYBDA	4	1	3	0	5
Jun-97	28-8-2 LGBDA	5	1	2	1	4, 6, 7
Jun-97	28-9-1 B MYBDA	5	1	2	1	4, 6, 7
Jun-97	28-9-1 LGBDA	5	1	3	2	3, 4
Jun-97	28-10-1 LGBDA	5	1	3	2	3, 4
Jun-99	28-1-1 LGBDA	?	0		0	3
Jun-99	28-1-1 MYBDA	9	0	3	1	3
Jun-99	28-1-2 LGBDA	5	1	2	0	4
Jun-99	28-1-2 MYBDA	3	1	3	1	5, 7
Jun-99	28-1-3 LGBDA	10	0	1	2	0, 5
Jun-99	28-1-4 MYBDA	3	1	3	1	5, 6, 7
Jun-99	28-2-1 MYBDA	4	1	2	1	3
Jun-99	28-2-1 LGBDA	5	3	2	1	3,9
Jun-99	28-2-2 LGBDA	?	0	3	0	4

Collection Date	Fungal Isolate [U]	GAA/TAA [V]	M [W]	GR [X]	Color [Y]	Texture [Z]
Jun-99	28-3-2 MYBDA	?	0	1	1	0, 5
Jun-99	28-3-3 MYBDA	3	0	1	0	0, 3
Jun-99	28-3-4 MYBDA	2	0	1	0	3
Jun-99	28-4-1 LGBDA	2	0	1	0	3
Jun-99	28-4-2 LGBDA	5	1	2	0	4
Jun-99	28-4-3 MYBDA	?	1	2	0	
Jun-99	28-4-4 MYBDA	?	0	2	0	
Jun-99	28-4-5 MYBDA	5	1	2	0	4
Jun-99	28-5-1 MYBDA	6	2	3	2	3
Jun-99	28-5-1 LGBDA	8	1	2	0	4
Oct-99	28 M1 1A	1	0	2	1	3
Oct-99	28 M1 1B	1	0	1	1	3
Oct-99	28 M1 3B	1	0	1	1	3
Oct-99	28 M2 2	3	0	2	1	0
Oct-99	28 L3 1	5	1	3	2	2, 7
Oct-99	28 L3 2	3	0	2	1	0
Oct-99	28 T2 1	1	0	3	1	5, 6
Oct-99	28 T2 2	5	0	2	1	3
	Site # 29					
Jun-97	29-1-4 LGBDA	5	1	2	0	3
Jun-97	29-1-4 MYBDA	5	1	3	0	3
Jun-97	29-2-1 MYBDA	5	1	3	2	3, 4
Jun-97	29-2-2 LGBDA	5	1	2	1	3
Jun-97	29-2-3 (5) MYBDA	5	1	2	2	3, 4
Jun-97	29-3-1 LGBDA	5	1	3	1	0
Jun-97	29-3-2 MYBDA	5	1	3	1	5
Jun-97	29-4-3 MYBDA	5	0	3	1	5
Jun-97	29-4-4 LGBDA	5	0	3	1	0
Jun-97	29-5-1 LGBDA	5	1	3	1	0
Jun-97	29-5-1 MYBDA	5	2	2	1	3
Jun-97	29-5-3 MYBDA	5	1	2	1	5
Jun-97	29-6-3 MYBDA	5	0	3	1	5
Jun-97	29-6-3 LGBDA	5	1	2	1	3
Jun-97	29-6-4 MYBDA	8	1	2	1	3
Jun-97	29-7-1 A MYBDA	5	0	2	1	5
Jun-97	29-7-2 LGBDA	5	1	2	1	3
Jun-97	29-8-2 LGBDA	5	1	3	1	
Jun-97	29-8-4 MYBDA	5	0	3	1	0
Jun-97	29-9-1 MYBDA	5	1	3	1	5
Jun-97	29-9-1 LGBDA	5	0	2	1	3
Jun-97	29-10-2 LGBDA	5	0	3	1	0
Jun-97	29-10-3 MYBDA	5	0	3	1	0
Jun-97	29-10-4 LGBDA	5	0	3	1	

Collection Date	Fungal Isolate [U]	GAA/TAA [V]	M [W]	GR [X]	Color [Y]	Texture [Z]
Jun-99	29-1-1 LGBDA	?	0	3	0	9
Jun-99	29-1-2 MYBDA	3	1	3	0	9
Jun-99	29-1-2 LGBDA	?	0	3	1	3, 9
Jun-99	29-1-3 LGBDA	?	0	2	0	3, 9
Jun-99	29-1-4 LGBDA	3	0	3	1	5
Jun-99	29-2-1 LGBDA	5	1	2	1	3
Jun-99	29-2-2 LGBDA	5	1	2	1	3
Jun-99	29-2-4 MYBDA	5	1	2	1	3
Jun-99	29-3-2 LGBDA	3	1	3	0	3, 9
Jun-99	29-3-3 MYBDA	3	1	3	0	3, 9
Jun-99	29-3-3 LGBDA	3	1	3	0	9
Jun-99	29-4-2 MYBDA	3	1	3	0	9
Jun-99	29-4-3 LGBDA	3	1	3	0	9
Jun-99	29-5-3 LGBDA	3	1	3	0	9
Jun-99	29-5-3 MYBDA	3	1	3	0	9
Oct-99	29 M1 1	8	0	3	1	0
Oct-99	29 M2 1	3	1	3	1	3, 6
Oct-99	29 M3 1	?	1	3	1	6
Oct-99	29 M3 2	3	1	3	1	6, 7
Oct-99	29 L2 1A	3	1	3	1	6, 7
Oct-99	29 L2 1B	3	1	3	1	6, 7
Oct-99	29 L3 1	3	1	3	1	6, 7
Oct-99	29 T1 1	3	0	3	1	6
Oct-99	29 T2 1	3	1	3	1	6, 7
Oct-99	29 T3 1	3	1	3	1	6, 7

U: Fungal Isolate: First number = the site number. Media type (L= LGBDA, T = LGBA, M = MYBDA). Last numbers = the plate and isolate number.
V: Oxidase reaction (Table 4, p. 41)
W: Microscopic characteristics (Table 11, p. 80)
X: Growth rate at 2 weeks (Table 11, p. 80)
Y: Morphological color (Table 11, p. 80)
Z: Morphological texture (Table 11, p. 80)

Results of bole chip fungal isolates for all research sites, show that 62% were white rotters (GAA/TAA groups 4-9) and 38% were brown rotters (GAA/TAA groups 1-3). Of the white rotters, 47% were isolated on LGBDA/LGBA media and 53% on MYBDA medium. Of the brown rotters, 44% were isolated on LGBDA/LGBA media and 56% on MYBDA medium. For all research sites, by collection date, the percent of white and brown rotters for June 1997 was 83%

and 17%, June 1999 was 29% and 71%, and October 1999 was 30% and 53%, respectively.

No white rotters were isolated from the research site # 22 during the June 1999 and October 1999 collections. One hundred percent of the fungal isolates from research site # 22 were brown rotters from the June 1999 collection. No fungal isolates were kept from the October 1999 collection of research site # 22 because no suspected basidiomycetes were isolated. For the research sites # 26, 28, and 29, 100% of the fungal isolates were white rotters for the June 1997 collection. For the research site # 26, 100% of the fungal isolates were brown rotters for the June 1999 collection.

A selected number of soil fungal isolates were sequenced, primarily from one research site, # 28, although a few fungal isolates from other research sites also were sequenced. Tables 24-27 list the GenBank BLAST identities of these isolates. They are listed by research site, collection date, primers used in sequencing, and include the GenBank accession number.

Table 24. GenBank BLAST results of bole chip fungal isolates from site # 19.

	Fungal Isolate [Z]	Collection Date	Primers	Blast results	Accession #
BC	19-5-1 M	June 1999	ITS 1&2	*Tyromyces chioneus*	AJ006676
BC	19-2-3 M	June 1999	ITS 1-3	*Coprinus cothurnatus*	AF345820

Z: Fungal Isolate: First number = the site number. Media type (L= LGBDA, T = LGBA, M = MYBDA). Last numbers = the plate and isolate number.

Fungal isolate 19-5-1 M from the June 1999 collection had basidia and spores, which, according to Stalpers (1978) is characteristic of *Tyromyces* species in culture.

Table 25. GenBank BLAST results of bole chip fungal isolates from site # 26.

	Fungal Isolate [z]	Collection Date	Primers	Blast results	Accession #
BC	26-9-3 MYBDA	June 1997	ITS1	*Panellus serotinus*	AY265847
BC	26-2-2 MYBDA	June 1999	ITS1-3	*Sistotrema brinkmannii*	AY089729
BC	26 M3 2A	Oct. 1999	ITS1	*Panellus serotinus*	AY265847
BC	26 T3 2	Oct. 1999	ITS1-3	*Trametes versicolor*	AY309018

Z: Fungal Isolate: First number = the site number. Media type (L= LGBDA, T = LGBA, M = MYBDA). Last numbers = the plate and isolate number.

Table 26. GenBank BLAST results of bole chip fungal isolates from site # 28.

	Fungal Isolate [z]	Collection Date	Primers	Blast results	Accession #
BC	28-1-3 M	June 1997	5.8SR, ITS1	*Tomentella* sp.	AY382822
BC	28-1-3 B M(m)	June 1997	5.8SR, LR3	*Basidioradulum radula*	AJ406474
BC	28-3-1 LGBDA	June 1997	ITS1-4	*Trametes versicolor*	AY309018
BC	28-4-1 MYBDA	June 1997	5.8SR, ITS1	*Stereum sanguinolentum*	AY089730
BC	28-5-4 LGBDA	June 1997	ITS1-4	*Tomentella* sp.	AY382822
BC	28-6-1 LGBDA	June 1997	ITS1-4	*Tomentella* sp.	AY382822
BC	28-7-1 B LGBDA	June 1997	5.8SR, ITS1-4	*Panellus stypticus*	AF289071
BC	28-7-2 LGBDA	June 1997	ITS1-4	*Panellus stypticus*	AF289071
BC	28-8-2 B MYBDA	June 1997	ITS1-3	*Hyphoderma setigerum*	AJ534283
BC	28-9-1 LGBDA	June 1997	ITS1-3	*Trametes versicolor*	AY309018
BC	28-1-1 MYBDA	June 1999	ITS1-3	*Irpex lacteus*	AB079265
BC	28-1-2 MYBDA	June 1999	ITS1-3	*Coprinus cothurnatus*	AF345820
BC	28-1-3 LGBDA	June 1999	ITS1-3	*Papiliotrema bandonii*	AF472626
BC	28-1-4 MYBDA	June 1999	ITS2 & 3	*Coprinus cothurnatus*	AF345820
BC	28-2-1 LGBDA	June 1999	5.8SR, ITS1-4	*Hypholoma* sublateritium	AY534117
BC	28-2-2 LGBDA	June 1999	ITS1 & 3	Ascomycete sp.	AY354279
BC	28-3-2 MYBDA	June 1999	ITS1-3	*Entoloma nitidum*	AY228340
BC	28-3-3 MYBDA	June 1999	ITS1 & 3	*Entoloma nitidum*	AY228340
BC	28-4-1 LGBDA	June 1999	ITS1-3	*Entoloma nitidum*	AY228340
BC	28-4-2 LGBDA	June 1999	ITS1 & 3	*Panellus stypticus*	AF289071
BC	28-4-3 MYBDA	June 1999	ITS1-3	*Athelia neuhoffii*	U85798
BC	28-5-1 LGBDA	June 1999	ITS1-3	Basidiomycete from bamboo	U65614
BC	28 M1 1A	Oct. 1999	ITS1-3	*Marasmius* species	AY216476
BC	28 M1 3B	Oct. 1999	ITS1-3	*Thanatephorus cucmeris*	AF455461
BC	28 M2 2	Oct. 1999	ITS1-3	*Chaunopycnis alba*	AF389192
BC	28 L3 1	Oct. 1999	ITS1-3	*Trametes versicolor*	AY309018
BC	28 L3 2	Oct. 1999	5.8SR, ITS1	Ascomycete	

	Fungal Isolate [z]	Collection Date	Primers	Blast results	Accession #
BC	28 T2 1	Oct. 1999	ITS1-3	Basidiomycete sp.	AJ279465
BC	28 T2 2	Oct. 1999	ITS1-3	*Trichaptum biforme*	U63473

Z: Fungal Isolate: First number = the site number. Media type (L= LGBDA, T = LGBA, M = MYBDA). Last numbers = the plate and isolate number.

Table 27. GenBank BLAST results of bole chip fungal isolates from site # 29.

	Fungal Isolate [z]	Collection Date	Primers	Blast results	Accession #
BC	29-2-1 MYBDA	June 1997	ITS1-3	*Panellus serotinus*	AY265847
BC	29-2-3 (5) MYBDA	June 1997	ITS1 & 3	*Coprinus cothurnatus*	AF345820
BC	29-4-3 MYBDA	June 1997	ITS1-3	*Panellus serotinus*	AY265847
BC	29-5-1 M	June 1997	ITS1-3	*Panellus serotinus*	AY265847
BC	29-7-2 L	June 1997	ITS1-3	*Panellus serotinus*	AY265847
BC	29-8-2 LGBDA	June 1997	ITS1-3	*Panellus serotinus*	AY265847
BC	29-1-1 LGBDA	June 1999	ITS1-3	*Irpex lacteus*	AB079265
BC	29-1-3 LGBDA	June 1999	ITS2 & 3	*Schizophyllum commune*	AF350925
BC	29-1-4 LGBDA	June 1999	ITS1-3	*Thanatephorus cucmeris*	AF455461
BC	29-3-2 L	June 1999	ITS1-3	*Coprinus cothurnatus*	AF345820
BC	29 M3 2	Oct. 1999	ITS1 & 2	*Sistotrema brinkmannii*	AY089729
BC	29 L2 1A	Oct. 1999	ITS1 & 3	*Sistotrema brinkmannii*	AY089729

Z: Fungal Isolate: First number = the site number. Media type (L= LGBDA, T = LGBA, M = MYBDA). Last numbers = the plate and isolate number.

Bole chip basidiomycete isolates identified by sequencing are listed below by collection date, order, and family by research site (Table 28). Order and family names selected using Hawksworth et al (1995).

Table 28. Bole chip isolates by research site (# 26, 28, and 29), collection date, order and family.

Site # 26		
Collection Date	Order	Family
June 1997		
Panellus serotinus	Agaricales	Tricholomataceae
June 1999		
Sistotrema brinkmannii	Stereales	Sistotremataceae
October 1999		
Panellus serotinus	Agaricales	Tricholomataceae
Trametes versicolor	Poriales	Coriolaceae

Site # 28

Collection Date	Order	Family
June 1997		
Tomentella sp.	Thelephorales	Thelephoraceae
Basidioradulum radula	Stereales	Hyphodermataceae
Hyphoderma setigerum	Stereales	Hyphodermataceae
Trametes versicolor	Poriales	Coriolaceae
Panellus stypticus	Agaricales	Tricholomataceae
Stereum sanguinolentum	Stereales	Stereaceae
June 1999		
Athelia neuhoffii	Stereales	Atheliaceae
Coprinus cothurnatus	Agaricales	Coprinaceae
Hypholoma sublateritium	Agaricales	Strophariaceae
Entoloma nitidum	Agaricales	Entolomataceae
Papiliotrema bandonii	Tremellales	Tremellaceae
Panellus stypticus	Agaricales	Tricholomataceae
Irpex lacteus	Stereales	Steccherinaceae
October 1999		
Trichaptum biforme	Poriales	Coriolaceae
Marasmius species	Agaricales	Tricholomataceae
Trametes versicolor	Poriales	Coriolaceae
Thanatephorus cucmeris	Ceratobasidiales	Ceratobasidiaceae

Site # 29

Collection Date	Order	Family
June 1997		
Panellus serotinus	Agaricales	Tricholomataceae
Coprinus cothumatus	Agaricales	Coprinaceae
June 1999		
Irpex lacteus	Stereales	Steccherinaceae
Schizophyllum commune	Schizophyllales	Schizophyllaceae
Thanatephorus cucmeris	Ceratobasidiales	Ceratobasidiaceae
Coprinus cothumatus	Agaricales	Coprinaceae
October 1999		
Sistotrema brinkmannii	Stereales	Sistotremataceae

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Tables 29 (basidiomycete) and Table 30 (non-basidiomycete) summarize the GenBank identities for the bole chip fungal isolates and include GenBank accession numbers and the number of isolates with this identification.

Table 29. GenBank sequence identification of basidiomycete bole chip fungal isolates.

Identification	GenBank Accession #	# of isolates
Athelia neuhoffii	U85798	1
Basidiomycete sp.	AJ279465	1
Basidiomycete from bamboo	U65614	1
Basidioradulum radula	AJ406474	1
Coprinus cothurnatus	AF345820	5
Entoloma nitidum	AY228340	3
Hyphoderma setigerum	AJ534283	1
Hypholoma sublateritium	AY534117	1
Irpex lacteus	AB079265	2
Marasmius species	AY216476	1
Panellus serotinus	AY265847	7
Panellus stypticus	AF289071	3
Papiliotrema bandonii	AF472626	1
Schizophyllum commune	AF350925	1
Sistotrema brinkmannii	AY089729	3
Stereum sanguinolentum	AY089730	1
Thanatephorus cucumeris	AF455461	2
Tomentella sp.	AY382822	3
Trametes versicolor	AY309018 , 17	4
Trichaptum biforme	U63473	1
Tyromyces chioneus	AJ006676	1

Table 30. GenBank sequence identification of non-basidiomycete bole chip fungal isolates.

Identification	GenBank Accession #	# of isolates
Chaunopycnis alba	AF389192	1
Ascomycete	-	2

Discussion

The calcium and phosphorus levels decreased between the June 1997 and June 1999 collections. This agrees with other researchers who have shown

that bark contains more calcium and phosphorus than sound wood (Lambert et al. 1980). After the June 1997 collection and removal of bole wood, the bark on the research boles fell off. Therefore, the chemical analyses of bole wood samples for the June 1999 collection did not include bark.

Eighty percent of the bole wood fungal isolates had clamp connections. Only three bole chip fungal isolates identified by sequencing were not basidiomycete fungi. None of these three isolates had clamp connections. Sixty percent of the fungal isolates were white rotters, GAA/TAA positive (groups 4-9). Less than one percent of the fungal isolates had oidia.

The basidiomycete isolates from the bole chip wood that were identified by sequencing are from seven orders: Agaricales (25), Ceratobasidiales (2), Poriales (4), Schizophyllales (1), Stereales (9), Thelephorales (3), and Tremellales (1). Agaricales is the dominant order of the basidiomycete fungi isolated from bole chip wood and identified by sequencing. Families and orders were determined using Hawksworth et al (1995).

CHAPTER V

FUNGAL FRUITING BODY ISOLATES

Introduction

The presence of fruiting bodies of decay fungi varies with the season, although most decay fungi fruit in the autumn. For example, Allen et al. (2000) showed that taxa richness of sporocarps found on CWD increased with an autumn collection as compared to a spring collection and by log volume (size of the bole) in a New Zealand temperate mountain beech forest. Miller (1995) also observed more diversity in macro-fungal fruiting bodies in the fall season in temperate forests of the United States. Therefore, during this research, fungal fruiting bodies were collected for isolation during the August to October season.

Although several published keys are available to identify basidiomycete cultures from basidiocarps and to identify basidiocarp fruiting bodies (Appendix 22), exact identifications are often difficult and elusive when working to identify unknown basidiomycetes in culture from wood and soil substrates.

Recently, molecular techniques have been used to investigate phylogenetic relationships between taxa of specific genera of fungi (e.g. Banik et al. 1996 [*Armillaria*], Banik et al.1998 [*Laetiporus*], Erland 1995 [*Tylospora*], Frontz et al. 1998 [*Armillaria*], Garbelotto et al. 1996 [*Heterobasidion*], Johnson and Vilgalys 1998 [*Lepiota*], Nakasone 1991 [*Phlebia*], Roy et al. 1997

[*Phlebiopsis*], and Theodore et al. 1995 [*Serpula*]). Bruns et al. (1998) developed a sequence database for 80 genera of ectomycorrhizae in the group Hymenomycetes. Molecular techniques used by researchers to study fungi were reviewed by Bruns et al. (1991). Cullen (1997) focused his review relating to fungal systematics on ligninolytic fungi and Palfreyman (1998) reviewed molecular techniques to identify and detect wood decay fungi.

Materials and Methods

Fungal fruiting bodies within a one-meter radius of each research bole were collected in wax paper bags and returned to the laboratory for culturing. Five visits to the research sites were conducted for collecting fungal fruiting bodies: August 19, 1997 (M1), September 7, 1997 (M2), October 2, 1997 (M3), October 3, 1998 (M4), and August 2, 1999 (M5). Also, during the 5th collection date (M5), fungal fruiting bodies were collected in a larger area around the boles and were labeled as Area 1 (sites # 26, 28, & 29) and Area 2 (sites # 19, 21, & 22). During additional trips to the research sites (2001-2002), while gathering plant species data, fungal fruiting bodies growing at the research areas were identified using morphological keys and photographed. These fungal fruiting bodies were not cultured, but they are included in the list of fungi found at the research sites.

In the laboratory, fungal fruiting bodies were surface sterilized and then cut open to expose the inner tissue. Techniques described by Ainsworth (1995), Hutchison (1991), Thorn (1991), Treu and Agerer (1990), and Watling (1977) were modified and used to sample tissue and spores from the fruiting bodies. A

2 mm square piece of inner tissue (stipe or pileus) was removed aseptically and placed in the center of a petri dish containing the following media: water agar medium (WA), water agar with antibiotics (WA +), malt yeast extract agar with antibiotics (MYA +), malt extract agar with antibiotics (MEA +) (Appendix 23), MYBDA, and LGBDA. The antibiotics used in these media were: chlortetracycline-HCl, streptomycin sulfate, and penicillin G. With some of the basidiocarps, a piece of the hymenium was glued (Elmers® school glue) onto the top of the petri dish to facilitate spore dispersal onto the culture medium. This helped reduce contamination of these cultures. After culturing the fruiting bodies, taxonomic keys (Bessette et al. 1997, Baron 1999) were used to identify them.

Results

Preliminary identifications of fungal fruiting bodies collected at the research sites and summary cultural data by research site and collection date are shown in Table 31.

Table 31. Summary cultural data of fungal fruiting body isolates by collection date and research site.

Collection [S]	Fungal Isolate [T]	GAA/TAA [U]	M [V]	GR [W]	Color [X]	Texture [Y]	Identification [Z]
M1	19-3 A MYBDA	non					*Stereum ostrea*
M2	19-2 MYBDA	5					*Trametes versicolor*
M3	19-1 A MYBDA	4					*Panellus serotinus*
M3	19-1 B MYBDA	4					*Panellus serotinus*
M3	19-6 MYBDA	5	1				*Trametes versicolor*
M5	19-1 spores MYBDA	4	1	2	1	0	*Trametes cervina*
M5	19-5 spores MYBDA	4	0	1	2	0	*Phellinus chrysoloma*
M5	19-7 wood MYBDA	9	2	3	1	2, 3	? *Coniophora*
M5	19-8 spores MYBDA	5	1	3	2	2	*Trametes versicolor*
M5	19-8 spores MEA +	5	1	3	2	1	*Trametes versicolor*
M5	19-8 wood LGBDA	5	1	3	2	2	*Trametes versicolor*

Collection [S]	Fungal Isolate [T]	GAA/TAA [U]	M [V]	GR [W]	Color [X]	Texture [Y]	Identification [Z]
M3	21-4 MYBDA	5	0	3	0		cream gill w/ annulus
M3	21-5 WA	non					*Clavalina cinerea*
M3	21-9 A MYBDA	4					cream gill
M3	21-9 B MYBDA	4					cream gill
M3	21-10 MYBDA	5	0	3	1	1, 3	? *Cortinarius*
M3	21-11 WA	3					*Bisporella citrina*
M3	21-11 MYBDA	non					*Bisporella citrina*
M4	21-1 MYBDA	5	0	3	1	3	
M2	22-4 MYBDA	5	1				? *Coniophora*
M3	22-5 MYBDA	4	0	1	2	3	*Russula*
M4	22-1 MYBDA	?	0	2	1	2, 3	gill
M4	22-2 MYBDA	5	1				*Panellus serotinus*
M4	22-2 MYA +	5	0				*Panellus serotinus*
M1	26-2 A MYBDA	5					*Trichaptum*
M1	26-7 MYBDA	5	1				*Cortinarius*
M2	26-6 MYBDA	8	1	2	1	3	*Chlorociboria aeruginoscens*
M2	26-12 MYBDA	9					? *Coniophora*
M3	26-5 MYBDA	3					*Panellus serotinus*
M3	26-8 MYBDA	5	1	2	2		*Panellus stypticus*
M4	26 MYBDA	5	0	3	0	1	
M4	26-2 MYBDA	6	1				Panellus serotinus
M4	26-5 WA	6	0				*Trametes*
M4	26-8 MYBDA	non	0	2	0		*Trichoglossum*
M4	26-10 MYA +	4	0	3	1	4	*Hygrophoropsis*
M5	26-3 wood MYBDA	5	1	2	1	5	*Panellus stypticus*
M1	28-5 MYBDA	2	0	3	1	5	*Trichaptum*
M2	28-7 A MYBDA	5	1				*Panellus stypticus*
M2	28-7 B MYBDA	5	1				*Panellus stypticus*
M2	28-8 WA	5	1	2	1	3	
M2	28-9 MYBDA	3	0	2	1	1	*Clavulinopsis fusiformis*
M2	28-11 MYBDA	9					*Bisporella ctirina*
M3	28 ? WA	5	1	2	2	3	
M3	28-9 MYBDA	non	0	3	0	0	gray cap, tan gills
M3	28-11 MYBDA / WA	5					*Panellus stypticus*
M3	28-11 WA	5					*Panellus stypticus*
M3	28-12 MYBDA	8	0	2	2	3	light brown M.
M3	28-13 MYBDA	non	1	2	1	3	cream gill, fringe margin
M4	28-1 MYA +	5	1				*Panellus serotinus*
M4	28-1 1 WA	2	1				Panellus serotinus

Collection [S]	Fungal Isolate [T]	GAA/TAA [U]	M [V]	GR [W]	Color [X]	Texture [Y]	Identification [Z]
M4	28-1 2 WA	2	1				Panellus serotinus
M4	28-2 MYBDA	?	1				*Phyllotopsis nidulans*
M4	28-2 WA	?	0				*Phyllotopsis nidulans*
M4	28-3 MYA + / MYBDA	?	0	1	2	3	gill
M4	28-4 MYA+	5	1				gill
M4	28-4 MYBDA	5	?1				gill
M5	28-1 tissue MYBDA	4	1	2	1	0	*Panellus stipticus*
M1	29-2 MYBDA	6	1				*Trametes versicolor*
M1	29-3 MYBDA	7					gill, ? *Coprinus*
M2	29-3 MYBDA	5	0		0	0	*Trametes*
M3	29-2 MYBDA	4	1		1	5, 6	little brown gill
M3	29-5 MYBDA	5	1				*Trametes versicolor*
M3	29-6 MYBDA / WA	?			2	1, 8	Cortinarius
M3	29-7 MYBDA / WA	4	1		1	5, 6	little brown gill
M3	29-11 MYBDA	non			0	3, 6	med brown gill
M3	29-11 A WA	non	?1		0	3, 6	gill
M3	29-13 MYBDA	2					*Lycoperdon*
M3	29-14 MYBDA	7			1	1	*Ascocoryne cylichnium*
M3	29-16 MYBDA	4			1	1, 7	tan brown gill
M3	29-17 MYBDA	5			2	8	brown gill
M4	29-1 WA	5	1		2	1	gill
M4	29-1 MYBDA	5	0		2	1	gill
M4	29-1 MYA +	5	1				gill
M4	29-3 MYA +	non	0				gill
M4	29-3 MYA + ?	non	1				gill
M4	29-3 1 MYA +	7	0				gill
M4	29-3 2 MYA +	5	0				gill
M4	29-3 1 WA +	5	0				gill
M4	29-3 2A WA +	5					gill
M4	29-3 2B WA +	5	0	1	2	3	gill
M4	29-3 4 MYA +	non	1				gill
M5	29-2 tissue MYBDA	5	0	3	1	3	*Mycena leaiana*
M5	29-3 spores MYBDA	5	1	2	1	2, 3	*Panellus stypticus*
M5	29-7 spores MYBDA	7	1	2	2	0	*Auricularia auricula*
M5	29-8 tissue MYBDA	4	1	2	1	3	*Collybia confluens*
M5	A1-1 spores MYBDA	9	0	2	1	1	*Stereum ostrea*
M5	A1-1 spores MEA +	9	0	2	2	1	*Stereum ostrea*
M5	A1-3 wood MYBDA	1	1	2	1	1	*Fomitopsis pinicola*
M5	A1-10 tissue MEA +	1	non	non			metallic gill
M5	A1-11 tissue MYBDA	5	0	2	2	0	*Hygrophorus*

Collection [S]	Fungal Isolate [T]	GAA/TAA [U]	M [V]	GR [W]	Color [X]	Texture [Y]	Identification [Z]
M5	A1-15 tissue MYBDA	7	0	3	2	2	? *Pholiota*
M5	A1-15 spores MYBDA	7	0	2			? *Pholiota*
M5	A1-16 spores MEA +	4	1	2	0	0	*Trichaptum*
M5	A1-16 spores MYBDA	5	0	2	1	0	*Trichaptum*
M5	A1-16 wood MYBDA	3	0	2	2	3, 4	*Trichaptum*
M5	A1-16 wood LGBDA	9 ?	1	1	1	2	*Trichaptum*
M5	A1-17 A wood MEA +	3	1	3	1	0	*Trametes*
M5	A1-17 B wood MEA +	2	1	3	0	6	*Trametes*
M5	A2-5 tissue MYBDA	non	non	1		2	yellow, gill decurrent
M5	A2-8 tissue MYBDA	1	non	non			*Irpex lacteus*
M5	A2-13 wood MYBDA	5	1	3	2	1	*Trametes versicolor*
M5	A2-15 wood MEA +	1	non	2			yellow fan rhizomorphs

S: Collection date: M1 Aug. 1997, M2 Sept. 1997, M3 Oct. 1997, M4 Oct. 1998, M5 Aug. 1999.
T: Fungal Isolate: first number = the site number, second number = the isolate number.
A1 were collected in Area 1, A2 were collected in Area 2. Media type used by collection date (M1 MYBDA, M2 MYBDA & WA, M3 MYBDA & WA, M4 MYBDA, WA, WA+ MYA+, M5 MYBDA, MEA+ LGBDA)
U: Oxidase reaction (Table 4, p. 41)
V: Microscopic characteristics (Table 11, p. 80)
W: Growth rate at 2 weeks (Table 11, p. 80)
X: Morphological color (Table 11, p. 80)
Y: Morphological texture (Table 11, p. 80)
Z: Identification by using morphological keys

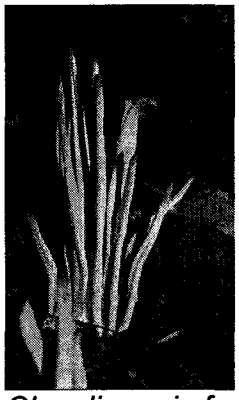
Most of the fungal isolates from basidiocarp fruiting bodies were white rotters, with GAA/TAA reactions of 4-9. Not all fungal isolates were evaluated for microscopic or macroscopic characteristics or for growth rate. Some of the fungal fruiting bodies were photographed at the research sites (Fig. 55).

Panellus serotinus (Fr.) Kunh.

Panellus stypticus (Bull.:Fr.) Karst.

Clavulina cinerea (Fr.) Schroet.

Trichoglossum hirsutum (Pers.:Fr.) Boud.

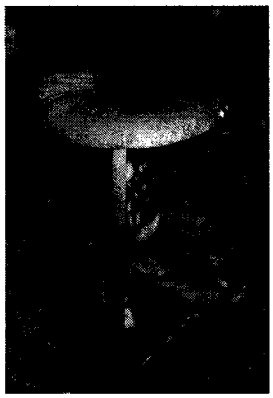


Clavulinopsis fusiformis (Sow.:Fr.) Corner

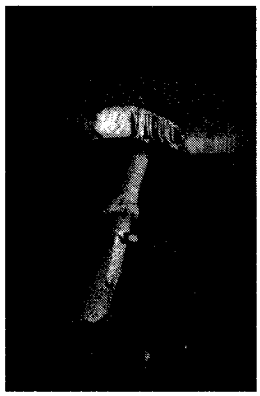
Cantharellus infundibuliformis Fr.

Lycoperdon perlatum Pers.

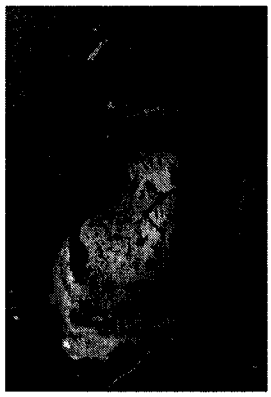
Ganoderma applanatum (Pers.) Pat.



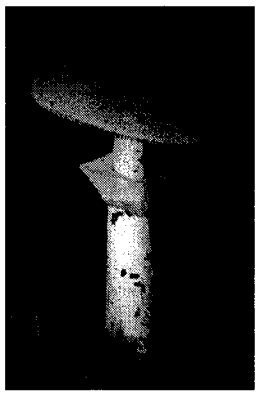
Xerula furfuracea (Pk.) Redhead



Amanita flavoconia Atk.



Cortinarius armillatus (Fr.) Kumm.



Amanita citrina (Schaeff.) Gray

Fig. 55. Examples of fungal fruiting bodies found at the research sites.

The fungal fruiting bodies found at each research site, which were identified by using taxonomic keys, are listed in Appendix 24. Two percent of the identified basidiomycetes were families in the order Poriales (Coriolaceae, Lentinacae, and Polyporaceae) and 39% were in the order Agaricales (Amanitaceae, Coprinaceae, Entolomataceae, Hygrophoraceae, Strophariaceae, and Tricholomataceae). The most common fungal families found at these research sites were Coriolaceae (12 species) and Tricholomataceae (13 species). Families and orders were determined using Hawksworth et al (1995).

A selected number of soil fungal isolates were sequenced, primarily from one research site, # 28. A few fungal isolates from other research sites were also sequenced. Table 32 lists the GenBank BLAST identities for the isolates.

Table 32. GenBank BLAST results of basidiocarp fungal isolates from site # 26, 28, 29 and Area 1.

Fungal Isolate [z]	Collection Date	Primers	Blast results	Accession #
Site # 26				
M1 26-2A MYBDA	Aug. 1997	ITS1-3	*Trametes versicolor*	AY309017
M3 26-5 MYBDA	Oct. 1997	ITS1 & 3	*Sistotrema brinkmannii*	AY089729
M3 26-8 MYBDA	Oct. 1997	ITS1-3	*Panellus stypticus*	AF289071
M4 26-2 MYBDA	Oct. 1998	ITS1-3	*Hypholoma sublateritium*	AY534117
M4 26-5 WA	Oct. 1998	ITS1-3	*Trametes versicolor*	AY309017
M5 26-3 wood M	Aug. 1999	ITS1-3	*Panellus stypticus*	AF289071
Site # 28 and Area 1				
M2 28-7 B MYBDA	Sept. 1997	ITS1-3	*Panellus stypticus*	AF289071
M2 28-11 MYBDA	Sept. 1997	ITS1-3	*Phanerochaete sordida*	AF475150
M3 28 ? WA	Oct. 1997	ITS1-4	*Hypholoma sublateritium*	AY534117
M3 28-11 WA	Oct. 1997	ITS1 & 3	*Panellus stypticus*	AF289071
M4 28-1 MYA +	Oct. 1998	ITS1-3	*Panellus serotinus*	AY265847
M4 28-1 WA 1	Oct. 1998	ITS1-3	*Fomitopsis pinicola*	AY354214
M4 28-1 WA 2	Oct. 1998	5.8Sr, ITS1-3	*Fomitopsis pinicola*	AY354214
M4 28-2 MYBDA	Oct. 1998	LR3, 5.8SR, ITS1-3	*Rectipilus natalensis*	AY571058
M4 28-2 WA	Oct. 1998	ITS1-3	*Henningsomyces* sp.	AY571047
M4 28-4 MYA+	Oct. 1998	ITS1-4	*Hypholoma sublateritium*	AY534117
M4 28-4 MYBDA	Oct. 1998	ITS1-4	*Hypholoma sublateritium*	AY534117
M5 28-1 tissue MYBDA	Aug. 1999	ITS1 & 3	*Panellus stypticus*	AF289071
M5 A1-3 wood MYBDA	Aug. 1999	ITS1-3	*Fomitopsis* sp.	AF509233
M5 A1-16 spores MEA+	Aug. 1999	ITS1-3	*Trichaptum biforme*	U63473
M5 A1-16 spores MYBDA	Aug. 1999	ITS1-3	*Trichaptum biforme*	U63473
M5 A1-17 A wood MEA+	Aug. 1999	ITS1-3	glacial ice Basidiomycete	AF261656
Site # 29				
M1 29-2 MYBDA	Aug. 1997	ITS1-3	*Trametes versicolor*	AY309017
M2 29-3 MYBDA	Sept. 1997	ITS1-3	*Trametes versicolor*	AY309018
M3 29-5 MYBDA	Oct. 1997	ITS1-3	*Trametes versicolor*	AY309017

Z: Fungal Isolate: first number = the site number, second number = the isolate number. A1 were collected in Area 1, A2 were collected in Area 2. Media type used by collection date (M1 MYBDA, M2 MYBDA & WA, M3 MYBDA & WA, M4 MYBDA, WA, WA+ MYA+, M5 MYBDA, MEA+ LGBDA)

Table 33 summarizes the number of isolates of each species identified from basidiocarp fruiting bodies.

Table 33. GenBank sequence identification of basidiocarp fungal isolates.

Identification	GenBank Accession #	# of isolates
Trametes versicolor	AY309018	1
Sistotrema brinkmannii	AY089729	1
Hypholoma sublateritium	AY534117	4
Panellus serotinus	AY265847	1
Panellus stypticus	AF289071	5
Phanerochaete sordida	AF475150	1
Henningsomyces sp.	AY571047	1
Fomitopsis sp.	AF509233	1
Fomitopsis pinicola	AF347106	2
Trametes versicolor	AY309017	4
Trichaptum biforme	U63473	2
Rectipilus natalensis	AY571058	1
glacial ice Basidiomycete	AF261656	1

Discussion

A few of the GenBank identifications did not agree with their basidiocarp fruiting body identifications. These included M3 26-5, M2 28-11, and M4 28-2. This could be because GenBank is not complete and my techniques to culture from basidiocarps varied. But, basidiocarp morphological identifications were confirmed by sequencing in GenBank for *Trametes versicolor* (Fr.) Pil., *Panellus serotinus* (Fr.) Kunh., *Panellus stypticus* (Bull.:Fr.) Karst., *Trichaptum biforme* (Fr.) Ryv., and *Fomitopsis* sp. Karst.

BLAST results revealed that most of the basidiocarp fungal isolates were identified in the orders Agaricales (10) in the families Strophariaceae (4), and Tricholomataceae (6), and in the order Poriales (10) in the family Coriolaceae. Two basidicarp fungal isolates were in each of the orders Stereales (Meruliaceae and Sistotremataceae) and Schizophyllales (Schizophyllaceae). Basidiocarps

identified using morphological keys show the greatest number of species in the order Agaricales (29) as compared to Poriales (15). Families and orders were determined using Hawksworth et al (1995).

CHAPTER VI

SITE # 28 *HYPHOLOMA SUBLATERITIUM* FUNGAL ISOLATES

Introduction

Logging techniques used in our forests have significantly decreased the amount of coarse woody debris on the forest floor. Wood decay fungi are becoming increasingly rare as a result (Berg et al. 1995). Little is known of the distribution of ramets or genets of wood decay basidiomycetes or how many genets exist in a single bole. Multiple infections have been observed in other studies (Holmer et al. 1994, Kay and Vilgalys 1992, Garbelotto et al. 1999). Population structure of wood decay basidiomycete fungi at a given site needs to be further elucidated.

Genets on a single log were studied by Kauserud and Schumacher (2002) and they found congruent results between somatic incompatibility and PCR-RFLP. Similar studies have also demonstrated that genets of wood decaying fungi may coexist in the same bole, for example, *Phellinus tremula* (Holmer et al. 1994), *Pleurotus ostreatus* (Jacq. ex Fr.) Kumm. (Kay and Vilgalys 1992), *Stereum gausapatum* (Boddy and Rayner 1982), and *Heterobasidion annosum* (Garbelotto et al. 1999). These studies suggest that there may be intraspecific competition for space and nutrients among genets.

Homokaryotic and heterokaryotic mycelia were found when studying *Heterobasidion annosum* (Garbelotto et al. 1999), yet, a single genet of *Armillaria* was found to occupy an entire woodland (Smith et al. 1992).

Kauserud and Schumacher (2002), while looking at the population structure of *Phellinus nigrolimitatus* (Romell) Galzin in three logs, saw similar results with SI and PCR-RFLP and could distinguish between genets. Each genet had unique PCR-RFLP patterns. They also observed different morphological cultural characteristics among genets. In fact, in hindsight, they could distinguish between genets by morphological characteristics alone.

Wood decay fungi can take up mineral nutrients (Wells et al. 1990) and their hyphae can translocate these nutrients over long distances (Cairney 1992) forming a nutrient cycle. Lindahl et al. (1999) demonstrated translocation of ^{32}P from a wood-decomposing saprotrophic fungus *Hypholoma fasciculare* (Huds.:Fr.) Kummer to an ectomycorrhizal fungus *Suillus variegates* (Sw.) Kuntze and their plant hosts. Saprotrophic fungi have a higher enzymatic potential than ectomycorrhizal fungi (Lindahl et al. 1999) making them a more effective decomposer. *Lepista* sp., a saprotrophic fungus, was shown to decompose beech leaves faster than ectomycorrhizal fungi (Colpaert and van Tichelen 1996).

Species of *Hypholoma* are cord-forming basidiomycete wood decay fungi. Mycelial cord systems can translocate nutrients long distances. Cord-forming saprotrophic fungi take up nutrients from the soil and litter and are a major pathway for nutrient relocation (Wells et al. 2001). Cords of the saprotrophic

wood decaying basidiomycete *Phanerochaete velutina*, translocated [^{32}P] orthophosphate many meters to tree roots, living plants, leaf litter, and wood (Boddy 1993).

Species of *Hypholoma* are useful to science. *Hypholoma capnoides* (Fr.) Karst. was studied in wood as a biological control against *Heterobasidion annosum* (Holdenrieder 1984). Several species of *Hypholoma* were tested for antibiotic production (Backens et al. 1984). Holmer and Stenlid (1996) used wood blocks inoculated with *Hypholoma capnoides* to test if this fungus would invade through soil and replace other fungal species in wood inoculated with other wood decomposing basidiomycete fungi. Studying the succession of fungi in pine stumps, Runge (1986), observed *Hypholoma capnoides* during the middle stages of decay and observed *Hypholoma fasciculare*. and *Hypholoma capnoides*. during the final stages of decay. In Finland, *Hypholoma capnoides* was found on stumps of spruce, pine, birch and aspen for twelve years after the cutting of these trees (Hintikka 1993).

Members of Strophariaceae, the family containing species of *Hypholoma*, form a paraphyletic group when compared to other agaric fungi. However, genera of *Hypholoma*, *Stropharia*, and *Pholiota* form a monophyly (Moncalvo et al. 2000). Moncalvo et al. (2002) put the genus *Hypholoma* into Clade 109, which is part of the strophariod Clade 107, but, in their analysis, they concluded that this genus is polyphyletic. Bruns et al. (1998) prepared a sequence database of basidiomycetes with a bias towards ectomycorrhizal taxa and grouped Strophariaceae with Cortinariaceae and Entolomataceae.

Hypholoma sublateritium may have bioaccumulation value in cleaning up the environment. *Hypholoma sublateritium* has methyl mercaptan (MeSH) capturing properties (Negishi et al. 2001). Mercury has been quantified from soil and mushrooms including *Hypholoma sublateritium* in Poland (Falandysz 2002).

Somatic Incompatibility

Many researchers have used SI testing, but the results of these crosses are not fully understood. Many journal papers have been reviewed (Appendix 25) and the terms used for antagonism between pairings include barrage (aerial hyphae wall), pigment or discoloration formation, "bow tie" reaction, and gaps. "Line gaps" of sparse mycelium were distinct between pairs of isolates (McCallum et al. 2004) and those with slight or no reaction were considered vegetatively compatible. On self-pairings, a slight barrage was seen that was thinner and with less aerial mycelium than between incompatible isolates. Field isolates of basidiomycete fungi (heterokaryons), when paired, are often incompatible (Kauserud and Schumacher 2002).

Somatic compatibility traditionally has been used to look at population structure in basidiomycetes (Vasiliauskas and Stenlid 1999). Monokaryon pairings of *Serpula lacrymans* between progeny of dikaryotic isolates showed few mating factors suggesting little genetic diversity. These homokaryotic isolates produced oidia (sometimes called arthrospores), where the heterokaryotic isolates produced clamp connections morphologically (Schmidt and Moreth-Kebernik 1991). Vegetative compatibility groups have lost their ability to recognize self from non-self because of the sharing of similar alleles in *Serpula*

lacrymans (Kauserud 2004). Self and non-self recognition is regulated by genes at one or more independent loci (Hansen and Hamelin 1999). No genetic variation (identical sequences) was found among isolates when sequencing the ITS1-5.8SR-ITS2 nrDNA using the primers ITS4 and ITS5. But, isolates had highly variable growth and cultural morphology, which indicates that some genetic variation is present. Genetic variation was found among isolates of *Serpula himantioides* using ISSR analyses (Kauserud 2004).

Sequencing

The nrDNA ITS1-5.8S-ITS2 region has been used in molecular phylogenetic studies in Agaricales, e.g., (Aanen et al. 2000 [*Hebeloma crustuliniforme*], den Bakker et al. 2004 [*Leccinum*], Guzman-Davalos et al. 2003 [*Gymnopilus*], Hoiland and Holst-Jensen 2000 [*Cortinarius*], Hughes et al. 1999 [*Flammulina*], Peintner et al. 2001 [*Cortinarius*], and Seidl 2000 [*Cortinarius*]).

Other agarics have been studied for phylogenetic and taxonomic relationships, e.g., *Agaricus* (Mitchell and Bresinsky 1999) and *Macrolepiota* (Vellinga et al. 2003). In these studies the nrDNA ITS region and 28S gene were classified.

Microsatellites

Microsatellites are tandemly repeated simple DNA sequences that are dispersed throughout the genome and are becoming widely used from individual to population studies (Douhan and Rizzo 2003). This approach involves the amplification of DNA fragments using GC-rich primers to detect DNA

polymorphisms. These fragments are separated on an agarose gel and detected with ethidium bromide.

Microsatellites are highly abundant and evenly dispersed throughout eukaryotic genomes (Weising et al. 1995). This method of DNA fingerprinting has been used by Ali et al. (1986), Epplen (1988), Georges et al. (1988), Meyer et al. (1993), Nubom et al. (1990), and Vassart et al. (1987). The total number of articles published in specialized sections of the journal Molecular Ecology using microsatellites went from 20 in March 1999 to 95 in March 2001. This illustrates that microsatellite markers are being used more and more when studying fungi, plants, and animals (Zane et al. 2002).

Microsatellites were used to study evolutionary recombination, selection, migration, and drift under field conditions (Douhan and Rizzo 2003). Intersequence simple repeats (ISSRs) were used to compare geographic populations of *Trichaptum abietinum* (Kauserud and Schumacher 2003). SSR's are present in both coding and noncoding regions. Microsatellite-primed PCR detected interspecific and intraspecific variation in *Actinidia* species (Weising et al. 1995). Random amplified microsatellite markers (RAMS) combines several characteristics of RAPD and microsatellite analysis as a variation of ISSR was used to distinguish between closely related isolates of *Heterobasidion annosum* (Fr.) Bref. in Europe (Vainio and Hantula 1999).

Populations of *Fomitopsis pinicola* were studied by arbitrary primed PCR AP-PCR using M13 and $(CAGA)_4$ primers (Hogberg et al. 1999). The genetic diversity of *Botryosphaeria dothidea* in pistachios was investigated using

microsatellite-primed PCR (MP-PCR) (Ma et al. 2001). Longer primers are used for MP-PCR and, therefore, these primers are considered more robust than RAPDs (McDonald 1997). They allow more stringent annealing temperatures and reaction conditions for reproducibility and prevent partial/mismatch primer binding (Sunnucks 2000, Weising et al. 1995).

Usually, microsatellite isolation involves screening genomic libraries with probes. Williams et al. (1990), Wu et al. (1994), and Cifarelli et al. (1995) have avoided this time consuming and costly task by modifying RAPD methods. By selecting primers used by other researchers, the need to design flanking primers was eliminated. For this research, the following microsatellite primers were used: $(GACA)_4$, M13 (5' – GAG GGT GGC GGT TCT – 3'), and $(GTG)_5$.

The tetranucleotide primer $(GACA)_4$ was used in studying animal genomes (Epplen 1988) and in studying plant, human, yeast, and *Escherichia coli* DNA (Weising, et al. 1995). The primer M13 (5' – GAG GGT GGC GGT TCT – 3') is a core sequence of the M13 phage. *Gigaspora* intersporal genetic variation was discovered using M13 primer (Zeze et al. 1997). Vassart et al. (1987) used the minisatellite M13 primer to study human and animal DNA and Holmer et al. (1994) used the minisatellite M13 when studying *Phellinus tremulae*. The trinucleotide primer $(GTG)_5$ was used by Nurnberg et al. (1989) to study human somatic tissue and peripheral blood and in studying plant, human, yeast, and *Escherichia coli* DNA (Weising et al. 1995).

Three ISSR-PCR amplifications were performed using the primers $(GTG)_5$, $(CAC)_5$, or $(GACA)_4$ to understand the tolerance of heavy metals Zn, Cd, Cu, and

Ni of *Suillus luteus* (L.:Fr.) Roussel (Colpaert et al. 2000). This testing showed that 90% of the isolates were from different individuals. The genetic variation was greater in the unpolluted site than in the polluted site.

When studying *Botryosphaeria dothidea* Ma et al. (2001), used all three of these microsatellite primers. These three primers were highly reproducible and exhibited variation at the species, subspecies, and individual strain levels when compared to other microsatellite primers from all strains of *Cryptococcus* and *Rhodotorula* (Meyer et al. 1993). Tetra- and tri-nucleotide primers can detect polymorphisms in eukaryotic taxa, and can show interspecific and intraspecific variation in a species (Weising et al. 1995).

Material and Methods

Somatic Incompatibility

Macroscopic observations of crosses were made to determine if controls of *Hypholoma capnoides* and *Hypholoma fasciculare* from the Forest Products Lab (WI) were similar to the *Hypholoma* isolates obtained in the current research and to see if the bole chip isolate was similar to the soil and basidiocarp isolates. Self-crosses were run as controls. All crosses were done on MEA medium with the actively growing culture inoculum plugs placed three centimeters apart from each other, two isolates per petri dish. The crosses were observed every week for four weeks and photographed.

Sequencing

The nrDNA ITS1-5.8S-ITS2 region was sequenced including part of the flanking nuc-LSU (25S) and nuc-SSU (18S) genic regions. The forward and

reverse strand of genomic DNA of these fungal isolates was derived using ITS1-4 primers by sequencing. The SeqMan package of DNA-star software was used to prepare multiple sequence alignment contig files of all the primers of each *Hypholoma* isolate and to compare their contig's of all *Hypholoma* isolates.

Microsatellite-primed PCR

The microsatellite primers used in this research were: $(GACA)_4$, M13 (5' – GAG GGT GGC GGT TCT – 3'), and $(GTG)_5$, which were purchased commercially. PCR was performed in 50 µL reaction volumes containing 35.8 µL sterile molecular water, 5 µL buffer –Mg, 2 µl $MgCl_2$, 1 µL dNTP's, 1 µL primer, 0.2 µL of *Taq* DNA polymerase; and approx. 100 ng of template DNA. Reaction conditions were the following for amplification on an Eppendorf Mastercycler thermocycler. Initial denaturation at 94°C for 2 min followed by 35 cycles of 30 sec. denaturation at 94°C, 30 sec. annealing at 52°C and extension at 72°C for 1 min. After a final extension at 72°C for 7 min the samples were kept at 4°C until electrophoresis. Negative controls without template, positive control of an unknown fungal isolate, *Hypholoma capnoides* (collection number OKM-1523-T from the Forest Products Lab in WI), and *Hypholoma fasciculare* (collection number OKM-2932-T from the Forest Products Lab in WI) were included in all runs. Both of these *Hypholoma* control isolates obtained from the Forest Products lab were collected in Idaho, USA; *H. capnoides* from a stump base and *H. fasciculare* from an *Acer glabrum* var. *douglasii* stump. All amplifications were duplicated for verification of bands.

The amplified DNA was separated by electrophoresis on 2.0% agarose in 1X TBE buffer for ca. 2 hours, stained with 0.5 mg/L ethidium bromide (EtBr) for 15 min, soaked in water for 15 min, and then visualized under UV light and photographed. The gels were manually analyzed for absence or presence of bands. The DNA amplification, followed by electrophoresis, resulted in 19 scorable bands. Primer $(GACA)_4$ produced 4-5 bands, primer M13 produced 6-7 bands, and primer $(GTG)_5$ produced 4-7 bands.

The morphological and molecular methods used to analyze the fungal isolates are described in Chapter 2.

Results

Morphological Characteristics

The *Hypholoma* isolates were of two distinct types in regards to morphological characteristics. All *Hypholoma* isolates were GAA/TAA oxidase positive. All October jar wood bait, three of the soil pit wood baits (SPB 28-1 M 1A, SPB 28-2 M 1A, SPB 28-2 T 1A), and two of the basidiocarp culture isolates (M3 28 ? WA, M4 28-4 MYA+) had clamp connections, a fast growth rate, and zonate macroscopic growth (Fig. 56). The bole chip, all direct soil, one soil pit wood bait (SPB 28-2 T 2A), and one basidiocarp (M4 28-4 MYBDA) culture isolates had oidia, a slow growth rate, and a feathery/powdery macroscopic growth (Fig. 57). (The first morphological observation of M4 28-4 MYBDA and SPB 28-2 T 2A isolates did not have oidia and M4 28-4 MYBDA had questionable clamps.) Summary data of *Hypholoma* isolates are grouped by growth rate (Table 34).

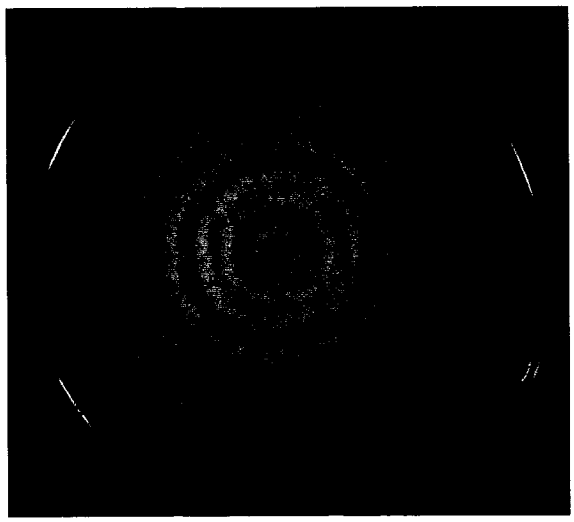


Fig. 56. *Hypholoma* fungal isolate showing the zonate pattern and fast growth, full plate in 2 weeks. These fungal isolates had clamp connections.

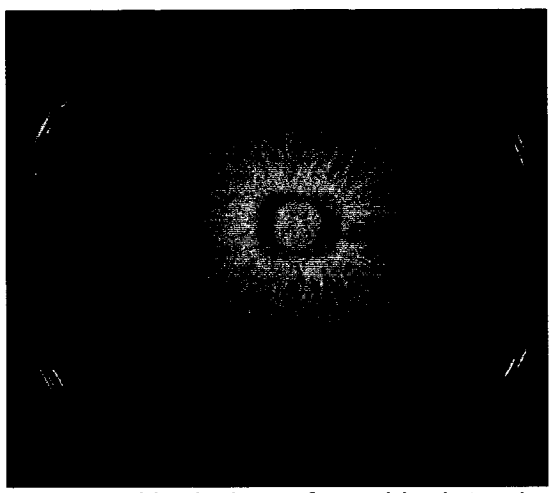


Fig. 57. *Hypholoma* fungal isolate showing the feathery/powdery pattern and slower growth, 1/2 plate in 2 weeks. These fungal isolates had oidia.

Table 34. Summary cultural data of *Hypholoma* isolates.

Fungal Isolate [V]	Soil Horizon	GAA/TAA [W]	M [X]	GR [Y]	Texture [Z]
Fast growing					
10JB 28-1 WP 2 2	Oe	1	1	1	1
10JB 28-2 WP 2 1	E	1	1	1	1
10JB 28-2 WP 8	E	1	1	1	1
10JB 28-2 WP 1 6	E	1	1	1	1
10JB 28-2 RM 1 1	E	1	1	1	1
10JB 28-3 WP 2 2	BC	1	1	1	1
SPB 28-1 MYBDA 1A	Oe	1	1	1	1
SPB 28-2 MYBDA 1A	E	1	1	1	1
SPB 28-2 Thorn 1A	E	1	1	1	1
M4 28-4 MYA+		1	1	1	1
M3 28 ? WA		1	1	1	1
Slow growing					
BC 28-2-1 LGBDA 6/99		1	0	0	0
Soil 28-1 OSL L2 2 B	Oe	1	0	0	0
Soil 28-1 OSL L2 2	Oe	1	0	0	0
Soil 28-1 OSL T1 1	Oe	1	0	0	0
Soil 28-1 OSL L2 2 A	Oe	1	0	0	0
SPB 28-2 Thorn 2A	E	1	0	0	0
M4 28-4 MYBDA		1	0	0	0

V: Fungal Isolate: Method/source 10JB = October jar bait, SPB = soil pit bait, M3 & M4 basidiocarp, Soil, BC = bole chip
W: Oxidase reaction (0 = negative, 1 = positive)
X: Microscopic characteristics (0 = oidia, 1 = clamps)
Y: Growth rate at 2 weeks (0 = slow, 1 = fast)
Z: Morphological texture (0 = powdery, 1 = zonate)

Sequencing

Table 35 indicates the number of sequence runs that were done per culture isolate per primer (ITS1-4, LR3, 5.8SR).

Table 35. Number of sequence runs per primer for each *Hypholoma* isolate. See Table 34 for fungal isolate abbreviations.

Fungal Isolate		ITS1	ITS2	ITS3	ITS4	LR3	5.8SR
	Fast growing						
10JB	10JB 28-1 WP 2 2	2	2	2	1	1	1
10JB	10JB 28-2 WP 2 1	2	2	2	1	0	0
10JB	10JB 28-2 WP 8	2	2	2	1	0	0
10JB	10JB 28-2 WP 1 6	2	2	2	1	0	0
10JB	10JB 28-2 RM 1 1	2	2	2	1	0	0
10JB	10JB 28-3 WP 2 2	2	1	2	1	0	1
SPB	SPB 28-1 M 1A	2	2	2	1	0	1
SPB	SPB 28-2 M 1A	2	2	2	1	0	1
SPB	SPB 28-2 T 1A	1	0	1	1	0	0
M3	M3 28 ? WA	1	1	1	1	0	0
M4	M4 28-4 MYA+	1	1	1	1	0	1
	Slow growing						
BC	BC 28-2-1 L 6/99	2	2	2	1	0	1
Soil	Soil 28-1 OSL L2 2B	2	2	3	0	0	1
Soil	Soil 28-1 OSL L2 2	1	1	1	1	1	0
Soil	Soil 28-1 OSL T1 1	1	1	1	1	0	1
Soil	Soil 28-1 OSL L2 2A	1	1	1	1	0	1
SPB	SPB 28-2 T 2A	2	2	2	1	0	0
M4	M4 28-4 MYBDA	1	1	1	1	0	0

The aligned sequence data for all isolates listed in Table 35 were exactly the same for every base pair, (Fig. 58). Significantly, after cleaning up the sequences of all 18 *Hypholoma* isolates from research site # 28, they were identical and had a length of 700bp.

The sequence data (contig's) of all the *Hypholoma* isolates were BLASTed in GenBank. The result selected had the highest percent homology, *Hypholoma sublateritium*, with an accession number of AY534117, score bit 1302, E value 0.0, 99% identity. The common name of this species is brick caps or brick top.

The classification is Strophariaceae, Agaricales, Basidiomycota, and Fungi. Family and order was determined using Hawksworth et al (1995).

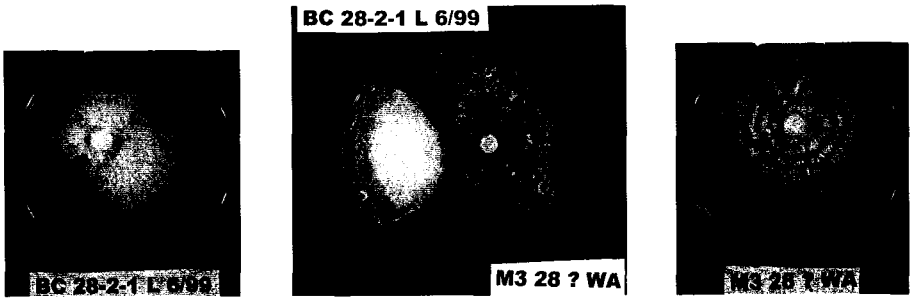
Synonym names are *Nematoloma sublateritium* (Schaeff.) Karst., and *Agaricus sublateritius* Schaeff.

GAACCTGCGGAAGGATCATTATTGAATGAACTTGACTTGGTTGCTGCTGGTCTTTTCGA
AGGCATGTGCACACTTTGTCATCTTTATATCTCCACCTGTGCACCTTTTGTAGACCTGA
AACCAAATTTCCGAGGCAACTCGGTCGTGAGGAAATGCTTAACAGCTTTCCTTGTTAGG
TTCCAGGGCTATGTTTTCATATACACCATAAGAATGTAACAGAATGTCATTATTAGGCT
TAGTTGCCTTATAAACTATATACAACTTTCAGCAACGGATCTCTTGGCTCTC**GCATCGA**
TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA
ATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCA
TTAAATTCTCAACCTTTGCTTACTTTTTTGGTTAGCAAATGGCTTGGATGTGGGGGTAT
GCAGGTTTCTGTAATGAAATTAGCTCCCCTGAAATACATTAGCTGGTTGCCTTGTGTGA
ACCTGTCTATTGGTGTGATAATTATCTACGCCGTGGACTATTCGCCGTTTTTAGCACTG
CTTATAACCGTCTGTTCATTCGGACAATATATGACAATTTGACCTCAAATCAGGTAGGA
CTACCCGCTGAACTTAA**GCATATCAATAA**

Fig. 58. Aligned sequence (contig) for all the *Hypholoma* isolates, the bases highlighted represent the sequence of the primers (ITS1-4).

Somatic Incompatibility

Somatic incompatibility testing of the *Hypholoma* isolates showed a similar pattern to the cultural data: two different types of reactions. Figures 59-61 show a definitive golden discoloration reaction at the interface of the two hyphal fronts. The bole wood (chip) inocula are to the left and the other *Hypholoma* isolates are to the right. The pure culture of each isolate is included in each figure for comparison of the growth patterns.

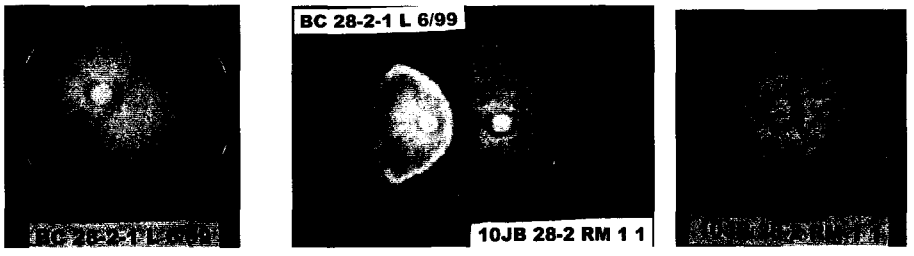


Bole chip **SI Cross** Basidiocarp

Fig. 59. The SI cross between the bole chip (BC 28-2-1 L) and basidiocarp (M3 28 ? WA) fungal isolates showing a discoloration at the interface of the two hyphal fronts.

Bole chip **SI Cross** Basidiocarp

Fig. 60. The SI cross between the bole chip (BC 28-2-1 L) and basidiocarp (M4 28-4 MYA+) fungal isolates showing a discoloration at the interface of the two hyphal fronts.



Bole chip **SI Cross** Jar Bait, E soil horizon

Fig. 61. The SI cross between the bole chip (BC 28-2-1 L) and jar bait (JB 28-2 RM 11) fungal isolates showing a discoloration at the interface of the two hyphal fronts.

The same SI reaction as shown in Figures 59-61 were seen on all Jar Bait and three of the Soil Pit Bait *Hypholoma* culture isolates (10JB 28-1 WP 22, 10JB 28-2 WP 21, 10JB 28-2 WP 8, 10JB 28-2 WP 16, 10JB 28-3 WP 22, SPB 28-1 M

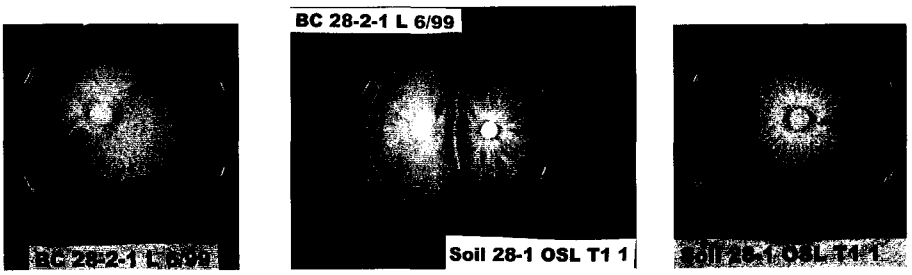
1A, SPB 28-2 M 1A, SPB 28-2 T 1A). These are the *Hypholoma* isolates that had the zonate texture, were fast growing, and had clamp connections.

Figure 62 illustrates a slight spacing at the interface of the two hyphal fronts of bole chip isolate, BC 28-2-1 L, and the direct soil isolate, Soil 28-1 OSL L2 2B.

Bole chip **SI cross** Direct Soil, Oe soil horizon

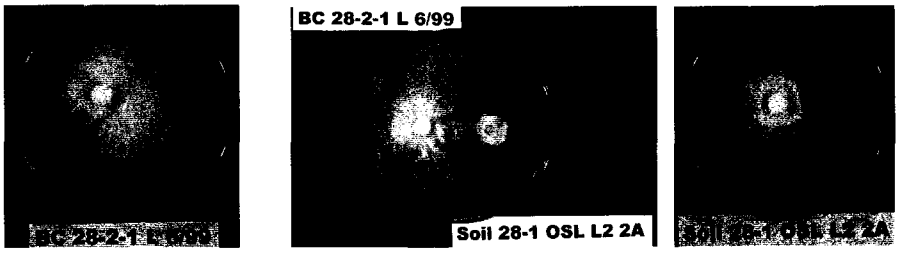
Fig. 62. The SI cross between the bole chip (BC 28-2-1 L) and direct soil (Soil 28-1 OSL L2 2B) fungal isolates showing a slight spacing at the interface of the two hyphal fronts.

Figures 63-65 illustrate the type of SI on all direct soil isolates and the soil pit bait isolate SPB 28-2 T 2A crossed with the bole chip isolate. These crosses are not clearly compatible and have some reaction taking place. This type of reaction occurred only with the slower growing *Hypholoma* isolates that had a feathery/powdery texture and had oidia.



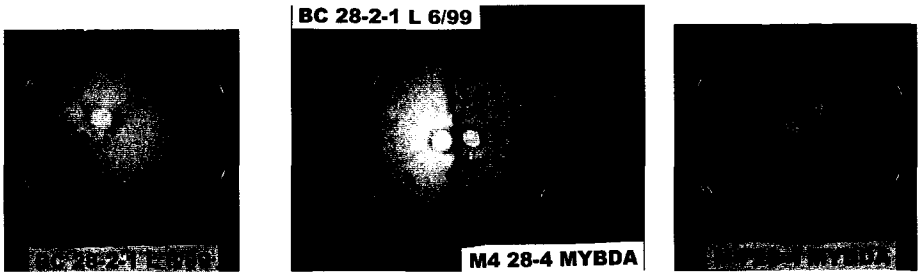
Bole chip **SI cross** Direct Soil, Oe soil horizon

Fig. 63. The SI cross between the bole chip (BC 28-2-1 L) and direct soil (Soil 28-1 OSL T11) fungal isolates showing a slight golden discoloration and barrage at the interface of the two hyphal fronts.



Bole chip **SI cross** Direct Soil, Oe soil horizon

Fig. 64. The SI cross between the bole chip (BC 28-2-1 L) and direct soil (Soil 28-1 OSL L22A) fungal isolates showing the direct soil isolate has stunted growth and has a golden discoloration.



Bole chip **SI cross** Basidiocarp

Fig. 65. The SI cross between the bole chip (BC 28-2-1 L) and basidiocarp (M4 28-4 MYBDA) fungal isolates showing a slight discoloration on the underside of the petri dish at the interface of the two hyphal fronts. Both inocula have a golden discoloration.

Two different fungal isolates were kept from basidiocarp M4 28-4 (Fig. 66). One was isolated on MYA+ medium and the other on MYBDA medium. Both media were identical in ingredients except that MYBDA contains two fungicides

(Benomyl and Dichloran). The fungal isolate on the MYA+ medium is fast growing, and has zonate texture and clamp connections. The fungal isolate on the MYBDA medium is slow growing and has a feathery/powdery texture and oidia.

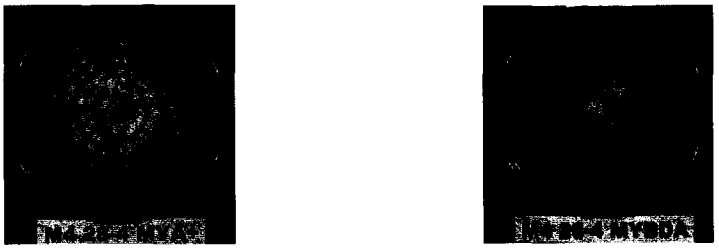
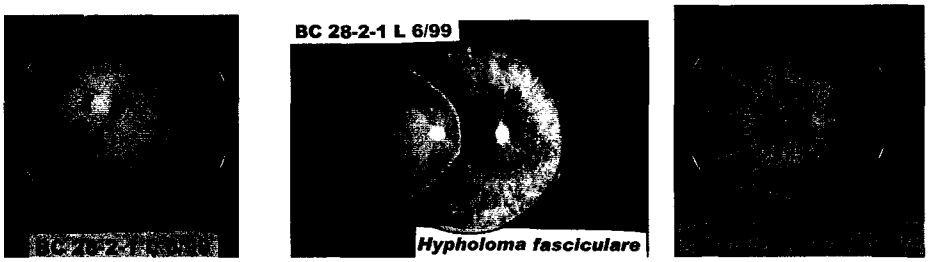


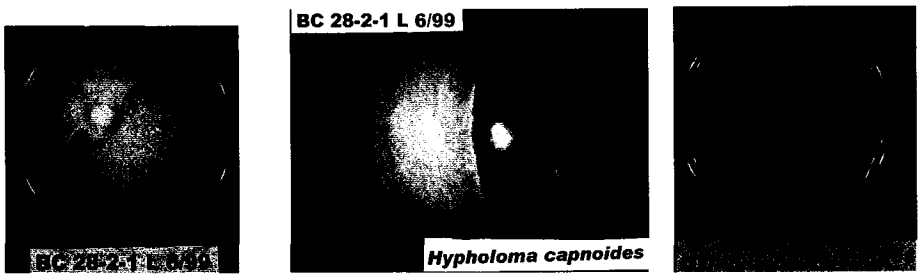
Fig. 66. Both of these basidiocarp isolates come from the same basidiocarp, but were isolated on different culture medium. One medium is MYA+, which includes the same proportion of malt and yeast extract, antibiotics, and agar as the other medium MYBDA, which also contains two fungicides (Benomyl and Dichloran).

Figures 67-68 show the somatic incompatibility crosses of the bole wood (chip) isolate with known fungal isolates from the Forest Products Lab (WI) *Hypholoma fasciculare* and *Hypholoma capnoides*. Figure 69 shows the somatic incompatibility cross of the known fungal isolates *Hypholoma fasciculare and H. capnoides*. Also the self-crosses of these known fungal isolates show that spacing at the hyphal front or slight barrage may not suggest incompatibility.



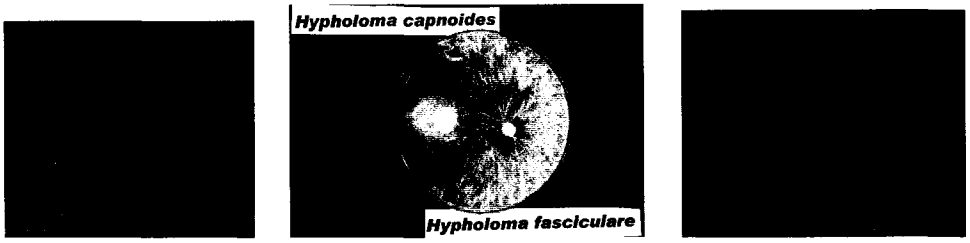
Bole chip **SI cross** *Hypholoma fasciculare*

Fig. 67. The SI cross between the bole chip (BC 28-2-1 L) fungal isolate and the known *Hypholoma fasciculare* shows a discoloration at the interface of the two hyphal fronts.



Bole chip **SI cross** *Hypholoma capnoides*

Fig. 68. The SI cross between the bole chip (BC 28-2-1 L) fungal isolate and the known *Hypholoma capnoides* shows a golden discoloration at the interface of the two hyphal fronts.



Self cross of *Hypholoma capnoides* **SI cross** Self cross of *Hypholoma fasciculare*

Fig. 69. The SI cross between the known isolates, *Hypholoma fasciculare* and *H. capnoides* shows a golden discoloration at the interface of the two hyphal fronts. *Hypholoma capnoides* growth is stunted and is golden in color. The self-crossing of *Hypholoma capnoides* shows a spacing at the hyphal front. The self-crossing of *Hypholoma fasciculare* shows a barrage at the hyphal front.

Because of the differences in the cultural data and somatic incompatibility crosses of these *Hypholoma* isolates, microsatellite primers were run. Table 36 shows the order in which the isolates and controls where placed in the wells per gel.

Table 36. Each agarose gel of microsatellite primers were set up in the same order of unknown *Hypholoma* isolates, known *Hypholoma fasciculare* and *Hypholoma capnoides* and negative and positive controls. The ladder on each end of these gels is 1KB+.

Microsatellite Electrophoresis Gels

Gel A well #	Isolate/ladder	Gel B well #	Isolate/ladder/controls
1	1 KB+ ladder	1	1 KB+ ladder
2	Soil 28-1 OSL L2 2B	2	M4 28-4 MYA+
3	BC 28-2-1 L 6/99	3	M4 28-4 MYBDA
4	10JB 28-1 WP 2 2	4	Soil 28-1 OSL L2 2
5	10JB 28-2 WP 2 1	5	Soil 28-1 OSL T1 1
6	10JB 28-2 WP 8	6	Soil 28-1 OSL L2 2A
7	10JB 28-2 WP 1 6	7	*Hypholoma fasciculare*
8	10JB 28-2 RM 1 1	8	*Hypholoma capnoides*
9	10JB 28-3 WP 2 2	9	SPB 28-2 T 1A
10	SPB 28-1 M 1A	10	negative control
11	SPB 28-2 M 1A	11	positive control
12	SPB 28-2 T 2A	12	1KB+ ladder
13	M3 28 ? WA		
14	1 KB+ ladder		

The absence or presence of only clear and reproducible bands was scored using the microsatellite primers. The amplification products of one primer at a time were compared on a gel by electrophoresis.

The M13 primer yielded 6-7 bands in a molecular mass range of 275-1900 bp. Figures 70 & 71 show the agarose gels using the primer M13.

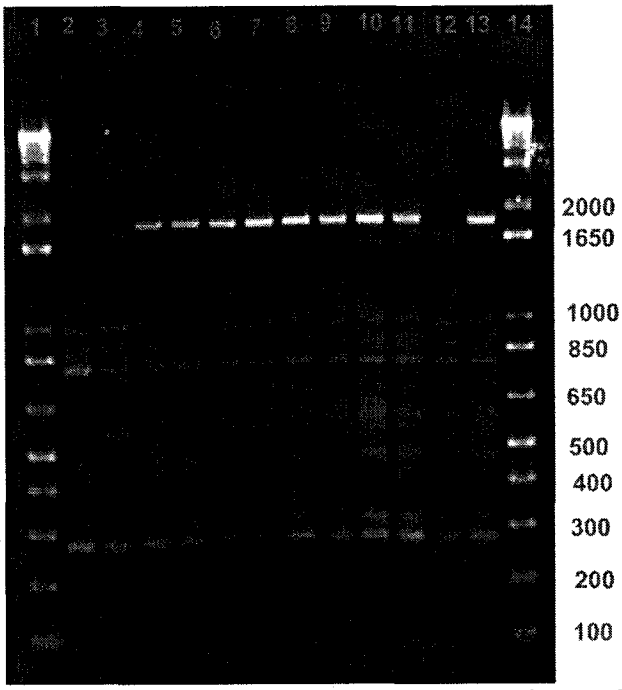


Fig. 70. Agarose gel A using M13 primer. The two end wells are the 1KB+ ladder (100-2000 bp). Satellite bands using the M13 primer included: 1900, 1650, 1000, 800, 475, 325, 275 bp.

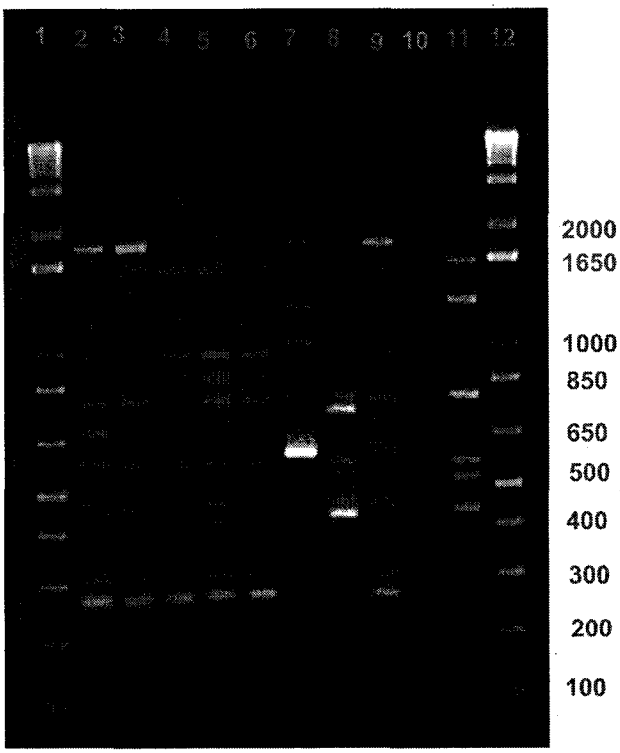


Fig. 71. Agarose gel B using M13 primer. The two end wells are the 1KB+ ladder (100-2000 bp). Satellite bands using the M13 primer included: 1900, 1650, 1000, 800, 475, 325, 275 bp.

Tables 37, 38, and 39 list the microsatellite bands per *Hypholoma* culture isolates using primers M13, (GACA)4, and (GTG)5, respectively.

Table 37. Site #28 *Hypholoma* fungal isolates satellite bands (bp) using the M13 primer

Well #	Gel A			Base Pair				
2	Soil 28-1 OSL L2 2B		1650	1000	800	475	325	275
3	BC 28-2-1 L 6/99		1650	1000	800	475	325	275
4	10 JB 28-1 WP 2 2	1900	1650	1000	800	475	325	275
5	10 JB 28-2 WP 2 1	1900	1650	1000	800	475	325	275
6	10 JB 28-2 WP 8	1900	1650	1000	800	475	325	275
7	10 JB 28-2 WP 1 6	1900	1650	1000	800	475	325	275
8	10 JB 28-2 RM 1 1	1900	1650	1000	800	475	325	275
9	10 JB 28-3 WP 2 2	1900	1650	1000	800	475	325	275
10	SPB 28-1 M 1A	1900	1650	1000	800	475	325	275
11	SPB 28-2 M 1A	1900	1650	1000	800	475	325	275
12	SPB 28-2 T 2A		1650	1000	800	475	325	275
13	M3 28 ? WA	1900	1650	1000	800	475	325	275
Well #	**Gel B**							
2	M4 28-4 MYA+	1900	1650	1000	800	475	325	275
3	M4 28-4 MYBDA	1900	1650		800	475	325	275
4	Soil 28-1 OSL L2 2		1650	1000	800	475	325	275
5	Soil 28-1 OSL T1 1		1650	1000	800	475	325	275
6	Soil 28-1 OSL L2 2A		1650	1000	800	475	325	275
9	SPB 28-2 T 1A	1900	1650	1000	800	475	325	275

The (GACA)4 primer yielded 4-5 bands in a molecular mass range of 475-1900 bp. Figures 72 & 73 are the agarose gels using the primer (GACA)4.

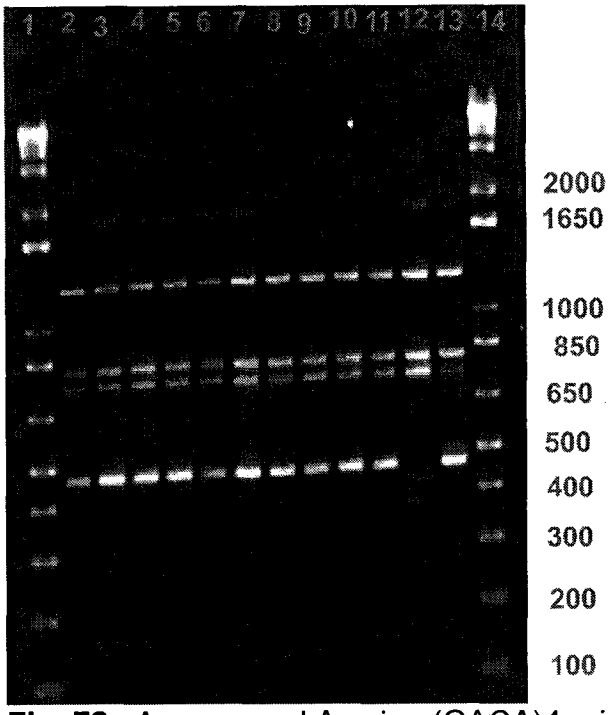


Fig. 72. Agarose gel A using (GACA)4 primer. The two end wells are the 1KB+ ladder (100-2000 bp). Satellite bands using this primer included: 1900, 1300, 800, 750, 475 bp.

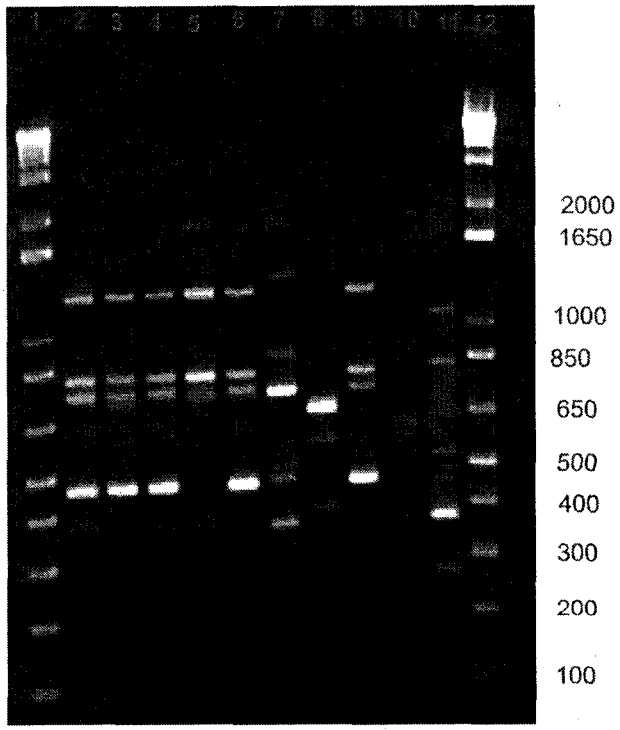


Fig. 73. Agarose gel B using (GACA)4 primer. The two end wells are the 1KB+ ladder (100-2000 bp). Satellite bands using this primer included: 1900, 1300, 800, 750, 475 bp.

Table 38. Site #28 *Hypholoma* fungal isolates satellite bands (bp) using the (GACA)4 primer

Well #	Gel A	Base Pair				
2	Soil 28-1 OSL L2 2B	1900	1300	800	750	475
3	BC 28-2-1 L 6/99	1900	1300	800	750	475
4	10 JB 28-1 WP 2 2	1900	1300	800	750	475
5	10 JB 28-2 WP 2 1	1900	1300	800	750	475
6	10 JB 28-2 WP 8	1900	1300	800	750	475
7	10 JB 28-2 WP 1 6	1900	1300	800	750	475
8	10 JB 28-2 RM 1 1	1900	1300	800	750	475
9	10 JB 28-3 WP 2 2	1900	1300	800	750	475
10	SPB 28-1 M 1A	1900	1300	800	750	475
11	SPB 28-2 M 1A	1900	1300	800	750	475
12	SPB 28-2 T 2A	1900	1300	800	750	
13	M3 28 ? WA	1900	1300	800	750	475
Well #	**Gel B**					
2	M4 28-4 MYA+	1900	1300	800	750	475
3	M4 28-4 MYBDA	1900	1300	800	750	475
4	Soil 28-1 OSL L2 2	1900	1300	800	750	475
5	Soil 28-1 OSL T1 1	1900	1300	800	750	
6	Soil 28-1 OSL L2 2A	1900	1300	800	750	475
9	SPB 28-2 T 1A	1900	1300	800	750	475

The (GTG)5 primer yielded 4-7 bands in a molecular mass range of 375-1325 bp. Figures 74 and 75 are the agarose gels using the primer (GTG)5.

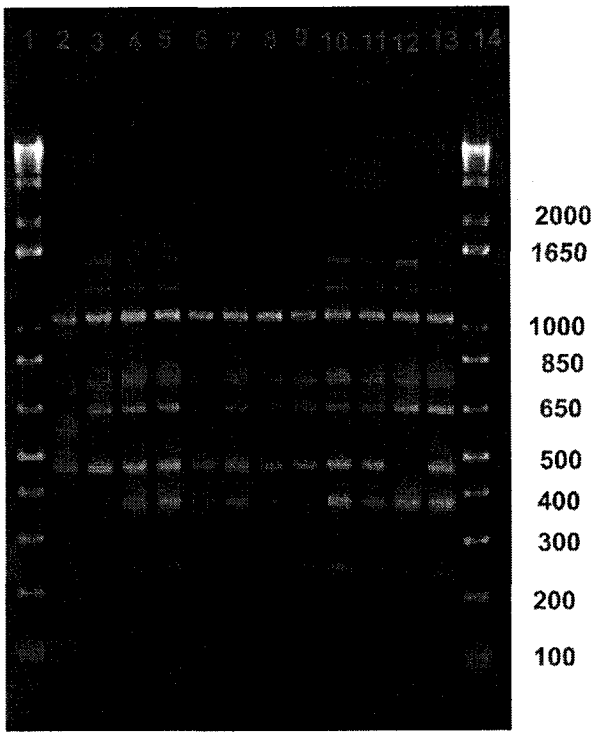


Fig. 74. Agarose gel A using (GTG)5 primer. The two end wells are the 1KB+ ladder (100-2000 bp). Satellite bands using this primer included: 1500, 1325, 1000, 750, 650, 475, 375 bp.

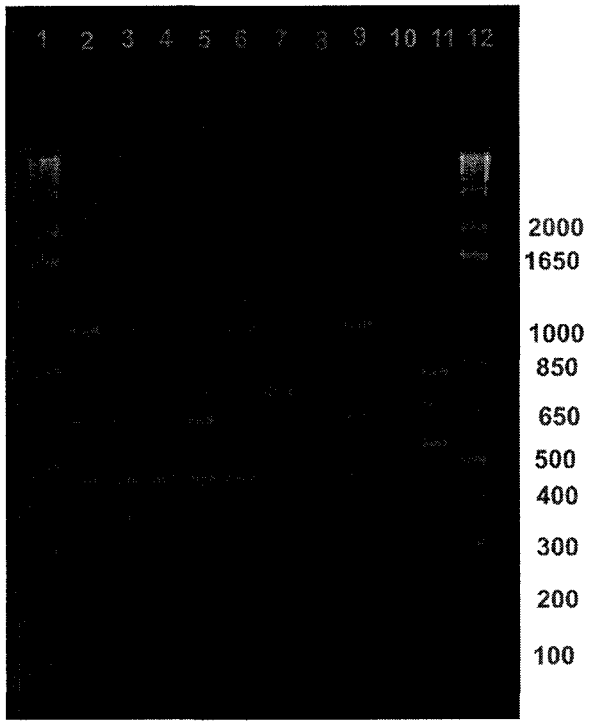


Fig. 75. Agarose gel B using (GTG)5 primer. The two end wells are the 1KB+ ladder (100-2000 bp). Satellite bands using this primer included: 1500, 1325, 1000, 750, 650, 475, 375 bp.

Table 39. Site #28 *Hypholoma* fungal isolates satellite bands (bp) using the (GTG)5 primer.

Well #	Gel A	Base Pair						
2	Soil 28-1 OSL L2 2B	1500	1325	1000			475	
3	BC 28-2-1 L 6/99	1500	1325	1000	750	650	475	
4	10 JB 28-1 WP 2 2	1500	1325	1000	750	650	475	375
5	10 JB 28-2 WP 2 1	1500	1325	1000	750	650	475	375
6	10 JB 28-2 WP 8	1500	1325	1000	750	650	475	375
7	10 JB 28-2 WP 1 6	1500	1325	1000	750	650	475	375
8	10 JB 28-2 RM 1 1	1500	1325	1000	750	650	475	375
9	10 JB 28-3 WP 2 2	1500	1325	1000	750	650	475	375
10	SPB 28-1 M 1A	1500	1325	1000	750	650	475	375
11	SPB 28-2 M 1A	1500	1325	1000	750	650	475	375
12	SPB 28-2 T 2A	1500	1325	1000	750	650		375
13	M3 28 ? WA	1500	1325	1000	750	650	475	375
Well #	**Gel B**							
2	M4 28-4 MYA+	1500	1325	1000	750	650	475	375
3	M4 28-4 MYBDA	1500	1325	1000	750	650	475	375
4	Soil 28-1 OSL L2 2	1500	1325	1000	750		475	
5	Soil 28-1 OSL T1 1	1500	1325	1000	750	650	475	
6	Soil 28-1 OSL L2 2A	1500	1325	1000	750		475	
9	SPB 28-2 T 1A	1500	1325	1000	750	650	475	375

The *Hypholoma* isolates that had fewer bands using the microsatellite primers are also the isolates that were slower growing, had feathery/powdery texture, and had oidia. There are very few informative characters in the morphological and microsatellite results. Therefore, PAUP phylogenetic analysis is not included in this publication.

Figure 76 is a photograph of *Hypholoma sublateritium* basidiocarp found on a hardwood bole at the research site, taken September 1999.

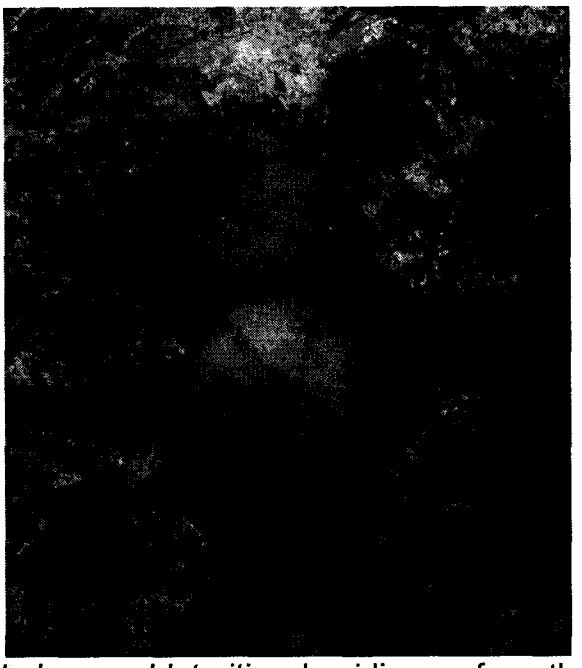


Fig. 76. *Hypholoma sublateritium* basidiocarp from the research site.

Hypholoma sublateritium was isolated from three soil horizons, Oe, E, and BC, at the research site # 28. Figure 77 illustrates these soil horizons. Isolates were cultured from 1) the organic (Oe) soil horizon by the direct soil, jar bait, and soil pit bait culture methods, from 2) the mineral soil horizon (E) by the jar bait and soil pit bait culture methods, and from 3) the mineral soil horizon (BC) by the jar bait culture method.

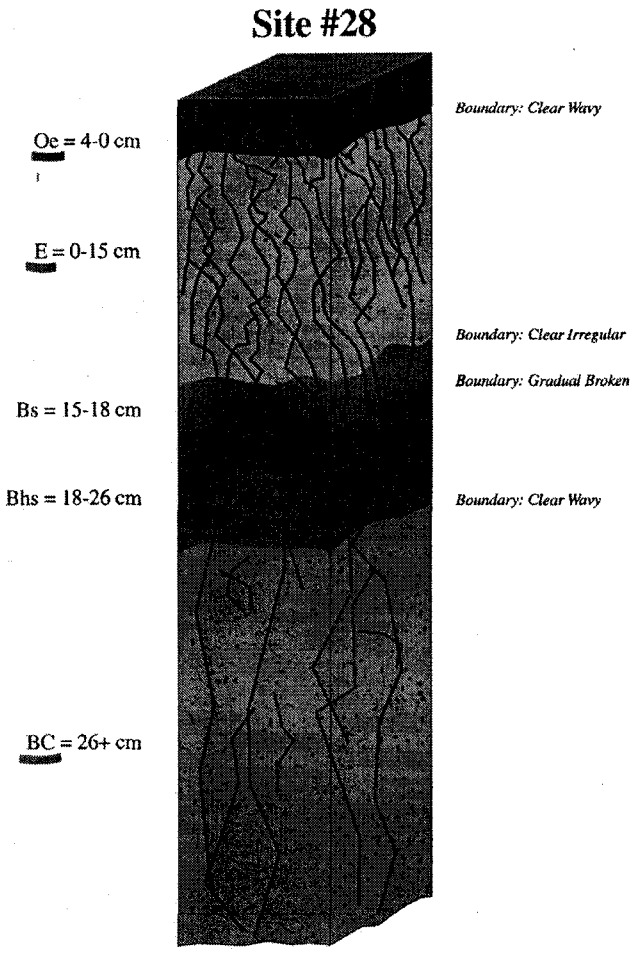


Fig. 77. Soil horizons at research site # 28, indicating (underlined in red) from which horizons *Hypholoma sublateritium* were isolated.

Discussion

At the research site # 28, the basidiocarp *Hypholoma sublateritium* was collected and isolated from soil in the October 1997 and October 1998 collection. *Hypholoma sublateritium* was isolated from bole wood in June 1999. Basidiocarps of this fungus were photographed at this research site in September 1999 (Fig. 76). The fungus was isolated from organic soil (Oe) by using the direct culture method, jar wood bait culture method in October 1999 and isolated again in October 2000 by the soil pit wood culture method. It was

isolated from mineral soils (E and BC soil horizons) in October 1999 by the jar wood bait culture method, and in October 2000 (E soil horizon) by the soil pit wood culture method.

Morphologically (see Table 34) isolates with oidia that are slower growing were isolated from the bole wood, organic soil (direct culture method), and mineral soil (E horizon) by the soil pit wood bait culture method and basidiocarp. Isolates that had clamp connections and faster growth habit were isolated from basidiocarp, organic soil, and mineral soils (E & BC horizons). The latter two substrates yielded isolates from jar wood bait and soil pit wood bait cultural methods.

Based on sequencing, the ITS region of the genome of these *Hypholoma* isolates from bole wood, soil, and basidiocarps indicate they are the same species. However, the morphological, somatic incompatibility testing and microsatellite testing indicates that the isolates with the clamp connections are probably heterokaryotic and the isolates with the oidia are homokaryotic. Dr. Rytas Vilgalys (personal communication) reported that he also saw morphological differences with *Hypholoma* fungal isolates. Other researchers have documented these phenomena among fungal isolates of the same species growing in close proximity to each other.

Hypholoma sublateritium also was found at another research site, # 26, near site # 28. Its basidiocarp was collected and isolated from in October 1998, it was isolated in mineral soil (E horizon) in June 1999 by jar wood bait culture method, and it was isolated directly from organic soil (Oe horizon) in October

1999. The basidiocarp and mineral soil isolates had clamp connections and the organic soil isolate had oidia. Very few bole chip fungal isolates from site # 26 were sequenced, so it is not know if this fungus was in the bole wood.

Further research with these fungal isolates may be necessary to confirm that a hyphal bridge connects the bole wood with the mineral soil. However, the data do confirm the presence of the same species, *Hypholoma sublateritium*, in these substrates, suggesting that this fungus taps the mineral soil for nutrients in the decay process.

CHAPTER VII

CONCLUSIONS

The purpose of this research was to determine, for the first time, whether the same species or organism of basidiomycete fungus is present in a continuum from the mineral soil to the decaying log, thus forming a hyphal bridge for the transport of minerals to aid in the decay process. The research was successful in demonstrating that the same species, *Hypholoma sublateritium*, was found in all of these substrates and the evidence strongly suggests that the isolates from these substrates are the same organism. The verification of this research was accomplished through specific cultural methods and media, and the use of DNA sequencing of the nrDNA ITS1-5.8S-ITS2 region of the genome.

Cord-forming fungal mycelia can acquire and translocate phosphorus from soil and they have the ability to polarize a new wood resource (Wells et al. 2001). A wood-decomposing saprotrophic fungus *Hypholoma fasciculare* translocated ^{32}P to an ectomycorrhizal fungus *Suillus variegates* and its plant hosts, (Lindahl et al. 1999). *Hypholoma* species are cord-forming fungi. Further studies with *Hypholoma sublateritium* will be required to prove that this fungus can translocate nutrients from the mineral soil to the coarse woody debris.

Since this research was initiated, other methods have been published to study fungi in soils. Ranjard et al. (2001) have developed an automated rRNA intergenic spacer analysis (F-ARISA) to characterize fungal communities from different soil types. Sobek and Zak (2003) have developed a Soil Fungi Log procedure to study the functional role of micro-fungi in soils. The Epicentre SoilMaster™ DNA extraction kit has been developed to provide PCR-ready DNA from microbial populations in soil and sediment samples. MO BIO UltraClean™ Soil DNA isolation kit (Cat. # 12800-100) will remove humic acid inhibitors and extract DNA from soil microbes.

Comparing the three media (LGBDA, MYBDA, & LGBA) used to isolate the fungi from all the research sites, MYBDA was more effective in obtaining putative basidiomycetous fungi from the various substrates. With bole wood: 100 culture isolates were obtained using LGBDA; 133 culture isolates were obtained using MYBDA; and 11 culture isolates were obtained using LGBA. With soil pit wood baits: 7 culture isolates were obtained using LGBDA; 20 culture isolates were obtained using MYBDA; and 6 culture isolates were obtained using LGBA.

Isolating directly from soil: 49 culture isolates were obtained using LGBDA, (26 from organic soil and 23 from mineral soil horizon); 35 culture isolates were obtained using MYBDA (8 from organic soil and 26 from mineral soil horizon); and 11 culture isolates were obtained using LGBA (10 from organic soil and 1 from mineral soil horizon). To isolate basidiomycete fungi from organic soil, LGBDA and LGBA media were more effective. To isolate basidiomycete fungi from mineral soil, MYBDA medium was most effective.

However, the bole chip and all of the direct soil *Hypholoma* isolates were isolated on LGBDA medium and two of the four soil pit bait isolates were isolated on LGDA (Thorn) medium. It also is interesting that five of the six jar bait *Hypholoma* isolates were isolated from white pine wood. White pine wood is less dense than hardwood red maple. White rot fungi decay both softwood and hardwood species. *Hypholoma sublateritium* prefers hardwood species, but other species of *Hypholoma* prefer coniferous wood, while some species are found on both hardwood and softwood and a few species grow among sphagnum moss.

Only MYBDA medium was used in the jar bait culture method. Over all the research sites, the June soil jar baits yielded only one putative basidiomycetous fungus from the organic soil horizon, but it did yield three from the upper mineral soil horizon and ten from the lower mineral soil horizon. Over all the research sites, the October soil jar baits yielded six organic soil horizon isolates, nine from the upper mineral soil horizon and one from the lower mineral soil horizon.

From the soil pit wood baits, of all the research sites, 19 putative basidiomycetous fungal isolates came from organic soil horizon, six from the upper mineral soil horizon, and eight from the lower mineral soil horizon.

Although only a few of the total number of fungal isolates were sequenced, the data is convincing that these methods and media are specific for basidiomycete fungi. One hundred percent of the soil pit wood bait and soil jar

wood bait isolates, 68% of the direct organic soil culture isolates, and 59% of the direct mineral soil culture isolates have been identified as basidiomycetous fungi.

The number of fungal isolates that were kept for further studies and the percentage of those isolates that have clamp connections microscopically are shown in Table 40.

Table 40. Number of culture isolates from various substrates and percent showing clamp connections.

	Number	% w/ Clamp Cxn.
Bole Wood	245	80
Soil (direct)	100	5
Soil Jar Wood Bait	30	73
Soil Pit Wood Bait	33	91

If the presence of clamp connections is a positive indicator of a basidiomycete when isolating from a substrate, then incubating soil with wood, as in the jar bait and soil pit bait methods, dramatically increased the percent of fungal isolates from soil that were putative basidiomycetes. Only 5% of the fungal isolates cultured directly from soil had clamp connections. The jar bait and soil pit bait yielded isolates with 73% and 91% clamp connections; respectively. This is compared to 80% from bole wood. The culture method of exposing the soil to wood and culturing the decaying wood worked exceptionally well. Exposing soil samples to wood in the soil pit wood baits and jar wood baits was shown to be effective by the number of fungal isolates that were identified from research site # 28 as *Hypholoma sublateritium*.

In addition to the selective cultural methods, the use of selective media, LGBDA, MYBDA, and LGBA (Thorn), helped isolate basidiomycete fungi. Isolating fungi directly from soil whether white (WR) or brown (BR) rotters, the LGBDA and LGBA media worked best with organic soils (WR 71%, BR 75%). MYBDA medium worked best with mineral soils (WR 67%, BR 93%). Using the soil pit bait cultural method, isolating white rotters from organic or mineral soil did not favor either of the selective media (organic soil 42% on LYBDA/LGBA and 58% on MYBDA, mineral soil 56% on LGBDA/LGBA and 44% on MYBDA). But, to isolate brown rotting fungi from mineral soil using the soil pit bait cultural method, MYBDA is the preferred medium to use (100%). In isolations from bole wood, both media, LGBDA/LGBA or MYBDA, were selective for white (LGBDA/LGBA 47%, MYBDA 53%) and brown rotters (LGBDA/LGBA 44%, MYBDA 56%).

No matter what method or medium was used, it proved to be very difficult to get pieces of basidiocarps to produce mycelium in pure culture. After this research was completed, Gail Dailey, a graduate student working in our research lab, developed a method (unpublished) to get pure isolates of basidiomycete fungi from fungal fruiting bodies by placing a piece of the basidiocarp into a solution of 20% bleach, 20% (95%) ethanol and 60% distilled water for one minute prior to placing it onto MYBDA medium.

Identification of unknown fungal isolates solely based on cultured mycelia is time consuming and problematic using keys by Nobles (1948 and 1965) and Stalpers (1978). With the advanced molecular methods this research showed

that a definitive name could be given to unknown fungal isolates. These results further affirm that effective research in this area can now be done.

With the basidiocarp, bole chip, and soil fungal isolates, the greatest number identified by sequencing was in the basidiomycete family Tricholomataceae of the order Agaricales. The second most common family was Strophariaceae also in Agaricales. The number of families found in the basidiocarp (6), bole wood (14), and soil (11) are listed in Table 41. Families and orders were determined using Hawksworth et al (1995).

Table 41. Families and Orders of sequence identified fungal isolates.

Order	Family	Basidiocarp	Bole Chip	Soil
Agaricales	Coprinaceae		5	1
	Entolomataceae		3	
	Strophariaceae	4	1	16
	Tricholomataceae	6	11	9
Ceratobasidiales	Certobasidiaceae		2	3
Ganodermatales	Ganodermataceae			1
Hymenochaetales	Hymenochaetaceae			2
Poriales	Coriolaceae	10	6	4
Schizophyllaceae	Schizophyllaceae	2	1	
Stereales	Atheliaceae		1	
	Echinodontiaceae			1
	Hyphodermataceae		2	
	Meruliaceae	1		4
	Sistrotremataceae	1	3	
	Steccherinaceae		2	1
	Stereaceae		1	1
Thelephorales	Thelephoraceae		3	
Tremellaceae	Tremellales		1	
# of families		6	14	11

Additional testing of the *Hypholoma sublateritium* fungal isolates should include mating tests between the monokaryon and dikaryon isolates. This could test if de-dikaryotization is occurring.

In a survey of the shrub and herbaceous layers of the forest, six species were found only in Area 2 (sites # 19, 21, & 22); fourteen species were found only in Area 1 (sites # 26, 28, & 29), and sixteen species were found in both research areas. The forest canopy of the research Area 2 that included the sites # 19, 21, & 22, had nine species of trees; research Area 1 that included the sites # 26, 28, & 29, had five species of trees. The two most dominant tree species, by number of trees, for Area 2, was *Acer rubrum* and *Abies balsamea* (L.) Miller and for Area 1, *Acer rubrum* and *Fagus grandifolia* were the most dominate tree species. Looking at the total basal area of all trees, the dominate tree species for Area 2 was *Acer rubrum* and *Fagus grandifolia*, and for Area 1 *Acer rubrum* and *Betula papyrifera*.

Several fungal fruiting bodies identified at the research sites are specific to the tree species also present at these research sites. In Area 1 twenty-six species and in Area 2 twenty species are associated with hardwoods (e.g. *Polyporus, Fomes, Trametes, Phyllotopsis, Irpex, Stereum, Xerula, Hypoxylan, Trichaptum, Chlorociboria, Panellus, Crepidotus, Phellinus, Stereum*). In Area 1 ten species and Area 2 nine species are associated with conifers (e.g. *Tyromyces, Auricularia, Clavulina, Coltricia, Peziza, Xeromphalina, Lacterius, Calocera, Neolecta, Xeromphalina, Boletus, Trichaptum*). The dominant trees (BA) at both research areas are hardwoods; therefore having more fungal fruiting

bodies that are specific for hardwoods would be expected. *Amanita, Suillus, Hygrophorus,* and *Tricholoma* are found under *Pinus*. *Climacodon, Russula,* and *Oxyporus* are specific for *Acer. Ascocoryne, Inonotus,* and *Marasmius* are specific for *Fagus*. *Piptoporus and Inonotus* are specific for *Betula*. *Lycogala, Hemitrichia, Hypocrea,* and *Hygrophoropsis* are found on well rotted wood. *Entoloma, Microglossum, Trichoglossum, Hygrophorus, Ramariopsis, Coprinus, Bisporella, Lycoperdo,* and *Scutellinia* are found on decaying wood. *Hypholoma sublateritium* is found in temperate northern hemisphere on dead hardwoods such as *Acer, Betula,* and *Quercus* (Farr et al. 1995).

Aligned sequence data for all fungal isolates can be found on my website. A good Internet resource for researchers interested in the value of coarse woody debris in the environment is listed in Appendix 26.

CHAPTER VIII

SUMMARY

Depletion of nutrients in forests around the world is a growing concern. Is the harvesting of trees from the forests depleting the nutrients for the next generation of plants? Should more trees be allowed to fall to the forest floor and decay? Course woody debris (CWD) in the forest ecosystem is vital for the recycling of nutrients either through decomposition of plant material or by the translocation of nutrients via fungal hyphae. During decomposition, minerals are imported via hyphae to aid in the decay process. Do fungi tap and transport mineral resources from beneath the organic layers of the soil? If so, what fungi are involved? If this nutrient cycling process is present in the ecosystem, then the absence of CWD in our forests due to current forest management practices eliminates the substrate for decay fungi and thereby cuts off the flow of nutrients, especially minerals, reentering the forest floor for the use by the next generation of plants.

The intent of this research was to determine if the same fungi were present in decaying wood boles and in contiguous soil horizons extending down to the mineral soil on the basis of cultural and molecular identifications. Data accumulated in this study confirm that Compartment 9 of the Bartlett Experimental Forest in New Hampshire is a typical northern mixed deciduous-

conifer forest in terms of vegetation and soil type. Although many species were identified from the various substrates sampled, one species, *Hypholoma sublateritium*, was found in three soil horizons and substrates: decaying bole wood, organic soil, mineral soil (2), and basidiocarp. DNA sequencing evidence strongly suggests that the isolates from these substrates are of the same species, supporting the hypothesis that a hyphal bridge does exist between mineral soils and decaying wood on the forest floor.

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APPENDICES

Appendix 1. Diameter at breast height (cm) of the trees for sites # 26, 28, & 29 (Area 1).

Sites # 26, 28, & 29

Tree	DBH (cm)	Tree	DBH (cm)
Acer rubrum	33.8	*Betula papyrifera*	33.8
Acer rubrum	35.8	*Betula papyrifera*	25.1
Acer rubrum	30.2	*Betula papyrifera*	30.2
Acer rubrum	32.0	*Betula papyrifera*	43.7
Acer rubrum	40.0	*Betula papyrifera*	32.8
Acer rubrum	32.0	*Betula papyrifera*	45.5
Acer rubrum	22.3	*Fagus grandifolia*	8.1
Acer rubrum	22.1	*Fagus grandifolia*	16.7
Acer rubrum	23.6	*Fagus grandifolia*	36.1
Acer rubrum	24.3	*Fagus grandifolia*	12.5
Acer rubrum	19.8	*Fagus grandifolia*	16.5
Acer rubrum	56.1	*Fagus grandifolia*	12.7
Acer rubrum	14.9	*Fagus grandifolia*	14.5
Acer rubrum	10.1	*Fagus grandifolia*	15.0
Acer rubrum	12.2	*Fagus grandifolia*	15.5
Acer rubrum	11.0	*Fagus grandifolia*	12.2
Acer rubrum	16.8	*Fagus grandifolia*	15.7
Acer rubrum	27.8	*Fagus grandifolia*	23.4
Acer rubrum	13.9	*Fagus grandifolia*	24.1
Acer rubrum	42.2	*Fagus grandifolia*	13.5
Acer rubrum	31.2	*Fagus grandifolia*	18.5
Acer rubrum	19.3	*Fagus grandifolia*	13.0
Acer rubrum	45.8	*Tsuga canadensis*	41.1
Acer rubrum	61.0	*Tsuga canadensis*	16.3
Acer rubrum	24.1	*Tsuga canadensis*	68.6
Acer rubrum	25.4	*Fraxinus americana*	24.6
Acer rubrum	10.1		
Acer rubrum	18.0		
Acer rubrum	18.5		
Acer rubrum	12.7		
Acer rubrum	28.0		
Acer rubrum	33.3		
Acer rubrum	25.7		

Appendix 2. Diameter at breast height (cm) of the trees for sites # 19, 21, & 22 (Area 2).

Sites # 19, 21, & 22

Tree	DBH (cm)	Tree	DBH (cm)	Tree	DBH (cm)
Acer rubrum	17.8	*Fagus grandifolia*	22.9	*Picea rubens*	15.2
Acer rubrum	17.8	*Fagus grandifolia*	35.6	*Picea rubens*	20.3
Acer rubrum	17.8	*Fagus grandifolia*	40.6	*Picea rubens*	20.3
Acer rubrum	17.8	*Fagus grandifolia*	40.6	*Picea rubens*	25.4
Acer rubrum	17.8	*Fagus grandifolia*	15.2	*Picea rubens*	30.5
Acer rubrum	17.8	*Fagus grandifolia*	15.2	*Picea rubens*	43.2
Acer rubrum	17.8	*Fagus grandifolia*	15.2	*Picea rubens*	25.4
Acer rubrum	20.3	*Fagus grandifolia*	15.2	*Picea rubens*	50.8
Acer rubrum	20.3	*Fagus grandifolia*	15.2	*Acer saccharum*	15.2
Acer rubrum	20.3	*Fagus grandifolia*	15.2	*Acer saccharum*	15.2
Acer rubrum	22.9	*Fagus grandifolia*	20.3	*Acer saccharum*	15.2
Acer rubrum	22.9	*Fagus grandifolia*	25.4	*Acer saccharum*	15.2
Acer rubrum	22.9	*Fagus grandifolia*	27.9	*Acer saccharum*	15.2
Acer rubrum	22.9	*Abies balsamea*	14.0	*Acer saccharum*	43.2
Acer rubrum	22.9	*Abies balsamea*	15.2	*Betula papyrifera*	27.9
Acer rubrum	25.4	*Abies balsamea*	15.2	*Betula papyrifera*	27.9
Acer rubrum	25.4	*Abies balsamea*	15.2	*Betula papyrifera*	53.3
Acer rubrum	27.9	*Abies balsamea*	15.2	*Pinus strobus*	22.9
Acer rubrum	27.9	*Abies balsamea*	15.2	*Pinus strobus*	22.9
Acer rubrum	27.9	*Abies balsamea*	15.2	*Pinus strobus*	27.9
Acer rubrum	30.5	*Abies balsamea*	15.2	*Pinus strobus*	40.6
Acer rubrum	30.5	*Abies balsamea*	15.2	*Pinus strobus*	45.7
Acer rubrum	33.0	*Abies balsamea*	15.2	*Pinus strobus*	50.8
Acer rubrum	35.6	*Abies balsamea*	15.2	*Quercus rubra*	15.2
Acer rubrum	38.1	*Abies balsamea*	15.2	*Quercus rubra*	15.2
Acer rubrum	15.2	*Abies balsamea*	15.2	*Quercus rubra*	27.9
Acer rubrum	15.2	*Abies balsamea*	15.2	*Quercus rubra*	17.8
Acer rubrum	15.2	*Abies balsamea*	15.2	*Tsuga canadensis*	15.2
Acer rubrum	15.2	*Abies balsamea*	15.2	*Tsuga canadensis*	22.9
Acer rubrum	15.2	*Abies balsamea*	15.2	*Tsuga canadensis*	50.8
Acer rubrum	15.2	*Abies balsamea*	15.2		
Acer rubrum	15.2	*Abies balsamea*	15.2		
Acer rubrum	15.2	*Abies balsamea*	15.2		
Acer rubrum	15.2	*Abies balsamea*	15.2		
Acer rubrum	15.2	*Abies balsamea*	15.2		
Acer rubrum	15.2	*Abies balsamea*	15.2		
Acer rubrum	15.2	*Abies balsamea*	15.2		
Acer rubrum	15.2	*Abies balsamea*	15.2		
		Abies balsamea	15.2		
		Abies balsamea	20.3		

Appendix 3. Vascular plants found in the understory of the research sites.

Binomial	Author	Common Name	Area 1 Site #'s 26, 28, 29	Area 2 Site #'s 19, 21, 22
Fagus grandifolia	Ehrh.	American Beech		X
Trillium undulatum	Willd.	Painted Trillium		X
Aralia nudicaulis	L.	Wild Sarsaparilla		X
Pteridium aquilinum	(L.) Kuhn	Braken Fern		X
Streptopus amplexifolius	(L.) DC.	Twisted Stalk		X
Lycopodium obscurum	L.	Tree Clubmoss		X
Viburnum alnifolium	Michx.	Hobblebush	X	X
Acer pensylvanicum	L.	Striped Maple	X	X
Clintonia borealis	(Ait.) Raf.	Bluebead	X	X
Cornus canadensis	L.	Bunchberry	X	X
Chimaphila umbellata	(L.) W. Bart.	Pipsissewa	X	X
Huperzia lucidula	(Michx.) Trevisan	Shining Clubmoss	X	X
Gaultheria procumbens	L.	Checkerberry	X	X
Aster acuminatus	Michx.	Whorled Aster	X	X
Trientalis borealis	Raf.	Star Flower	X	X
Maianthemum canadense	Desf.	Canada Mayflower	X	X
Tusga canadensis	(L.) Carriere	Eastern Hemlock	X	X
Lonicera canadensis	Marshall.	Fly Honeysuckle	X	X
Abies balsamea	(L.) Miller	Balsam Fir	X	X
Betula papyrifera	Marshall	Paper Birch, White Birch	X	X
Mitchella repens	L.	Partridge-berry	X	X
Medeola virginiana	L.	Indian Cucumber Root	X	X
Epifagus virginiana	(L.) W. Bart.	Beechdrops	X	
Coptis trifolia	(L.) Salisb.	Goldthread	X	
Tiarella cordifolia	L.	Foamflower	X	
Oxalis acetosella	Raf.	Common Wood Sorrel	X	
Goodyera tesselata	Lodd.	Checkered Rattlesnake Plantain	X	
Monotropa uniflora	L.	Indian Pipe	X	
Dryopteris carthusiana	(Vill.) H.P. Fuchs	Spinulose Woodfern	X	
Cypripedium acaule	Aiton	Pink Lady Slipper	X	
Uvularia sessilifolia	L.	Bellwort	X	
Trillium erectum	L.	Purple Trillium	X	
Smilacina racemosa	(L.) Desf.	False Solomon's Seal	X	
Aralia nudicaulis	L.	Wild Sarsaparilla	X	
Pyrola elliptica	Nutt.	Shinleaf	X	

Appendix 4. Temperatures (°C) of soil horizons. (* soil horizon not cultured)

Site #	Collection Dates	Temp. degrees C	
Horizon	June 1997	June 1999	Oct. 1999
Site # 19			
Oe	15.0	13.4	6.6
E	*13.4	11.9	
Oeb			
Bhsb	12.6		8.0
BCb	11.6	9.6	8.5
Site # 21			
Oe	14.3	14.8	6.3
E	*13.4	13.7	
Bhs	13.0	12.1	7.8
BC	11.9		9.2
Site # 22			
Oe	12.2	13.1	6.6
Bhs	12.0	11.5	7.1
BC	10.9	9.1	8.8
Site # 26			
Oe	16.3	14.9	6.2
E	*13.9	13.4	
Bw	13.1	11.7	7.0
BC	12.7		8.5
Site # 28			
Oe	14.3	14.3	6.2
E	13.9	13.7	6.3
Bs	*12.9		
Bhs	11.8	10.5	
BC	11.0		8.4
Site # 29			
Oe	14.2	13.0	6.1
E1	*14.2		
E2	*13.2		6.6
Bw	12.3	11.6	7.9
BC	11	9.8	

Appendix 5. Manufacturers of supplies and equipment used in this research.

DNA extraction
DNeasy® Plant Mini Kit, Cat. # 69106, Qiagen
Chelex-100 (5% soln.) Cat. # C-7901, Sigma
Molecular water, Cat. # W4502, Sigma
Micropestle, disposable pellet pestles, Cat. # KT49521-1590, VWR
Microcentrifuge tubes (1.7 mL), Cat. # 20170-650, VWR
Pipettors & tips:
- 0.1-2 µL, (P2), Gilson Pipetman
- 2-20 µL, (P20), Gilson Pipetman
- 10-100 µL, (P100), Gilson Pipetman
- 20-200 µL, Cat. # 40000-204, VWR
- 100-1000 µL, Cat. # 40000-208, VWR
- tips to fit 20-1000 µL, Cat. # 53508-819
- tips to fit 1-200 µL, Cat. # 53509-815

Ready-To-Go ™ PCR Beads, product # 27-9555-01, Amersham Pharmacia
Tube racks, VWR
- 80 place storage system, Cat. # 30128-282
- 96 place storage system, Cat. # 30128-346
- 96 place storage system with covers, Cat. # 30128-350

Gel Electrophoresis
Agarose, Cat. # 2120, EM Science Omni Pur
dNTP mix, Cat. # D7295, Sigma-Aldrich
eliminator® Dye Removal System, Cat. # 400480, Stratagene
Ethidium bromide (100µL/L of water), Cat. # EM-4410, VWR
Gel horizontal electrophoresis apparatus, Model B1, Owl Easycast™, Cat. # 27372-200, VWR
Gel-loading buffer Type II (0.25% bromophenol blue, 0.25% xylene cyanol FF, 15% Ficoll in water)
Gel reader, NucleoTech's GelExpert 3.5
Low DNA Mass Ladder, Cat. # 10068-013, Invitrogen
TAE buffer (for PCR), Cat. # 15558-042, Invitrogen
TBE buffer (for Microsatellite), Cat. # 15581-044, Invitrogen
1KB+ ladder, Cat. # 12308-011, Life Technologies

Media
Acetone, Cat. # AX0126-1, EM Science
Agar Bacto™, Cat. # 214010, Becton Dickinson & Co.
Ammonium nitrate, NH_4NO_3, EM Science
Benomyl (50% W.P.), Agway
Methyl 1-(butylcarbamoyl) - 2 - benzimiduzolecarbamate
Calciumnitrat-4-hydrat, $Ca(NO_3)^2 \cdot 4H_2O$, ER Reagents
Chlortetracycline-HCl, 80% Cat. 271802-256 Aldrich
Dichloran, $Cl_2C_6H_2(NO^2)NH^2$ (97%), Cat. # D6,782-0, Aldrich

2, 6 – Dichloro – 4 – nitroaniline,
p-Dioxane, Cat. # 9231-04, JT Baker
$FeSO_4 \cdot 7H_2O$
Gallic acid, Cat. # M685-07, JT Baker
Guaiacol, Cat. # G-5502, Sigma
Indulin AT (Alkali lignin), Cat. # I-6384, Sigma
Magnesium sulfate heptahydrate crystals, $MgSO_4 \cdot 7H_2O$, EM Science
Malt extract, Cat. # 218630, Becton Dickinson & Co.
Penicillin G (K-salt), Sigma
Potassium chloride, KCL, Cat. # P217-500, Fisher
Potassium phosphate monobasic crystal, KH_2PO_4, Cat. # PX1564-1, EM Science
Sodium pyrophosphate crystal, $Na_4P_2O_7 \cdot 10H_2O$, Cat. # S390-500, Fisher
Streptomycin sulfate, Kodak Eastman
Tannic acid, Cat. # T-0125, Sigma
Yeast extract, Cat. # 0886-17-0, Difco

Misc. Supplies and Equipment
Dry sterilizer, Inotech Biosystems International
French square jars (16 oz), Cat. # 115-50351, Cynmar Corp. or Cat. # 34601-60, Cole Parmer, or Cat. # OBA 12896 F40, Yankee Containers
Microbiology flow hood, (Microvoid), Air Control Inc.
Micro-centrifuge, Fisher Scientific
Vortex-2 Genie, VWR
Bel-Art Products Mini-sieve micro sieve set # F37845-0000, VWR

PCR
Oligodeoxyribonucleotides primers, Integrated DNA Technologies, Inc.
Platinum® *Taq* DNA polymerase, Cat. # 10966-034, Invitrogen
Included with the polymerase are the Buffer minus Mg and MgCL:
Buffer minus Mg (10X PCR buffer, 200 mM Tris-HCL (pH 8.4 & 500 mM KCL)
MgCl (50 mM $MgCl_2$)
QIAquick™ Spin PCR Purification Kit, Cat. # 28106, Qiagen
PCR machine, Eppendorf Mastercycler gradient
PCR strip tubes & cap (0.2 mL), Cat. # 20170-004, VWR
Racks for strip tubes, Cat. # 0554155, Fisher

Software
DNA-Star software (EdSeq & SeqMan), version 5
CHROMAS software version 2.23, Technelysium Pty Ltd.
NucleoTech's GelExpert 3.5
PAUP version 4.061a

Stains
Lactophenol Cotton Blue, Cat. # VW3427-0, VWR
Phloxine B, $C_{20}H_2Br_4Cl_4Na_2O_5$, Cat. # U029-03, JT Baker

Appendix 6. LGBDA medium

(lignin guaiacol with benomyl & dichloran & 3 antibiotics)

Thompson TA. 1998. *Development of techniques for the selective isolation of basidiomycetes from coarse woody debris and contiguous soil horizons.* M.S. Thesis, University of New Hampshire.

distilled water	964 mL
KH_2PO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	0.2 g
NH_4NO_3	0.1 g
KCl	0.1 g
$FeSO_4 \cdot 7H_2O$	0.02 g
$Ca(NO_3)_2 \cdot 4H_2O$	0.05 g
malt extract	2.0 g
agar	15 g

After autoclaving and cooled to 55°C, add the following, with sterile equipment:

1 M KOH	5 mL (5.6 g in 100 mL sterile water)
guaiacol	0.4 mL
indulin AT	(0.5 g suspended and partially dissolved in 5 mL dioxane)
* chlortetracycline-HCl	10 mL
* streptomycin sulfate	10 mL
* penicillin G	10 mL
* Benlate solution	2 mL
* dichloran solution	2 mL

* from Stock solutions:

chlortetracycline-HCl	0.6 g in 100 mL sterile water
streptomycin sulfate	0.3 g in 100 mL sterile water
penicillin G	0.3 g in 100 mL sterile water
benomyl	0.2 g in 50 mL acetone & 50 mL 70 % ETOH
dichloran	0.1 g in 100 mL 50 % ETOH

Final concentrations:

chlortetracycline-HCl	60 mg/L
streptomycin sulfate	30 mg/L
penicillin G	30 mg/L
benomyl	2 mg/L
dichloran	2 mg/L

Appendix 7. LGBA medium

(lignin guaiacol with benomyl & 3 antibiotics)

Thorn RG, Reddy CA, Harris D, Paul EA. 1996. Isolation of saprophytic basidiomycetes from soil. *Appl Environ Microbiol* 62(11):4288-4292.

distilled water	964 mL
KH_2PO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	0.2 g
NH_4NO_3	0.1 g
KCl	0.1 g
$FeSO_4 \cdot 7H_2O$	0.02 g
$Ca(NO_3)_2 \cdot 4H_2O$	0.05 g
malt extract	2.0 g
agar	15 g

After autoclaving and cooled to 55°C, add the following, with sterile equipment:

1 M KOH	5 mL (5.6 g in 100 mL sterile water)
guaiacol	0.4 mL
indulin AT	(0.5 g suspended and partially dissolved in 5 mL dioxane)
chlortetracycline-HCl	60 mg
streptomycin sulfate	30 mg
penicillin G	30 mg
* Benlate solution	2 mL

* from Stock solution:

benomyl 0.2 g benomyl in 50 mL acetone & 50 mL 70 % ETOH

Final concentrations:

chlortetracycline-HCl	60 mg/L
streptomycin sulfate	30 mg/L
penicillin G	30 mg/L
benomyl	2 mg/L

Appendix 8. MYBDA medium

(malt & yeast extract with benomyl & dichloran & 3 antibiotics)

Thompson TA. 1998. *Development of techniques for the selective isolation of basidiomycetes from coarse woody debris and contiguous soil horizons.* M.S. Thesis, University of New Hampshire.

malt extract	10 g
yeast extract	2 g
agar	20 g
distilled water	966 mL

After autoclaving and cooled to 55 °C, add the following, with sterile equipment:

* chlortetracycline-HCl	10 mL
* streptomycin sulfate	10 mL
* penicillin G	10 mL
* Benlate solution	2 mL
* dichloran solution	2 mL

* from Stock solutions:

chlortetracycline-HCl	0.6 g in 100 mL sterile water
streptomycin sulfate	0.3 g in 100 mL sterile water
penicillin G	0.3 g in 100 mL sterile water
benomyl	0.2 g in 50 mL acetone & 50 mL 70 % ETOH
dichloran	0.4 g in 100 mL 50 % ETOH

Final concentrations:

chlortetracycline-HCl	60 mg/L
streptomycin sulfate	30 mg/L
penicillin G	30 mg/L
benomyl	2 mg/L
dichloran	8 mg/L

Appendix 9. Gallic and Tannic acid media

Davidson RW, Campbell WA, Blaisdell DJ. 1938. Differentiation of wood-decaying fungi by their reactions on gallic and tannic acid medium. *J Ag Res* 57 (9):683-695.

malt extract	15 g
agar	20 g
distilled water	700 mL
gallic or tannic acid	5 g

Dissolve malt extract and agar in 700 mL of distilled water. Put 300 mL additional distilled water into a separate flask. Autoclave both flasks. Dissolve gallic or tannic acid in sterilized water. When medium is cooled down, add the dissolved gallic or tannic acid solution with the medium. Mix by swirling and pour plates.

Heating gallic or tannic acid with agar causes hydrolysis of the agar. Tannic acid medium will have a milk-white appearance.

Appendix 10. Stains for microscope slides.

Lactophenol Cotton Blue
VWR Cat. # VW3427-0
Ready to use solution

Phloxine-B
JT Baker Cat. # U029-03
$C_{20}H_2Br_4Cl_4Na_2O_5$

Phloxine-B	1 g
Distilled water	100 mL

Appendix 11. DNA extraction protocol.

DNeasy® Plant Mini Kit
Preheat buffer AE to 65°C in the waterbath
Label all tubes (Lilac & white columns, 3 sets of microcentrifuge tubes, one set w/o caps)

Cell Lyses
Into a microcentrifuge tube 400 μL Buffer AP1 & 4 μL RNase A stock soln.
Place 40 μL ground hyphae into the tube vortex vigorously
Incubate for 10 min. at 65°C, mix 2–3 times during incubation by inversion

Add 130 μL Buffer AP2, mix, incubate on ice for 5 min.
Apply ~ 574 μL of the lysate to the QIAshredder spin column (lilac) sitting in a 2 mL collection tube & centrifuge for 2 min.
Transfer flow-through fraction to a new microcentrifuge tube without disturbing the cell-debris pellet

Add 675 μL Buffer AP3/E to the lysate (~450 μL), immediately mix by pipetting
Apply 650 μL of this mixture (include precipitate) to the DNeasy mini spin column (white) sitting in a 2 mL collection tube & centrifuge for 1 min.
Discard flow through
Repeat the above step with remaining sample in same collection tube
Discard flow through & collection tube

Place DNeasy column in a new 2 mL collection tube, add 500 μL Buffer AW to the column & centrifuge for 1 min.
Discard flow through
Add 500 μL Buffer AW to the column & centrifuge for 2 min to dry the membrane

Elution
Transfer the column to a microcentrifuge tube (not supplied, cut off and save caps) & pipet 100 μL preheated Buffer AE onto the membrane
Incubate for 5 min at RT
Centrifuge for 1 min.
Repeat the above elution steps

Appendix 12. PCR protocol.

Total 50 µL
Make a master mix

	1 Rxn
Water	34.8 µL
Buffer-Mg	5 µL
dNTP's	1 µL
MgCl	2 µL
Primer ITS 1	1 µL
Primer ITS 4	1 µL
Taq	0.2 µL

Use 45 µL

DNA Template 5 µL

PCR profile:
Heat lid to 100°C
2 min. at 94°C

Denaturation	94°C	30 sec.
Annealing	55°C	30 sec.
Elongation	72°C	1 min.

35 cycles
Final Extension 72°C 7 min.
Hold at 4°C when done

Appendix 13. Electrophoresis protocol.

Use the 14 well, 1.5 mm, comb
Run one 1KB plus ladder and two Low DNA Mass ladders per gel

Make 1X TAE buffer
100 mL 10X TAE stock buffer into liter bottle
900 mL distilled water, mix

Make 1% Agarose gel
40 mL 1X TAE buffer in flask
0.4 g Agarose

Microwave to dissolve
Let flask cool to warm touch before pouring into gel apparatus
Place comb into gel
Let gel harden, approx. 20 min.
Turn gel in apparatus
460 mL 1X TAE buffer
Slightly wiggle and remove comb

Loading Gel
For each DNA/PCR product and Low Mass DNA ladder
1 μL loading dye buffer
4 μL template

For 1KB plus DNA ladder (loading dye included in ladder)
5 μL ladder

Set power source at 90 volts
Run until first blue dye reaches 4 on ruler on apparatus or approx. 1 hour

Wearing Nitrile gloves, place gel in Eth. Bromide Soln.
1 L distilled water pipette
100 μL Ethidium Bromide stock

Stain 15 min. in Eth. Bromide Soln.
Destain for 20 min. in distilled water
Photograph gel & save on Zip disc

Appendix 14. DNA purification protocol.

Qiagen QIAquick™ Spin

230 μL Buffer PB 5 vol. for a 50 μL rxn.
mix with PCR product, transfer to spin column
centrifuge 1 min.
discard flow-through

750 μL Buffer PE to column
centrifuge 1 min.
discard flow-through
centrifuge again 1 min.

place column into a clean 1.5 mL microcentrifuge tube
let air dry for 1 min.
50 μL Buffer EB to the center of the membrane
let stand for 1 min.
centrifuge 1 min.

Appendix 15. Prepare template samples for sequencing protocol.

Request Premix and NaOAc from UNH sequencing lab
PCR
Put rack on Ice
Make Master Mix
4 μL mixed Premix
1 μL Primer
Mix Master Mix
Into 0.2 mL PCR tubes (add in this order)
_____ μL water
__5__ μL Master Mix
_____ μL purified PCR product (100 ng)
Total 20
Mix well, cap

PCR for Cycle Sequencing
Lid 100°C
96°C for 20 sec
50°C for 15 sec
60°C for 1 min
25 cycles
4° C hold

Run ethanol precipitation on samples
1.5 mL tubes (add in this order)
2 μL NaOAc
20 μL PCR product
80 μL 95% EtOH
Vortex briefly
Place tubes into Ice w/o rack for 15 min
centrifuge 15 min

Decant off onto Kimwipe
600 μL 70% EtOH
centrifuge briefly

Decant off onto Kimwipe
600 μL 70% EtOH
centrifuge briefly
Decant off onto Kimwipe
centrifuge briefly
Speed Vac 10 min., freeze samples, send to sequencing lab

Appendix 16. June 1999 soil processing method.

Using sterile spatulas,
Add 3.5 g of one soil sample into a flask containing 250 mL sterile 0.1% Sodium pyrophosphate
Place flask onto an orbital shaker at 250 rpm for 1 hour at room temperature
Pour the soil solution into a stack of two sieves (250 µm and 45 µm)
Rinse through with a brief shower of water
Wash the particles remaining on the 45 µm mesh sieve for 5 min. with the eyewash at the sink at a flow rate of approximately 20 liters/min
Collect the remaining solids at one edge of a tilted sieve to separate the suspended organic particles from the settled mineral particles
Remove 1 mL of a dense suspension of the organic particles using a sterile Pasteur pipette and dilute in 100 mL sterile water, mix by swirling flask
Using another sterile Pasteur pipette place 0.5 mL of diluted sample onto the center of the culture medium
The culture plates are placed onto a turntable, using a sterile glass hockey stick, spread the soil solution inoculum over the entire medium
In addition, an additional plate of each culture medium are inoculated with 1 ml of diluted soil solution
The sieves are rinsed with water and surface sterilized with 70% ethanol between soil samples
Control plates of LGBDA and MYBDA are inoculated with rinse solution between samples to confirm cleanliness of sieves

Appendix 17. Dilution tests with the August 1999 organic and mineral soils.

Using sterile spatulas,
Add 2.5 g of organic soil into a flask containing 250 mL sterile 0.1% Sodium pyrophosphate
Place the flask onto an orbital shaker at 250 rpm for 1 hour at room temperature
Pour the soil solution into a stack of two sieves (250 µm and 45 µm)
Rinse through with a brief shower of water
Wash the particles remaining on the 45 µm mesh sieve for 5 min. with the eyewash at the sink at a flow rate of approximately 20 L/min
'Liquid' (suspended organic particles) and 'grains' (settled mineral particles) of washed soil from tilted sieve, are used as an inoculum, using a turntable and sterile hockey stick, as follows:
One mL of 'liquid' from the 45 µm sieve is placed into a flask containing 199 mL sterile distilled water. Half mL and 1 mL of this dilution is streaked onto the culture medium
One mL of 'grains' from the 45 µm sieve is placed into a flask containing 199 mL sterile distilled water. Half ml and 1 mL of this dilution is streaked onto the culture medium

Add 3.5 and 5 g of mineral soil into two separate flasks containing 250 mL 0.1% sterile Sodium pyrophosphate
Place the flasks onto an orbital shaker at 250 rpm for 1 hour at room temperature
Process each soil sample as follows:
Pour one soil solution into a stack of two sieves (250 µm and 45 µm)
Rinse through with a brief shower of water
Wash the particles remaining on the 45 µm mesh sieve for 5 min. with the eyewash at the sink at a flow rate of approximately 20 L/min
'Liquid' and 'grains' of this washed soil are used as an inoculum, using a turntable and a sterile hockey stick, as follows:
Undiluted 0.5 mL of washed 'liquid'
Undiluted 0.5 mL of washed 'grain'
Two mL of 'liquid' from the 45 µm sieve is placed into a flask containing 98 mL sterile distilled water. Half mL and 1 mL of this dilution is streaked onto the culture medium
Four mL of 'liquid' from the 45 µm sieve is placed into a flask containing 96 ml sterile distilled water. Half ml and 1 ml of this dilution is streaked onto the culture medium

Two mL of 'grains' from the 45 µm sieve is placed into a flask containing 98 mL sterile distilled water. Half mL and 1 mL of this dilution is streaked onto the culture medium

Four mL of 'grains' from the 45 µm sieve is placed into a flask containing 96 mL sterile distilled water. Half mL and 1 mL of this dilution is streaked onto the culture medium

Appendix 18. October 1999 soil processing method.

Using sterile spatulas,
Add 2.5 g of an organic soil to a flask containing 250 mL sterile 0.1% sodium pyrophosphate
Place the flask onto an orbital shaker at 250 rpm for 1 hour at room temperature
Pour the soil solution into a stack of two sieves (250 µm and 45 µm)
Rinse through with a brief shower of water
Wash the particles remaining on the 45 µm mesh sieve for 5 min. with the eyewash at the sink at a flow rate of approximately 20 L/min
'Liquid' (suspended organic particles) and 'grains' (settled mineral particles) of washed soil from tilted sieve, are used as an inoculum, using a turntable and sterile hockey stick, as follows:
Two mL of 'liquid' from the 45 µm sieve is diluted with 198 mL of water, 1 mL of this dilution is streaked onto the culture medium
Also, 0.5 mL 'grains' from the 45 µm sieve is diluted with 199.5 mL of water, 0.5 mL of this dilution is streaked onto the culture medium

Add 5 g of a mineral soil to 250 mL sterile sodium pyrophosphate, and
Place the flask onto an orbital shake at 250 rpm for 1 hour at room temperature.
Pour the soil solution into a stack of two sieves (250 µm and 45 µm)
Rinsed through with a brief shower of water
Wash the particles remaining on the 45 µm mesh sieve for 5 min. with the eyewash at the sink at a flow rate of approximately 20 L/min
'Liquid' and 'grains' of this washed soil are used as an inoculum as follows:
Four mL of 'liquid' from the 45 µm sieve is diluted with 196 mL of water, 1 mL of this dilution is streaked onto the culture medium
Also, 2 mL 'grains' from the 45 µm sieve is diluted with 98 mL of water, 0.5 mL of this dilution is streaked onto the culture medium

Appendix 19. June 1997 soil chemical analysis.

Cultured Soil Horizons Chemical Analysis (ppm)

	Horizon	pH	Mg	Ca	K	P	% OM
Site # 19	Oe	3.6	56	392	88	7	53.19
	Bhsb	5	14	68	16	4	10.46
	BCb	5.1	12	66	8	1	3.29
Site # 21	Oe	3.8	28	300	66	10	25.72
	Bhs	4.6	14	72	14	6	10.03
	BC	4.9	12	64	10	2	2.62
Site # 22	Oe	3.7	52	290	90	4	33.23
	Bhs	4.8	14	70	16	6	6.86
	BC	5.1	12	62	14	8	3.46
Site # 26	Oe	3.8	34	136	82	4	44.46
	Bw	4.7	14	82	24	4	11.39
	BC	5	12	70	8	1	3.18
Site # 28	Oe	3.7	50	456	64	4	73.7
	E	4	14	90	16	4	1.44
	Bhs	4.9	12	78	14	4	12.86
Site # 29	Oe	3.9	32	254	74	4	36.23
	Bw	5.1	10	72	10	4	9.28
	BC	5.2	10	76	8	3	1.69

Appendix 20. Published decay classes used by other researchers (listed by year published).

Author	Research location	Tree species studied
Lang & Forman 1978	NJ	*Quercus* (*alba, borealis,* & *velutina*)
Triska & Cromack 1979	OR	*Pseudotsuga menziesii*
Lambert, et al. 1980	NH	*Abies balsamea*
Sollins 1982	OR	*Pseudotsuga menziesii*
Graham & Cromack 1982	WA	*Tsuga heterophylla* & *Picea sitchensis*
Fahey 1983	WY	*Pinus contorta* ssp. *latifolia*
Means, et al. 1985 & 1992	OR	*Pseudotsuga menziesii*
Harmon, et al. 1986	OR	*Abies balsamea*
Sollins, et al. 1987	OR	*Pseudotsuga menziesii*, *Tsuga heterophylla*, *Thuja plicata*
Spies, et al. 1988	OR & WA	*Pseudotsuga menziesii*, *Abies amabilis*, *Thuja plicata*
Crawford, et al. 1990	OR	*Pseudotsuga menziesii*
Arthur & Fahey 1990	CO	*Picea engelmannii, Abies lasiocarpa*
Keenan, et al. 1993	Vancouver Island	*Thuja plicata*, *Tsuga heterophylla*
Busse 1994	OR	*Pinus contorta* ssp. *latifolia*
Niemela, et al. 1995	Finland	*Picea abies, Pinus sylvestris*
Renvall 1995	Finland	conifers
Marra & Edmonds 1996	OR	*Tsuga heterophylla, Pseudotsuga menziesii*
Krankina et al. 1999	Russia	*Pinus sylvestris, Picea abies, Betula pendula*
Kruys & Jonsson 1999	Sweden	*Picea abies*

Appendix 21. Chemical analysis of soil pit wood bait, prior to insertion in soil horizons, and bole wood.

	ppm				
June 1997	**Mg**	**Ca**	**K**	**P**	**% OM**
Soil Pit Wood Bait	0.01	0.10	0.02	0.10	99.29
Bole # 19	0.01	0.20	0.02	0.20	99.32
Bole # 21	0.01	0.10	0.02	0.10	99.48
Bole # 22	0.01	0.30	0.04	0.10	99.22
Bole # 26	0.01	0.20	0.02	0.10	99.67
Bole # 28	0.01	0.10	0.02	0.10	99.40
Bole # 29	0.01	0.20	0.02	0.10	99.26
June 1999	**Mg**	**Ca**	**K**	**P**	**% OM**
Bole # 19	0.02	0.20	0.10	0.01	98.67
Bole # 21	0.02	0.20	0.10	0.04	99.29
Bole # 22	0.02	0.10	0.10	0.01	99.02
Bole # 26	0.02	0.10	0.10	0.01	99.46
Bole # 28	0.02	0.10	0.10	0.01	99.42
Bole # 29	0.02	0.10	0.10	0.01	99.51

Appendix 22A. Published keys to identify basidiomycetes in culture or as fungal fruiting bodies.

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Gilbertson RL. Phylogenetic relationships of Hymenomycetes with resupinate, hydnaceous basidiocarps. pp. 275-307.
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Appendix 22E.

Warcup JH, Talbot PHB. 1962. Ecology and identity of mycelia isolated from soil. *Trans Brit Mycol Soc* 45(4):495-518.

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Appendix 23. MEA + antibiotics

Nobles MK. 1948. Identification of cultures of wood-rotting fungi. *Can J Res* 26:281, 290-312, 414-431.

malt extract	12.6 g
agar	20 g
distilled water	970 mL

After autoclaving and cooled to 55°C, add the following, with sterile equipment:

* chlortetracycline-HCl	10 mL
* streptomycin sulfate	10 mL
* penicillin G	10 mL

* from Stock solutions:

chlortetracycline-HCl	0.6 g in 100 mL sterile water
streptomycin sulfate	0.3 g in 100 mL sterile water
penicillin G	0.3 g in 100 mL sterile water

Final concentrations:

chlortetracycline-HCl	60 mg/L
streptomycin sulfate	30 mg/L
penicillin G	30 mg/L

WA medium

agar	20 g
distilled water	1000 mL

Appendix 24A. Fungal fruiting bodies found in the research areas or sites.

	Family	Area or Site [z]
Basidiomycetes		
Amanita citrina (Schaeff.) Gray	Amanitaceae	2
Amanita flavoconia Atk.	Amanitaceae	1
Amanita porphyria (A.& Schw.: Fr.) Secr.	Amanitaceae	22
Amanita species Pers.	Amanitaceae	1, 2, 26
Auricularia auricula (Hooker) Underwood	Auriculariaceae	29
Boletus spadiceus Fr.	Boletaceae	2
Boletus species Fr.	Boletaceae	19, 21, 26
Suillus americanus (Pk.) Snell	Boletaceae	2
Cantharellus infundibuliformis Fr.	Cantharellaceae	1
Cantharellus xanthops (Pers.) Duby	Cantharellaceae	1
Clavulinopsis fusiformis (Sow.:Fr.) Corner	Clavariaceae	1, 2, 19, 21, 22, 26, 28, 29
Ramariopsis crocea	Clavariaceae	29
Ramariopsis laeticolor	Clavariaceae	28
Clavulina cinerea (Fr.) Schroet.	Clavulinaceae	1, 21, 29
Coniophora species DC.	Coniophoraceae	22
Coprinus micaceus (Bull.:Fr.) Fr.	Coprinaceae	2
Coprinus species Pers.	Coprinaceae	29
Fomes fomentarius (L.:Fr.) Kickx	Coriolaceae	1, 2, 19, 22, 28
Fomitopsis pinicola (Sw.:Fr.) Karst.	Coriolaceae	1
Hapalopilus nidulans (Fr.) Karst.	Coriolaceae	2
Oxyporus populinus (Schum.:Fr.) Donk	Coriolaceae	2
Perenniporia species Murr.	Coriolaceae	22
Piptoporus betulinus (F.) Karst.	Coriolaceae	1
Poria species Pers.	Coriolaceae	22
Trametes pubescens (Schum.:Fr.) Pil.	Coriolaceae	1, 2, 19, 26
Trametes versicolor (Fr.) Pil.	Coriolaceae	1, 2, 19, 21, 22, 26, 28, 29
Trichaptum abietinum (Fr.) Ryv.	Coriolaceae	2, 19, 21
Trichaptum biforme (Fr.) Ryv.	Coriolaceae	1, 26, 28, 29
Tyromyces chioneus	Coriolaceae	26
Cortinarius armillatus (Fr.) Kumm.	Cortinariaceae	2, 19, 22, 26, 28, 29
Phaeocollybia species Heim	Cortinariaceae	29
Crepidotus mollis (Fr.) Staude	Crepidotaceae	21
Calocera viscosa (Pers.:Fr.) Fr.	Dacrymycetaceae	28
Entoloma abortivum (Berk.:Curt.) Donk	Entolomataceae	28
Nolanea murraii (B & C.) Sacc.	Entolomataceae	1
Nolanea quadrata Berk. & Curt.	Entolomataceae	2
Nolanea species (Fr.) Kumm.	Entolomataceae	2

Appendix 24B.

	Family	Area or Site [z]
Basidiomycetes		
Ganoderma applanatum (Pers.) Pat.	Ganodermataceae	2, 26
Climacodon septentrionale (Fr.) Karst.	Hydnaceae	26
Hygrophoropsis aurantiaca (Wulf.:Fr.) Mre.	Hygrophoropsidaceae	26
Hygrophorus cantharellus (Schw.) Fr.	Hygrophoraceae	22
Hygrophorus miniatus (Fr.) Fr.	Hygrophoraceae	2
Hygrophorus species Fr.	Hygrophoraceae	1, 2, 28, 29
Coltricia perennis (Fr.) Murr.	Hymenochaetaceae	29
Coltricia species Gray	Hymenochaetaceae	1
Hymenochaete tabacina (Sow.) Lev.	Hymenochaetaceae	2
Inonotus obliquus (Pers.:Fr.) Pil.	Hymenochaetaceae	22
Phellinus chrysoloma	Hymenochaetaceae	19
Phyllotopsis nidulans (Fr.) Sing.	Lentinaceae	1, 26, 28
Lycoperdon perlatum Pers.	Lycoperdaceae	26, 29
Polyporus alveolaris	Polyporaceae	28
Polyporus varius Pers.:Fr.	Polyporaceae	1
Lacterius species Pers.	Russulaceae	1, 2, 26
Russula pulchra Burlingham	Russulaceae	2
Russula tenuiceps Kauffman	Russulaceae	1
Russula species Pers.	Russulaceae	1, 21, 22
Irpex lacteus (Fr.:Fr.) Fr.	Steccherinaceae	1, 2, 19
Stereum gausupatum (Fr.) Fr.	Stereaceae	19
Stereum ostrea (B & N) Fr.	Stereaceae	1, 19
Stereum species Pers.	Stereaceae	2, 26
Hypholoma species (Fr.) Kumm.	Strophariaceae	26, 29
Hypholoma sublateritium (Fr.) Quelet	Strophariaceae	28
Pholiota species (Fr.) Kumm.	Strophariaceae	1
Pholiota squarrosa (Pers.:Fr.) Kumm.	Strophariaceae	29
Clitocybe species (Fr.) Staude	Tricholomataceae	2, 22
Collybia confluens (Pers.:Fr.) Kumm.	Tricholomataceae	29
Collybia dichrous (Berk. & Curt.) Gilliam	Tricholomataceae	2
Collybia species (Fr.) Staude	Tricholomataceae	26
Marasmius rotula (Scop.:Fr.) Fr.	Tricholomataceae	2
Mycena leaiana (Berk.) Sacc.	Tricholomataceae	29
Mycena species (Pers.) Roussel	Tricholomataceae	2
Panellus serotinus (Fr.) Kuhn.	Tricholomataceae	19, 21, 22, 26, 28, 29
Panellus stypticus (Bull.:Fr.) Karst.	Tricholomataceae	26, 28, 29
Tricholoma saponaceum (Fr.) Kumm.	Tricholomataceae	22

Appendix 24C.

	Family	Area or Site [z]
Basidiomycetes		
Tricholoma species (Fr.) Staude	Tricholomataceae	1, 29
Xeromphalina campanella (Batsch:Fr.) Mre.	Tricholomataceae	19, 29
Xerula furfuracea (Pk.) Redhead	Tricholomataceae	1, 29
Myxomycetes		
Lycogola epidendrum	Lycogalaceae	19, 28, 29
Stemonitis		22
Hemitrichia clavata	Trichiaceae	28
Ascomycetes		
Microglossum rufum (Schw.) Underwood	Geoglossaceae	1
Trichoglossum hirsutum (Pers.:Fr.) Boud.	Geoglossaceae	1, 2, 26, 29
Hypocrea gelatinosa (Tode:Fr.) Fr.	Hypocreaceae	2, 28
Ascocoryne cylichnium (Tul.) Korf	Leotiaceae	19, 21, 22, 26, 29
Bisporella citrina (Batsch:Fr.) Korf. Carpenter	Leotiaceae	19, 21, 22, 26, 28, 29
Chlorociboria aeruginascens (Nyl.) Kanouse	Leotiaceae	2, 19, 26, 28, 29
Neolecta irregularis (Pk.) Korf & Rogers	Neolectaceae	21
Scutellinia scutellata (L.:Fr.) Lamb.	Otideaceae	26
Peziza badia Pers.:Merat	Pezizaceae	29
Hypoxylan species Bull.	Xylariaceae	1

Z: Research Area 1, 2, Site # 19, 21, 22, 26, 28, 29

Appendix 25A. Index of journal articles using somatic incompatibility testing.

Adaskaveg JE, Gilbertson RL. 1987. Vegetative incompatibility between intraspecific dikaryotic pairings of *Ganoderma lucidum* and *G. tsugae*. *Mycologia* 79(4):603-613.

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Banik MT, Burdsall Jr. HH. 2000. Incompatibility groups among North American populations of *Laetiporus sulphureus* sensu lato. *Mycologia* 92(4):649-655.

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Burrill EA, Worrall JJ, Wargo PM, Stehman SV. 1999. Effects of defoliation and cutting in eastern oak forests on *Armillaria* spp. and a competitor *Megacollybia platyphylla*. *Can J For Res* 29:347-355.

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Fries N. 1987. Somatic incompatibility and field distribution of the ectomycorrhizal fungus *Suillus luteus* (Boletaceae). *New Phytol* 107:735-739.

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Appendix 25B.

Hansen EM, Stenlid J, Johansson M. 1993a. Somatic incompatibility and nuclear reassortment in *Heterobasidion annosum*. *Mycol Res* 97(10):1223-1228.

Hansen EM, Stenlid J, Johansson M. 1993b. Genetic control of somatic incompatibility in the root-rotting basidiomycete *Heterobasidion annosum*. *Mycol Res* 97(10):1229-1233.

Hogberg N, Holdenrieder O, Stenlid J. 1999. Population structure of the wood decay fungus *Fomitopsis pinicola*. *Heredity* 83:354-360.

Holmer L, Nitare L, Stenlid J. 1994. Population structure and decay pattern of *Phellinus tremulae* in *Populus tremula* as determined by somatic incompatibility. *Can J Bot* 72:1391-1396.

Jacobson KM, Miller OK Jr, Turner BJ. 1993. Randomly amplified polymorphic DNA markers are superior to somatic incompatibility tests for discriminating genotypes in natural populations of the ectomycorrhizal fungus *Suillus granulatus*. *Proc Natl Acad Sci USA* 90:9159-9163.

Kauserud H, Schumacher T. 2002. Population structure of the endangered wood decay fungus *Phellinus nigrolimitatus* (Basidiomycota). *Can J Bot* 80:597-606.

Kauserud H, Schumacher T. 2003. Regional and local population structure of the pioneer wood-decay fungus *Trichaptum abietinum*. *Mycologia* 95(3):416-425.

Malik M, Vilgalys R. 1999. Somatic incompatibility in fungi. In: Worrall JJ, ed. *Structure and dynamics of fungal populations.* Dordrecht, The Netherlands: Kluwer Academic Publisher, p 123-138.

May G. 1988. Somatic incompatibility and individualism in the coprophilous basidiomycete, *Coprinus cinereus*. *Trans Br Mycol Soc* 91(3):443-451.

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Appendix 25C.

Nilsson RH, Hallenberg N. 2003. Phylogeny of the *Hyochnicium punctulatum* complex as inferred from ITS sequence data. *Mycologia* 95(1):54-60.

Otieno W, Sierra AP, Termorshuizen A. 2003. Characterization of *Armillaria* isolates from tea (*Camellia sinensis*) in Kenya. *Mycologia* 95(1):160-175.

Petersen RH, Bermudes D. 1992. *Panellus stypticus*: Geographically separated interbreeding populations. *Mycologia* 84(2):209-213.

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Appendix 26. User internet resource.

A great resource for researchers interested in the importance of coarse woody debris and the health of the forested ecosystem is the Dead Wood Ecology and Management Discussion List

http://groups.yahoo.com/group/dead_wood

View the members, calendar of events, and find recent literature. To subscribe to the email list serve dead_wood-subscribe@yahoogroups.com