ASPEN MYCORRHIZAE: ECOLOGY, SYNTHESES, AND GROWTH STUDIES

by

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(ABSTRACT)

The ectomycorrhizal communities of three aspen-covered sites in southwestern Montana and southeastern Idaho with varying stand age, conditions, and soil types were compared. In all, 39 species of ectomycorrhizal fungi were associated with P. tremuloides. Dominant fungal species varied by site. Species reported on acidic soils in Europe and other species reported to be "early colonizers" were found exclusively on the acidic soils of the Butte site. Many "late stage" fungi such as Amanitas and Cortinarii were found on the older and more productive sites. Nine isolates of ectomycorrhizal fungi were tested for their ability to form mycorrhizae with P. tremuloides. Amanita muscaria v. formosa, A. pantherina, Paxillus vernalis, and Pisolithus tinctorius formed mantles and Hartig nets. Inocybe lacera and Piloderma croceum formed mantles but no Hartig nets. Dry weight, stem diameter, height and number of roottips increased in the young aspen seedlings inoculated with a majority of the fungi listed above. The implications for aspen survival and growth are discussed.

ACKNOWLEDGEMENTS

This thesis is dedicated to Dr. O.K. Miller, Jr. whose devotion to both mycology and teaching has been a constant source of inspiration and to Hope Miller who made me feel at home "east of the Mississippi". Thank you both for shared knowledge, advice and support.

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for letting me study the area adjacent to their home in Butte and Albert and Chris Tilt for access to the Teton Ridge Ranch. Without the consent of these private home owners, this project would not have been possible.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Populus tremuloides is the most widely distributed tree in North America (Little 1971). Worldwide, only the closely - related Populus tremula has a more extensive range. P. tremuloides is a major component of the boreal forests of the western U.S., occupying millions of acres (DeByle 1985) and often existing in extensive pure stands. It spans a wide range of elevations; it has been found below 1,000 meters in Alaska up to 3,400 meters in Gunnison, Colorado (Langenheim 1962) and even as a component of the Krummholtz at 3,800 meters (Zwinger 1991). In the north central Rocky Mountains of Montana and Idaho aspen are usually found from 1,200 to 2,100 meters, just below or within the conifer belt. Within this broad area aspen are capable of surviving in a wide range of climatic and soil conditions. Aspen are found on shallow rocky soil, loamy sands, and even heavy clays (Fowells 1965).

Aspen is a rapidly colonizing pioneer species which can invade marginal sites such as coalspoils (Schramm 1966; Medve 1973; Shuffstall and Medve 1979; Meyer 1968) and phosphate spoils (Williams and Johnston 1984). I have personally observed it on copper tailings, talus slopes, avalanche chutes, smelter-devastated areas, and burns as well as on

rich, well-drained bottomland. Aspen are usually considered serel to conifers, but recent studies have suggested that climax or subclimax stands may exist in areas where conifers are not present or ecological factors preclude conifer survival (Mueggler 1985; Sheppard 1990).

Aspen generally reproduce by suckering producing genetically homogeneous stands called clones (Campbell 1984), but aspen trees are also capable of reproducing sexually by seed produced in catkins. Aspens are usually dioecious producing male and female clones, but trees with perfect flowers have been found (McDonough 1985). Seeds are not dormant so they either germinate within a few days of dispersal or lose their viability (McDonough 1979). P. tremuloides is predominantly ectomycorrhizal (Thomas 1943; Hackskaylo and Vozzo 1971; Malloch and Malloch 1981) although McDougal and Jacobs (1927) reported one tree in Utah to be both endo- and ectomycorrhizal, and Malloch and Malloch (1981) found endomycorrhizal colonization in 2 of 30 root samples. Anecdotal evidence in field guides suggests that a considerable number of fungal species may be mycorrhizal with P. tremuloides. However, Trappe (1962) lists only 3 putative fungal symbionts of P. tremuloides in his comprehensive review of previously reported ectomycorrhizal associates. The first report of mycorrhizal syntheses with P. tremuloides was by Godbout and Fortin (1985), who successfully synthesized

seedlings with isolates of 29 fungal species and of the 54 fungal species tested, 28 were found in P. tremuloides stands. This lack of interest in doing syntheses work with P. tremuloides may have been partly due to lack of a satisfactory method of seed sterilization. Early european workers sterilized seeds with quicksilver (mercury)(Melin 1923). New methods for the rapid propagation of aspen by cuttings have been developed (Ahuja 1984; Ahuja 1983; Schier 1978). In addition, cuttings have been sterilized by european mycorrhizal workers (Heslin 1986; Anselmi, Pirazzi and Giorcelli 1990), but both procedures are long and complicated. The pouch method used by Godbout and Fortin, while giving a rapid assessment of the ability to form mycorrhizae, is not accomplished aseptically and is not amenable to discovering if fungal inoculation enhances seedling growth.

Marx (1975) and many others have shown that mycorrhizal colonization enhances the growth of conifers. This has only recently been shown to be true for aspen. Anselmi, Pirazzi and Giorcelli (1990) found that the growth of some Populus species, other than P. tremuloides is enhanced by some fungal species as measured by differences in clone height, diameter, and volume. Lee and Koo (1985) found that P. alba x P. glandulosa cuttings inoculated with Pisolithus tinctorius increased 49% in dry weight over uninoculated controls under

nursery conditions. As of yet, however, no one has explored the effect of different mycorrhizal fungi on the early growth response of P. tremuloides seedlings. This would be useful information since P. tremuloides is fast becoming a tree of economic importance in the western U.S. due to its use in waferboard production (Sheppard 1990), and its potential for reforestation. Jones, Winokur, and Sheppard (1985) have pointed out the historical importance of aspen in the western U.S. for "wildlife habitat, livestock forage, watershed protection, esthetics and recreation".

Species composition of ectomycorrhizal fungal communities may vary by site with a specific host due to soil characteristics (Tyler 1984; Last, Dighton and Mason 1987) or tree age (Mason, Wilson, Last, and Walker 1983). Nothing is known about how the ectomycorrhizal fungal communities associated with P. tremuloides vary with site. Since P. tremuloides exists in large pure stands in western North America in a wide variety of soil and climatic conditions, it is highly likely that this tree species is associated with a variety of mycosymbionts in different environments.

The objectives of this research were:

1. to document the putative ectomycorrhizal species of fungi associated with P. tremuloides on three selected study sites.
2. to compare the species composition of the

ectomycorrhizal fungi on these sites as they relate to clone age, soil type, and other conditions.

3. to develop a satisfactory method for seed sterilization to make synthesis work feasible for this study.

4. to determine if isolates of culturable fungal species can form mycorrhizae with P. tremuloides seedlings under aseptic conditions in culture tubes and to characterize the mycorrhizae formed.

5. to determine if selected isolates of fungal species stimulate the growth of aspen seedlings "in vitro" and to determine how certain tree characteristics are affected by inoculation.

LITERATURE REVIEW

Early European observations and syntheses of Populus mycorrhizae

Early reports concerning the mycorrhizal status of the genus Populus are primarily european. After Frank (1885) coined the word mycorrhizae, Stahl (1900) used this term to describe the roots of Populus tremula L. Thessleff (1919) in Norway noted that Russula aeruginea Fr., R.integra (L.) Fr., Lactarius subdulcis (Bull.) Fr., L.flexuosus Fr., L.uvidus Fr., Cortinarius spp., and Boletus rufus Schaeff. are often found

fruiting near Populus tremula. Smotlacha (1912) in Czechoslovakia also noted that Boletus rufus was found with aspen. This is the same species, Boletus rufus (now Leccinum aurantiacum), found by Peyronel (1917) along with Amanitopsis (Amanita) vaginata, Russula virescens, Russula chloroides and Cortinarius collinitus in association with P. tremula. Peyronel (1922) also found a direct mycelial connection between B.rufus and P. tremula by excavating the soil. Tuber borchii (Mattirolo 1934) and Russula xerampelina (Melzer 1927) have also been found with aspen.

In 1923 Melin categorized the roots of P. tremula as ectendomycorrhizal. Walker (1984) suggested that Melin's picture is either an endo- and ectomycorrhiza on the same root or pseudomycorrhizal (no hartig net or well-developed mantle). Kelley (1950) contains a translation of part of Melins work. Melin reviews some of the earlier literature on fungal species associated with aspen and notes that aspen and birch seem to have many mycorrhizal associates in common. Melin (1923) accomplished the first mycorrhizal synthesis of Populus using seedlings of Populus tremula and the mycelium of B. rufus. His tests with B. edulis, B. badius and B. luteus were negative. Other syntheses followed in Europe: P. tremula and Cenococcum graniforme (Lihnell 1942); Populus euroamericana and Hebeloma longicaudum (Fontana 1961); Hebeloma hiemale (Fontana 1963); and Tuber albidum (Fontana and Palenzona

1969).

Dominik (1958) studied 186 root samples from 14 species of hybrid poplars and reported Populus to be primarily ectomycorrhizal. He found 3 types of trees to be endomycorrhizal when young but becoming ectomycorrhizal with age. Although Dominik found all species to be mycotrophic, under some peculiar ecological conditions, trees of some species (particularly P. serotina) existed autotrophically (without mycorrhizae). Dominik hypothesized that this may be one reason why Populus can survive on disturbed sites and suggests that seeds may bring in their own mycorrhizal fungi. He found a Cenococcum-type mycorrhizae in many of the root samples.

A literature review by Fontana (1961) confirms reports that Populus species associate with both endo- and ectomycorrhizae. The findings reported endomycorrhizae on one individual of P. nigra, and both endo and ectomycorrhizal on one P. euroamericana. In addition, P. tremula, P. alba and P. nigra had ectomycorrhizae. The review also presents descriptions of several types of mycorrhizae that occur naturally on P. tremula, P. alba and P. nigra.

North American Studies

Reports of endomycorrhizae on Populus spp. in North America

are mostly of P. deltoides (Lohman 1927; Thomas 1943; Vozzo 1969; and Vozzo and Hacskaylo 1974). However, P. tremuloides (McDougal and Jacobs 1927), hybrid poplars (Walker and McNabb 1984), and P. balsamifera (Malloch and Malloch 1982) have also been reported to be endo- as well as ectomycorrhizal.

North American species, like european species have been found to be predominantly ectomycorrhizal including P. tremuloides (McDougal and Jacobs 1927; Thomas 1943; Hackskaylo and Vozzo 1971), P. balsamifera (Malloch and Malloch 1982), P. grandidentata, P. heterophylla (Hackskaylo and Vozzo 1971), and hybrid poplars from Iowa (Walker and McNabb 1984).

Since aspen is a natural invader of disturbed sites, reports of its mycorrhizal condition often come from these areas. Schramm (1966) observed P. tremuloides to be ectomycorrhizal on coal spoils in Pennsylvania. He found Cenococcum graniforme (Sow.)Ferd. et Winge on the roots of aspen and observed Inocybe lacera Fr., Inocybe sp., Thelephora terrestris Ehrh. ex Fries, Pisolithus tinctorius (Pers.)Coker & Couch, Amanita rubescens (Fr.)S.F.Gray and Astreus hydrometricus (Pers.)Morgan. near mixed woods containing aspen, pine, oak, birch and willow. He noted that P. tinctorius was usually the first symbiont on the roots and that Amanita species existed only where leaf litter had accumulated. The association of P.tinctorius with Populus species has also been noted by Marx (1975; 1977), Meyer (1968)

and Grand (1976). Harris and Jurgensen (1977) reported that hybrid poplars became ectomycorrhizal on iron tailings, the trees showed good growth and the stunting of trees on copper tailings could be attributed to the failure of mycorrhizae to form. Shuffstall and Medve (1979) found that P. tremuloides naturally invaded mine spoils in western Pennsylvania where it grew better than on non-spoil sites although it was ectomycorrhizal under both conditions.

Trappe (1962) compiled reports of the putative mycorrhizal associates of most trees including aspen and noted syntheses that have given conclusive proof of symbiosis. References are cited for each report in an exhaustive bibliography. Three ectomycorrhizal species are listed under P. tremuloides: Cenococcum graniforme, Leccinum aurantiacum, and L.scabrum. One synthesis is recorded for Populus spp. with L. aurantiacum (Melin 1923); and none are listed for P. tremuloides.

Some other putatively mycorrhizal associates of P. tremuloides are: Paxillus vernalis (Watling 1969), Russula claroflava (Bills and Miller 1984), Amanita regalis (Fr.) (Miller 1982), Amanita armillariiformis Trueblood & Jenkins and Amanita aurantisquamosa Trueblood, Miller and Jenkins (Miller, Trueblood and Jenkins 1990). Tricholoma fulvomarginatum Ovrebo and Halling has been reported near aspen and cottonwood and is considered to be mycorrhizal with the latter (Ovrebo and Halling 1986).

Recent Studies

Godbout and Fortin (1985) tested the ability of isolates of 54 fungal species to form ectomycorrhizae with P. tremuloides. Twenty-four of these isolates were found near P. tremuloides. Of the 29 species which formed mycorrhizae including a Hartig net, 20 occurred in the field near P. tremuloides. Seeds were germinated on white sand under nonaseptic conditions for 30 days and placed in a growth pouch. The fungus was introduced one week later. It was not noted how long the symbionts were in the pouches before examination.

Heslin and Douglas (1986) tested the ability of 18 isolates (nine species of fungi) to form mycorrhizae with surface sterilized cuttings of P. trichocarpa torr. x Gray x P. tacamchace Mill. in vitro. C. geophilum Fr., Hebeloma crustulineforme (Bull:Fr.) Quel., H. sinapizans (Paul:Fr), Paxillus involutus, Tricholoma scalpturatum (Fr.) Quel. and Thelephora terrestris (Ehrh.)Fr. were found in P. sitchensis (Bong.)Carr woods in Ireland and poplar forests in France. Thelephora terrestris (Ehrh.)Fr. and P. involutus formed ectomycorrhizas with a mantle and Hartig net. Hebeloma crustuliniforme and P. tinctorius (Pers.)Coker & Couch formed mantles but no Hartig net.

Anselmi, Pirazzi, and Giorcelli (1990) listed the 17

fungal species screened for mycorrhizal synthesis with various Populus species. The isolates were from sporocarps found in Populus stands in Italy. These are: Boletus rubellus (Khrb.)Moser, B. rufus Schroef., Cortinarius saniosus Fr., Hebeloma populinum Romagn., Hebeloma testaceum, Hymenogaster griseus Vitt., Inocybe globocystis Bres., Laccaria laccata Fr. ex Scop., Lactarius controversus Fr. ex Pers., Melanogaster ambiguus, Paxillus involutus, Rhizopgon rubescens Tul., Thelephora terrestris (Ehrh.)Fr., Tricholoma suffocatum Rich. e Roze (=T.populinum J.Lange), Tuber albidum, T. maculatum and T. magnatum. All 17 species formed mycorrhizae with at least one of five hybrid poplar species. The tests were carried out in greenhouses or in the field using mini-cuttings which had been grown aseptically in pot culture. Trees inoculated with T. populinum, H. populinum, T. magnatum, M. ambiguus and I. globocystis increased in diameter, volume and height over uninoculated controls grown in field and nursery settings. Trees inoculated with P. involutus and H. testaceum did not show any response.

CHAPTER 1: STUDY SITES

INTRODUCTION

All three study sites are located in the northern Rocky

mountains of southwestern Montana and southeastern Idaho (Figure 1). The sites range from 1800 to 2000 meters in elevation and lie at a latitude of 45° and a longitude of 110° to 112°. The sites contain nearly pure stands of Populus tremuloides Michx., an important component of the northern boreal forests.

The selected areas had a combination of factors. The main criterian was Populus tremuloides stands as free of other ectomycorrhizal host plants as possible. Secondly, sites with a high potential for sporocarp production either because aspen covered an extensive area or because the trees were on a relatively moist site were necessary because the project depended upon locating and tissue culturing sporocarps of putatively mycorrhizal species. Thirdly, sites representing a subset of aspen stands that occur in southwestern Montana and southeastern Idaho within a certain elevation and precipitation range were chosen so that this subset could be characterized. Lastly sites were chosen that varied in soil characteristics in order to compare the ectomycorrhizal communities. Since public lands in the area are heavily grazed by cattle, all three sites are located on private land which is only lightly grazed by horses.

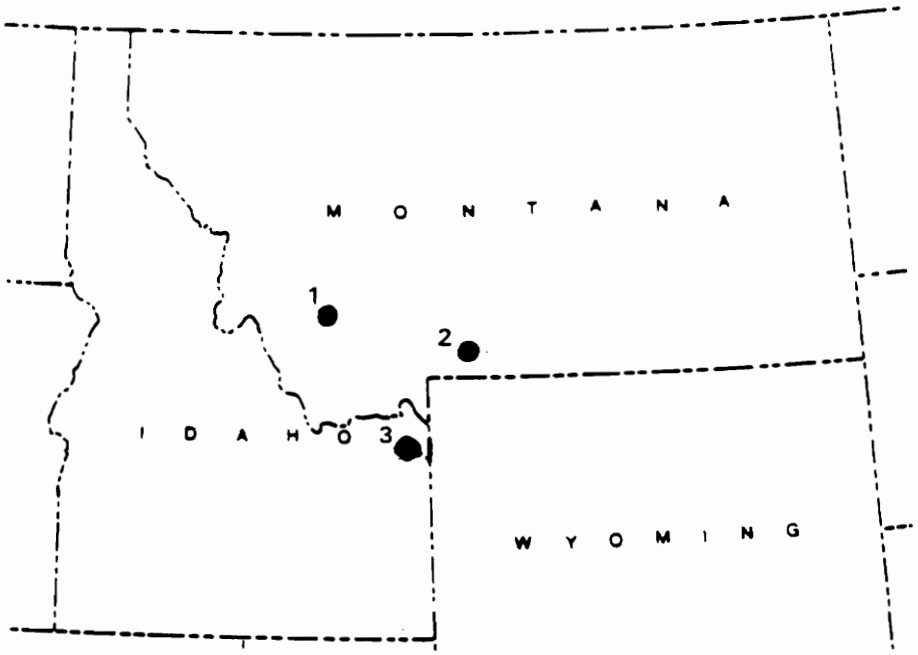


Figure 1. Study area in the northcentral Rocky mountains of the western U.S. and location of the study sites in southwestern Montana and southeastern Idaho: site 1 (Butte), site 2 (Cinnabar) and, site 3 (Teton).

METHODS AND MATERIALS

PRECIPITATION DATA: Records of monthly and annual precipitation for the collecting years of 1990 and 1991 were obtained from nearby weather stations. Precipitation data for Site 1 (Butte) came from the National Weather Service station located at the Butte airport located 8 km. from the site. Data for Site 2 (Cinnabar) site came from the SNOTEL site (Snow Survey) at Mammoth in Yellowstone National Park, 16 km. from the site, and information for Site 3 (Teton) came from the National Weather Service station at the Tetonia Experiment station 18 km. from the site.

MAXIMUM AGE OF ASPEN STANDS: The average maximum age of trees on each site was determined by coring the boles of ten of the largest P. tremuloides at breast height with an increment borer. It was assumed that trees with the largest diameters were the oldest trees. Cores were painted with iodine, surfaces sanded with emery cloth and rings counted with the aid of a dissecting scope. Values for each site were averaged and standard deviations calculated. The averages can be interpreted as the average age of the oldest trees on the site or, since aspen is a clonal species, as the average maximum stand age. No attempt was made to determine the age of the root system.

ASPEN COMMUNITY TYPE: Aspen community type was classified according to Mueggler (1988). Classification was determined by: 1) percent of aspen and other trees present, 2) presence or absence of a tall shrub, low shrub, tall forb, low forb and\or grass layer in the understory, and 3) the species composition of each layer. Dominant plant species were identified throughout the summers of 1990 and 1991 and identified according to Cronquist (1973). The per cent of each tree species present was estimated.

SOIL

Morphological characteristics: A pit was excavated to the parent material on each site. Soil horizons were described and samples collected from various depths. At least one sample was taken from each major horizon. Determination of the particle size distribution and all chemical tests were done by the VPI and SU soil testing lab. Soil samples were air-dried and particle size distribution was determined using a 10 mesh sieve to remove larger particles which were then weighed to determine the coarse fragment content (72 mm). The particle size distribution was determined for the fine fraction by the pipette method (Day, 1965). All chemical tests were performed on the < 2mm fraction. The depth, color, consistency and structure of each soil horizon was determined on site.

This information along with particle size distribution and element extraction by ammonium acetate was used to determine soil classification which was done by Dr. Lee Daniels of VPI and SU.

pH Determination: Equal volumes of soil and distilled water (20 ml:20 ml) were added to a beaker, stirred, and allowed to set for 15 min to 2 hrs. The solution was stirred again and a pH meter with a glass electrode used to determined the pH (Rhoades, 1982). The following classification from Brady (1990) is used in the discussion for the three soils:

Extremely acid	< 4.5
Very strongly acid	4.5-5.0
Strongly acid	5.1-5.5
Medium acid	5.6-6.0
Slightly acid	6.1-6.5
Neutral	6.6-7.3
Mildly alkaline	7.4-7.8
Moderately alkaline	7.9-8.4
Strongly alkaline	8.5-9.0
Very strongly alkaline	>9.1

Determination of Organic Matter: Acid-Dichromate digestion was used to determine organic matter percentage (Allison 1965; Peech 1947). A 0.67 M solution of dichromate was added to 1.5 mls. of soil (under a hood) and 20 ml. of H_2SO_4 was added after the solution cooled. The colorimeter reading on an aliquot of the solution was compared to a reference table to give percent organic matter.

Chemical analysis

1. Double acid nutrient extraction: P, Ca, Mg, K, Mn, Zn, Al, Cu and B were extracted in a dilute double-acid: 0.05 N HCl and 0.025 N H_2SO_4 (Donahue and Gettier, 1981) and analyzed by ICP (inductively coupled plasma) spectrophotometry. This gives the exchangeable plus acid soluble portions of the elements present.

2. Ammonium Acetate Extraction: The amounts of exchangeable Ca, Mg, K, and Al were determined by extraction with ammonium acetate, analyzed with an atomic absorption spectrophotometer (Thomas, 1982), and used to determine soil classification.

Exchangable hydrogen (H in Table 7) is the total reactive acidity to pH 8.2 which includes the exchangeable acidity and organic matter functional groups (C-OOH). CEC (Cation Exchange Capacity) used in soil classification is calculated by summing Ca + Mg + K + H resulting from the ammonium acetate extraction. The % base saturation is calculated using the formula:

$$\frac{Ca + Mg + K}{Ca + Mg + K + H}$$

Percent base saturation indicates the nonacid cation percentage and is inversely related to acidity. Chemical tests and OM and pH determination were done by the VPI and SU soil testing laboratory.

RECENT SITE HISTORY: Information on the recent history of the sites was gathered from local inhabitants: Mannie and Rosie Lisac lived on the Butte site for the last 70 years, Len and Sandy Sargent who have spent 40 years on the Cinnabar site and Lil and Phil Erikson are caretakers of the Sargent ranch, Al and Chris Tilt who manage the Teton Ridge Ranch and Dick Egbert who has lived in the Teton area for 80 years.

RESULTS AND DISCUSSION

BUTTE

The Butte site lies in one of the small drainages exiting the Rocky mountains just east of the continental divide near Butte, Montana, at an elevation of 1800 meters (Fig. 2). Granitic outcroppings are common on the site and the sandy soil is formed from colluvium on the steep slopes where runoff is rapid as evidenced by numerous sand washes and eroded gullies. The site covers 60 acres and consists of one steep northwest facing slope densely covered with aspen (Fig. 5b) and an almost barren southwest facing slopes populated with scattered stands of young aspen (Fig. 5a). These slopes are separated by a small stream in the valley bottom. There are a few male and female trees on the n-facing slopes, and seeds are produced on the site. Aspen are becoming reestablished on the S-facing slope but many are stressed by the unstable

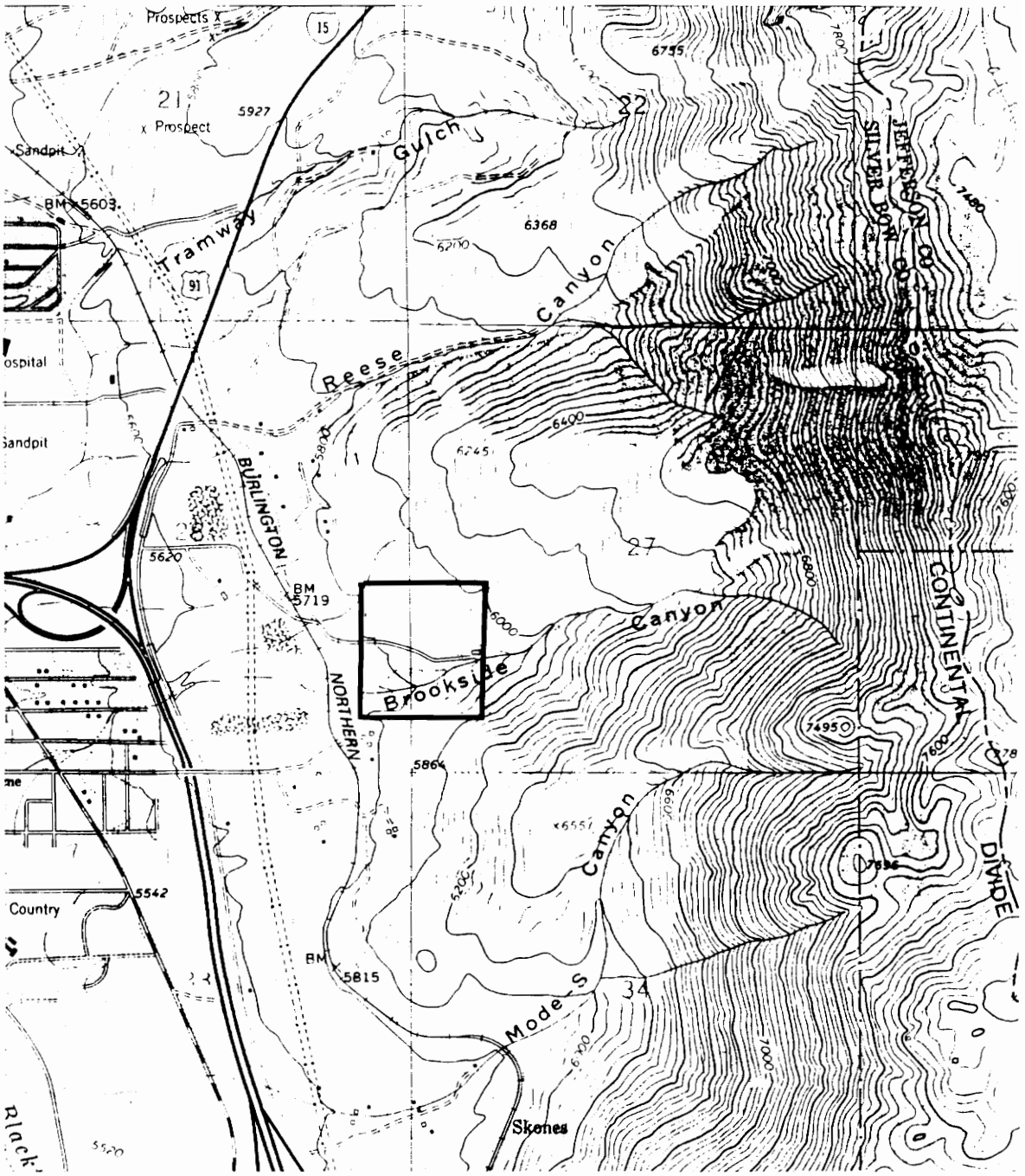


Figure 2. Location of site 1 (Butte), T.3.N, R.7.W, sec.27 and 28, Silverbow county, Montana; Homestake quadrangle. 2.6" = 1.6 km (1 mile).

slopes, erratic moisture and poor soil conditions. Diseased and insect damaged trees are common as are aspen which have toppled due to root exposure. A large open pit copper mine is visible from the site. Fumes from nearby copper smelters operating from the late 1800's drastically reduced the vegetation around Butte for many years. Untreated smelter emissions contain large amounts of sulfur dioxide capable of acidifying the environment (Quinn 1989) and may also contain various heavy metals. The country was denuded of timber because it was used in the mining process and because emissions killed the trees (James 1975). Mannie and Rosie Lisac, owners of the South Side dairy on which the site lies, relate that 70 years ago the site was devoid of trees. Gradually the smelters closed and trees began repopulating the area. Large acreages in the area are now covered with pioneering young aspen, although highly contaminated areas are still barren. Dairy cattle grazed the site for over 70 years, and it is now lightly grazed by horses and deer.

CINNABAR

The Cinnabar site just north of Yellowstone National Park lies in the basin at the head of Cinnabar canyon at an elevation of 1900 meters (Fig. 3). Local mountain glaciation during the pleistocene filled the valley with glacial till from which the deep gravelly loam soils formed. The dark

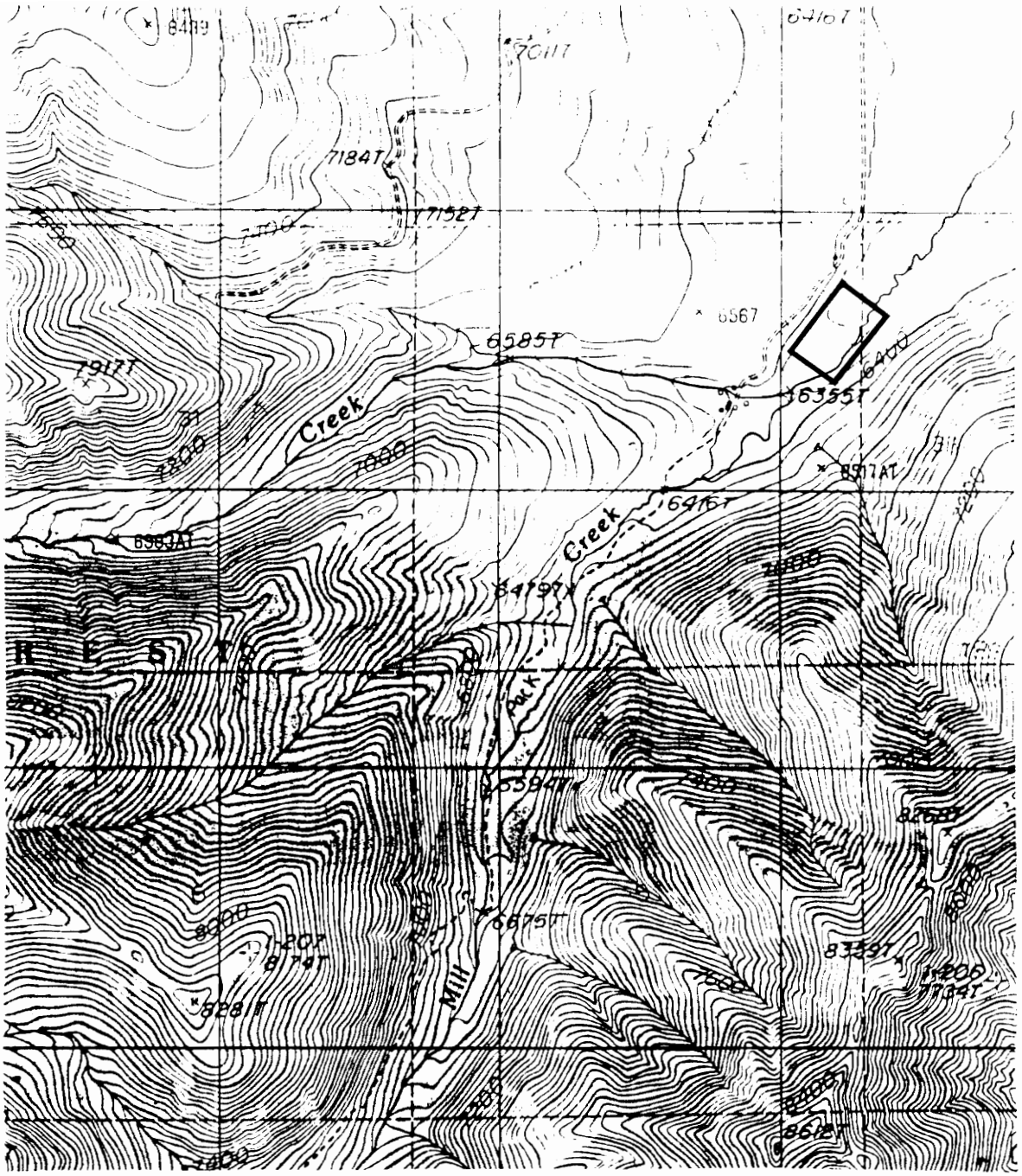


Figure 3. Location of site 2 (Cinnabar), T.8.S, R.7.E, sec. 32, Park county, Montana; Sportsman lake quadrangle. 2.6" = 1.6 km (1 mile).

alluvial soil is fertile and has a high moisture-holding capacity. The surrounding mountainous terrain is covered with Douglas fir at higher elevations, the site itself consists of a single aspen stand on the valley floor (Fig. 5d) bordered on the west by Mill creek and on the east by a low-lying wet area. The small, 8 hectare site is almost flat and the high organic content of the deep soil, the landscape position in the valley bottom, the dense canopy, and the wet areas combine to make this a moist site. There are no seed-producing trees on the site. Young clones are present beneath the older trees but are more prevalent at the stand perimeter. Unlike the young trees on the Butte site which have their own extensive root systems, young trees here are connected to the horizonal roots of the older trees and are delayed in developing their own root system. The site is in the initial stages of conifer invasion, but there were none in the root zone studied.

According to Len and Sandy Sargent, owners of the land on which the Cinnebar site sits, fire swept the area in 1910 and again in the 1960's. It is not known if either fire reached the study site. The meadow adjacent to the study site was flooded by beavers as recently as 25 years ago but dried out after the demise of the beavers. The site is now part of a privately owned ranch and is used as a winter grazing area for horses. In the summer deer and moose are found on the site.

TETON

The Teton site in southeastern Idaho lies in the undulating foothills just west of the Grand Tetons (Fig. 4). The forests at higher elevations to the east are dominated by lodgepole pine and at even higher elevations by spruce-fir. The vast acreages under cultivation to the west and north are punctuated with remnant aspen woodlands. The site itself, at an elevation of 1900 to 2000 feet, consists of gently rolling aspen-covered uplands interspersed with meadows often occupied by sagebrush (Fig. 5c). This is the largest of the three sites covering 55 hectares, and contains a large number of aspen stands. The condition of these stands varies greatly from degenerating stands to vigorously cloning ones. Over time this site has probably possessed aspen longer than the others. The well-drained soils which formed from loess associated with various interglacial periods, are relatively fertile but the upland position of the site and the late summer and early autumn dry spells can combine to cause a moisture shortage at that time of year. There are no significant streams on the site. Below the deep loess is ash flow tuff composed of rhyolite which is volcanic in origin.

Dick Egbert, who has lived in the area for 80 years, relates that aspen have covered the Teton site since at least the 1920's. He remembers evidence of a fire that was rumored to have swept the area in the 1860's. He did not think the

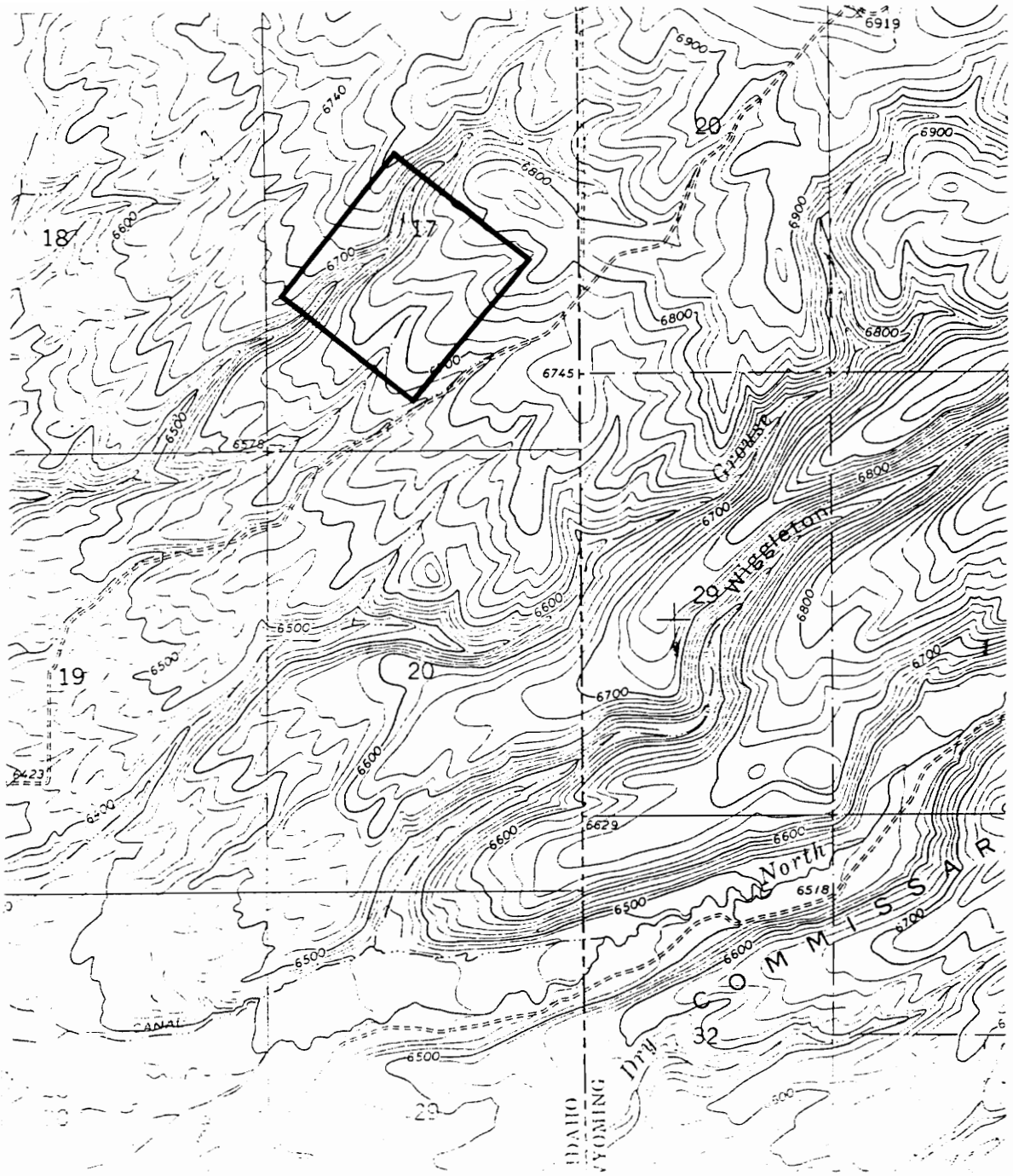
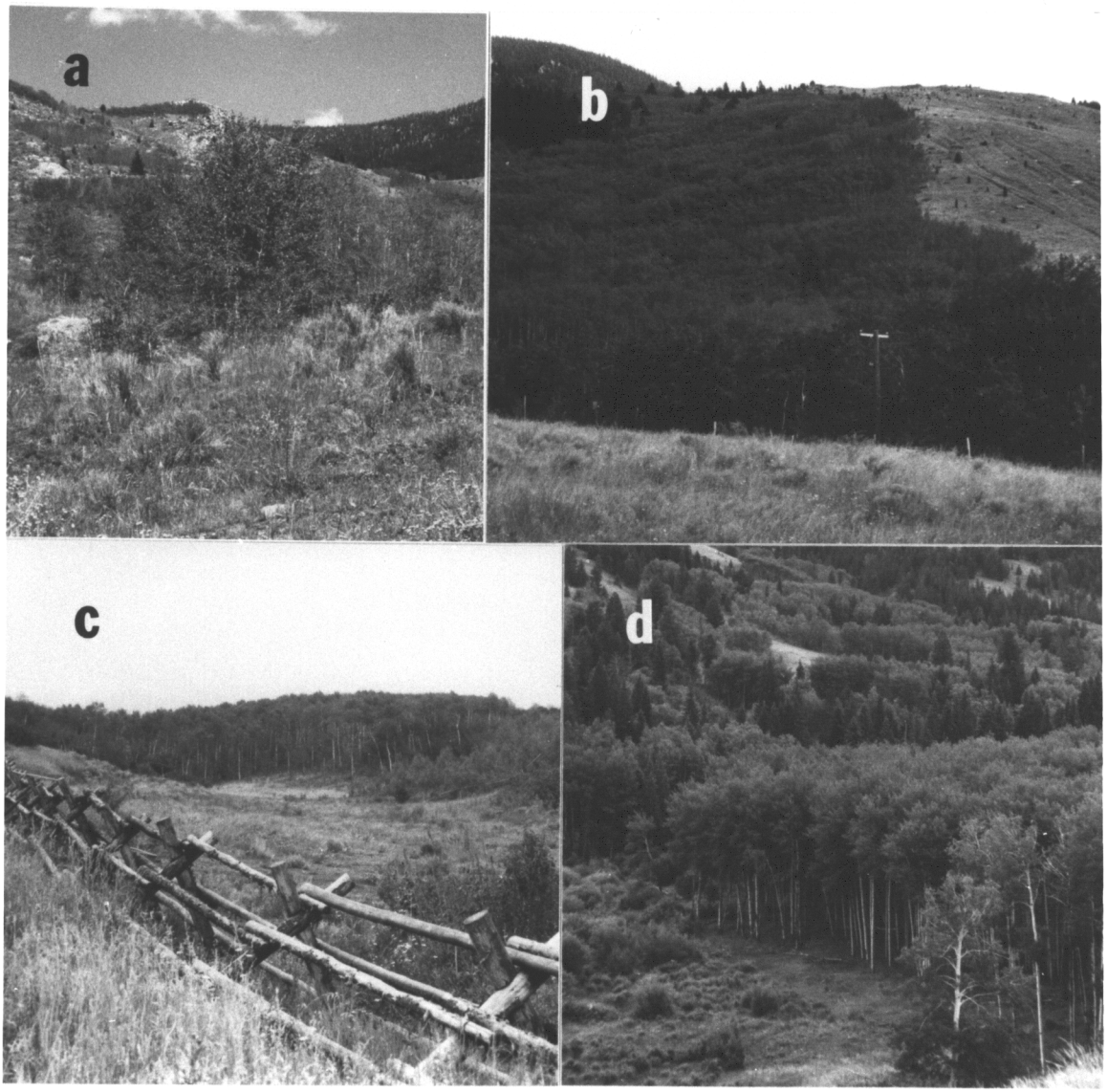


Figure 4. Location of site 3 (Teton), R.45.E, T.6.N, sec.18, Teton county, Idaho; Clawson Idaho-Wyoming quadrangle. 2.6" = 1.6 km (1 mile).

Figure 5. Photographs of sites, a) young aspen clones on the southwest-facing slopes of Butte site, b) older aspen on the northwest-facing slopes of Butte, c) rolling aspen-covered hills of the Teton site, and d) aspen stand on the valley floor of Cinnabar basin.


a
b
c
d

Bench creek fire of 1910 or the Darby fire of 1920 reached the site. The area is now a private guest ranch and is lightly grazed by horses and deer.

PRECIPITATION

Precipitation on all three sites is between 10 and 20 inches (Table 1). Butte, with a yearly normal of 30.91 cm, has the lowest with almost 40% falling in May and June. During 1990 and 1991, the field seasons for this study, precipitation was slightly below normal at the Butte site. The yearly normal listed for the Cinnabar site is 40.0 cm. and for the Teton site, 41.25 cm. Due to local variation rainfall recorded at the Yellowstone Weather Station for the Cinnabar site is probably somewhat lower than that on the site. The yearly average precipitation on the Teton site may be somewhat higher than that recorded at the Tetonia station because precipitation increases with elevation in this region.

AGE OF TREES ON THE SITES

The ages of the largest diameter trees found on each site are shown in Table 2. Aspen on the southwest-facing slope of the Butte site, younger than those on other sites, have a mean age of 36.5 years. Aspen on the valley floor of this site averaged 20 years older: the oldest tree cored was 66 years old. Tabulated tree-boring data correlates with information

Table 1. Precipitation in centimeters at weather stations closest to each site; yearly normal compared to precipitation for 1990 and 1991 collecting seasons.

	Butte			Cinnabar			Teton		
	normal	1990	1991	normal	1990	1991	normal	1990	1991
Jan	1.37	1.17	1.14	3.00	2.00	0.89	3.48	6.50	5.61
Feb	0.99	0.28	0.38	2.18	1.09	0.79	2.75	3.10	1.27
Mar	1.62	1.65	3.45	3.15	1.17	2.72	2.49	0.76	2.62
Apr	2.39	1.80	2.36	3.15	2.23	5.28	2.92	3.38	5.74
May	4.65	5.54	5.99	4.77	6.55	9.30	4.69	7.77	9.80
June	6.83	2.97	6.05	4.90	4.42	1.30	4.78	1.80	2.26
July	2.72	2.34	0.79	3.38	0.94	2.03	2.72	0.66	2.24
Aug	2.92	5.89	2.29	3.43	2.50	0.69	3.28	1.96	1.78
Sept	3.00	0.18	3.76	3.33	3.20	6.25	3.15	3.40	4.39
Oct	1.68	2.74	3.76	3.00	6.35	4.11	3.17	3.99	2.06
Nov	1.47	1.04	0.66	2.84	1.65	1.09	2.59	5.28	5.51
Dec	1.24	1.47	1.57	2.84	1.98	1.88	5.28	5.28	2.62
Ann	30.91	27.08	29.31	40.00	34.11	36.32	41.25	43.89	46.15

Location of weather stations

Site	lat.	long.	elev	dist.to site	site elev
Butte[a]	45° 57′	112° 30′W	1690 m	11 km	1800 m
Cinnebar[b]	44° 58′	110° 42′	1900 m	16 km	1920 m
Teton[c]	43° 51′	111° 16′W	1880 m	18 km	2050 m

[a] National Weather Service station at Butte, Montana airport
[b] SNOTEL site in Yellowstone National Park, Mammoth, Wyoming
[c] National Weather Service at Tetonia experiment station, Tetonia, Idaho

Table 2. Average Age of Aspen with greatest DBH (diameter at breast height) on each site.

Butte		Cinnabar	Teton
sw-slopes	valley floor		
34	62	75	64
29	49	90	56
46	58	70	59
36	64	114	60
46	66	91	67
42	64	93	63
37	62	85	56
24	44	68	57
35	54	69	69
36	39	56	64
x=36.5 yrs	x=56.2 yrs	x=81.1 yrs	x=61.5 yrs
s= 6.6 yrs	s= 9.4 yrs	s=16.7 yrs	s= 4.6 yrs
oldest tree:			
46 yrs	66 yrs	114 yrs	69 yrs

x = average tree age
s = standard deviation

indicating that there were no trees on the site 70 years ago and suggests that the sheltered, moist area along the stream was repopulated first. The average age of the biggest trees on the Cinnabar site was 81 years, by far the highest mean of all sites. One aspen was 114 years old. This site had the highest standard deviation in maximum age mean suggesting that the stand developed gradually from a few initial trees and has remained undisturbed for their lifetime. The average age of the largest trees on the Teton site is 61.5 years with the relatively low standard deviation of 4.6 years. The oldest tree was 69 years old. The implications of this are not clear, although fires occur regularly in the area. Soil features suggest aspen have populated the area for a long time.

UNDERSTORY VEGETATION OF BUTTE SITE

Over 99% of the trees on the Butte site are P. tremuloides (Table 3). Specimens of Juniperus scopulorum, Pseudotsuga menziesii, Picea englemanii, and Pinus contorta are rare and scattered. Alder, willow, and dogwood are found along the stream banks. No tall shrub understory exists although short shrubs such as Juniperus communis, Ribes spp., Amelanchier alnifolia, Rosa woodsii, Chrysothamneus nauseosus, and Artemesia tridentata are often found in the understory or in

Table 3. Vegetation on Butte site.

TREES	
Alnus Hill sp.	alder
Juniperus scopulorum Sarg.	Rocky mountain juniper
Picea engelmannii Parry ex Engelm.	Engelmann spruce
Pinus contorta Dougl. ex Loud	lodgepole pine
Populus tremuloides Michx.	quaking aspen
Pseudotsuga menziesii (Mirb.) Franc	Douglas-fir
Salix sp.	willow
SHRUBS	
Amelanchier alnifolia (Nutt.)Nutt.	serviceberry
Artemisia tridentata Nutt.	sagebrush
Berberis repens Lindl.	Oregon grape
Chrysothamneus nauseosus (Pall.)Britt	rabbitbrush
Cornus stolonifera Michx.	dogwood
Juniperus communis L.	common juniper
Ribes spp.	currant
Rosa woodsii Lindl.	wild rose
Rubus idaeus L.	rasberry
Rubus parviflorus Nutt.	thimbleberry
FORBS	
Achillea lanulosa Nutt.	yarrow
Centaurea sp.	knapweed
Equisetum arvense L.	horsetail
Eriogonium Michx. sp.	buckwheat
Eurotia lanata (Pursh)Moq.	winterfat
Iris missouriensis Nutt.	iris
Linaria vulgaris Hill	butter-and-eggs
Rumux sp.	curly dock
Smilacina stellata (L.) Desf.	starry solomon seal
Urtica dioica L.	nettle
GRASSES	
Agrostis spp.	bentgrass
Bromus spp.	brome
Phleum sp.	timothy

DATA FOR COMMUNITY TYPE DETERMINATION:
Trees: 99% aspen
Tall shrub layer: not present
Low shrub layer: present in areas, J. communis present
Tall forb layer: not present
Low forb layer: present in some areas
Grass: estimated at least 60% grass

adjacent meadows. The forb species are indicative of a xeric habitat. Eurotia lanata is found scattered on the sparsely vegetated southwestern slopes along with Erigonium spp., Berberis repens, and the knapweed, Centaurea. Very localized clusters of Iris missouriensis, Equisetum arvense, Achillea lanulosa, and Urtica dioica occur in the wetter areas of the valley floor. A variety of grasses including Phleum, Bromus and Agrostis constitute the dominant understory. This site combines a sparse low grassy undergrowth with scattered areas of low forbs and low shrubs, including J. communis. It does not fall into a major classification type, but the presence of the indicator species J. communis suggests it falls into a P. tremuloides/J. communis type of community. The pioneering Juniperus scopulorum dominates the landscape in adjacent areas and is found sporadically on the site; this species is not mentioned in any of the community classifications (Mueggler 1988). Many aspen communities containing juniper are considered to be grazing-altered and serel, often to a tall shrub understory type: and indeed the site has been grazed by cattle for the last 70 years.

UNDERSTORY VEGETATION OF THE CINNEBAR SITE

Over 99% of the trees on the Cinnabar site are P. tremuloides (Table 4). Pinus contorta and Picea englemannii

Table 4. Vegetation on Cinnabar site.

TREES	
Alnus Hill sp.	alder
Picea engelmannii Parry ex. Engelm.	Engelmann spruce
Pinus contorta Dougl. ex Loud	lodgepole pine
Populus tremuloides Michx.	quaking aspen
Salix spp.	willow
SHRUBS	
Berberis repens Lindl.	Oregon grape
Rosa woodsii Lindl.	wild rose
Rubus idaeus L.	Rasberry
Symphiocarpus albus (L.) Blake	snowberry
FORBS	
Achillea lanulosa Nutt.	yarrow
Angelica sp.	angelica
Aquilegia flavescens Wats.	yellow columbine
Castilleja Mutis ex L.f. sp.	paintbrush
Cirsium Mill. sp.	thistle
Equisetum arvense L.	horsetail
Galium boreale L.	bedstraw
Geranium richardsonii Fisch. & Trautv.	geranium
Heracleum lanatum Michx.	cow parsnip
Luzula parviflora (Erh.) Desv.	woodrush
Melilotus officinalis (L.)Lam.	sweet clover
Osmorhiza chilensis H.& A.	sweet cicely
Potentilla gracilis Dougl.	cinquefoil
Smilacina stellata (L.)Desf.	solomon's-seal
Thalictrum occidentale Gray	meadowrue
Urtica dioica L.	nettle
GRASSES	
Agrostis sp.	bentgrass
Bromus sp.	brome
Calamagrostis rubescens Buckl.	pinegrass

DATA FOR COMMUNITY TYPE DETERMINATION:
Trees: less than 1% conifers, 99% aspen
Tall shrub layer: not present
Low shrub layer: estimated at less than 10% cover
Tall forb layer: estimated at less than 10% tall forbs
Low forb layer: many herbaceous species, but none dominate
Grass: overall graminoid aspect with C. rubescens present

are sporadically present and, along with Pseudotsuga menziesii, are major components of the coniferous forests found at higher elevations. The site is small, and the presence of conifers must be kept in mind since the limitations of their root zones were not determined. Alder and willow are found on the wetter areas of the site. There is no tall shrub layer. A few short shrubs such as Rosa woodsii, Rubus idaeus and Symphiocarpus albus are found in localized areas but are not dominant understory components. A rich variety of forbs exists in the understory but none dominates. Heracleum lanatum, Achillea lanulosa, Castilleja sp., Urtica dioica, Thalictrum occidentale, Galium boreale, Geranium richardsonii, and Osmorhiza chilensis are scattered throughout the plot. In a low lying wet area Equisetum arvense is common. Calamagrostis rubescens and Agrostis and Bromus species make up the grass understory which is dominant on this site. Cinnabar falls into the P.tremuloides /Calamagrostis rubescens community type (Mueggler 1988), which has an overall graminoid aspect with incidental conifers. Other than grasses, the undergrowth contains many herbaceous species. This community type may be a climax community or one that is slowly serel to conifers, which seems to be the case here. It is also often grazing altered.

UNDERSTORY VEGETATION OF THE TETON SITE

Approximately 97% of the trees on the Teton site are P. tremuloides (Table 5). Pseudotsuga menziesii and Pinus contorta are rare, although they are the dominant forest vegetation in this area at elevations above 6800 feet. Willow and juniper occur occasionally. A tall shrub guild consisting of Prunus virginiana and Amelanchier alnifolia is dominant in many areas. A low shrub guild including Rosa woodsii and Symphiocarpus albus is present in localized areas, but the composition varies. Artemesia tridentata, Ribes sp., and Crataegus douglasii are occasionally present. Rudbeckia occidentalis (cone flower) constitutes a major understory component in some areas, and Lupinus spp. and Thalictrum occidentale can be prevalent in other areas. A variety of grasses are present including Calamagrostis rubescens, Elymus glaucus and Agropyron spicatum. The understory on the Teton site is the most diverse and complex of all the sites because of the presence of tall shrub, low shrub, and tall forb layers. It is a P. tremuloides/Amelanchier alnifolia-Symphiocarpus/tall forb community (Mueggler 1988) due particularly to the presence of Pinus virginiana and Alnus alnifolia. This is considered a climax community type, again indicating that probably aspen has persisted on this site through time.

Table 5. Vegetation on Teton site.

TREES	
Crataegus sp.	hawthorn
Juniperus scopulorum Sarg.	Rocky mt. juniper
Populus tremuloides Michx.	quaking aspen
Pinus contorta Dougl. ex Loud	lodgepole pine
Pseudotsuga menziesii (Mirb.)Franco	douglas-fir
Salix sp.	willow
SHRUBS	
Amelanchier alnifolia Nutt.	serviceberry
Artimesia tridentata Nutt.	sagebrush
Crataegus douglasii Lindl.	black hawthorn
Prunus virginiana L.	chokecherry
Ribes sp.	wild currant
Rosa woodsii Lindl.	wild rose
Symphiocarpus albus (L.) Blake	snowberry
FORBS	
Aster sp.	aster
Castilleja Mutis ex L.f. sp.	red paintbrush
Cirsium Mill. sp.	thistle
Clematis columbiana (Nutt.)T. & G.	clematis
Eurotia lanata (Pursh.) Moq.	winterfat
Heracleum lanatum Michx.	cow parsnip
Lupinus sp.	lupine
Rudbeckia occidentalis Nutt.	cone flower
Thalictrum occidentale Gray	meadowrue
Solidago sp.	goldenrod
GRASSES	
Agropyron spicatum (Pursh.)Scribn&Smith	wheatgrass
Bromus sp.	brome
Calamagrostis rubescens Buckl.	pinegrass
Elymus glaucus Buckl.	wildrye
Phleum sp.	timothy

DATA FOR COMMUNITY TYPE DETERMINATION:
Trees: canopy dominated by P. tremuloides
Tall shrub layer: present as A. alnifolia and P. virginiana exceeding 10% of canopy cover
Low shrub layer: present in areas
Tall forb layer: various tall forbs such as R.occidentalis and Lupinus sp.dominant in some areas

Although some authors have found conflicting results, Roberts and Christensen (1987) found that "vegetation patterns of successional aspen stands in Northern lower Michigan are clearly influenced by soil conditions." The stands studied fell into three shrub-tree classifications, each being found on a different soil type. The understory vegetation for the Michigan sites is not comparable to that found on the Montana and Idaho sites. The soil groups found with each of the three kinds of vegetation in Michigan were: A) sandy, low moisture, low nutrient availability; B) sandy, wet, low nutrient availability; and C) heavier-textured (often clay or calcareous), wet, high nutrient availability. Although correlation with individual soil variables was not high, there was some correlation in group A with bulk-density and organic matter and in group B with pH, Ca and Mg. Group C was too small for correlation analysis. Because there are only three plots in the present study, no attempt was made to correlate soil with understory type, but each of the Montana and Idaho sites had a different understory and soil characteristics.

SOILS FOUND ON THE THREE SITES

Steep slopes on the Butte site, combined with shallow soil of 70-84% sand (Table 6), the relatively low percent base saturation (Table 7), and low levels of macronutrients (Table

8), combine to make this a relatively unfertile site. The organic matter (Table 7) is well distributed with depth either because the soil is colluvium or because there was an earlier grass vegetation. The latter seems to be true from historical references. The soil is extremely acidic, as low as 4.3 at a depth of 15 cm. (Table 7). The lower pH's toward the surface suggest the acidity did not come from the parent material but from smelter-acidifying effects. High levels of Zn, Mn, Fe, Cu, and Al (Table 9) result from soil acidification and solubilization of these elements. The high acidity combined with the low amounts of P, K, Ca, and Mg give low base saturation (Table 7), as low as 11% at a depth of 20 cm., which is indicative of a stressed habitat. One surface sample taken from the valley floor of the Butte site indicated that organic matter, Ca, and Mg tended to increase and acidity tended to decrease in this area where the oldest aspen were found.

Landscape position in the center of the glaciated valley, high water table, deep soil, and high levels of the macronutrients P, K, Ca, and Mg (Table 8) combine to make Cinnabar the most productive of all three sites. Even though the soil contains much sand (57 to 70%), it also contains 25-28% silt (Table 6) and high organic matter (7.2% at 8 cm, Table 7). The high amount of organic matter may reflect the moist and productive conditions in this older forest. The soil

shows medium acidity at the surface (Table 7) due to the influence of organic matter and becomes slightly acid then neutral with increasing depth. Percent base saturation increases from 63.43% on the surface to as high as 93% at a depth of 94 cm. (Table 7). In general the micronutrients (Table 9) levels are much below that of Butte and there is an extreme disparity in copper levels which reach 142 ppm in Butte soil and are not found above 0.6 ppm for Cinnabar.

The soil parameters of the Teton site are typically intermediate between those of the Butte and Cinnabar sites. Even though the soil is relatively fertile as evidenced by reasonably high levels of macronutrients (Table 8) in the silt-rich soil, the upland location makes this a relatively dry site and therefore not as productive as the Cinnabar site. Soil characteristics suggest that the area has been covered by forest for a long while. The soil, a deep loess, is almost 80% silt (Table 6) near the surface. Organic matter, 2.4% at the surface, is as expected for forest soils and decreases with depth (Table 7). The soil is slightly acid at the surface, probably due to the organic matter but becomes almost neutral with depth (Table 7). Percent base saturation is relatively high 71-83% (table 7) and changes little with depth indicating a fertile soil. Micronutrients are comparable to those found at Cinnabar (Table 9) except that copper increases with depth.

Table 6. Morphological properties used to determine classification of soils on sites.

Horizon	depth in cm	color	structure	% sand	% silt	% clay
Butte						
A1	8	2.5Y 3/2	1 f, m, gr	82.0	15.3	2.8
A2	15	5Y 4/2	1 f, m, gr	81.3	13.5	5.2
Ab/Bw	20	10Y 3/3	1 f, gr, sbk	70.8	20.1	9.1
	58			74.2	17.6	8.2
	97			84.1	6.0	9.8
	97+			84.1	6.1	9.8

Soil type: Typic Cryorthent

				% sand	% silt	% clay
Teton						
A1, A2	15	10YR 3/2	1 f, gr	14.6	77.3	8.0
BA	36	10YR 4/3	1 m, gr, sbk	11.7	79.1	9.2
	56			11.7	79.1	9.2
Bw	84	10YR 4/3	2 f, m, sbk	11.4	67.1	21.4
	122			41.8	44.0	14.2
C	122+			55.6	36.5	7.9

Soil type: Typic Cryochrept

				% sand	% silt	% clay
Cinnabar						
A1	8	10YR 3/3	2 f,m, gr	65.4	27.4	7.2
	15			63.0	28.0	9.0
A2	20	10YR 4/3	1 f, gr, sbk	57.6	31.0	11.5
	33			64.4	25.1	10.5
C	43	10YR 4/3	0-M	67.0	25.8	7.2
	58			69.5	24.3	6.2
	94			67.4	24.0	8.5
	117			65.6	26.6	7.8
	119			65.4	27.2	7.3
	160			87.5	11.0	1.4

Soil type: Typic Cryumbrept

f = fine; m = medium; gr = granular; sbk = subangular blocky
0 = no structure, 1 = weak; 2 = moderate structure

Table 7. Chemical characteristics used to determine taxonomy of soil on sites.

depth in cm	pH	exchangable nutrients* Ca	Mg	K	H	%OM	%base sat.	CEC
Butte								
8	5.26	1.46	0.31	0.60	2.80	1.9	45.84	0.46
15	4.30	1.04	0.22	0.50	9.80	3.2	15.22	0.15
20	4.46	1.53	0.27	0.50	18.00	4.8	11.33	0.11
58	4.95	3.03	0.41	0.44	10.20	2.7	27.56	0.28
97	5.58	5.92	0.50	0.31	2.00	2.1	77.09	0.77
97+	5.74	4.23	0.60	0.20	2.80	1.2	64.24	0.64
Teton								
15	5.50	8.00	1.02	1.20	2.00	2.4	83.63	0.84
36	6.01	6.37	0.92	1.00	3.00	1.7	73.43	0.73
56	6.06	5.92	0.96	1.00	3.20	1.2	71.12	0.71
84	6.28	10.20	1.90	1.20	2.80	1.0	82.61	0.83
122	6.26	7.90	1.62	1.00	2.80	0.7	78.98	0.79
122+	6.23	5.02	1.02	0.90	2.00	0.7	77.63	0.78
Cinnabar								
8	5.46	12.60	3.30	1.10	9.80	7.2	63.43	0.63
15	5.70	11.80	3.00	1.20	8.40	5.7	65.57	0.66
20	5.80	13.70	3.40	1.20	10.00	5.5	64.66	0.65
33	6.04	11.10	3.00	1.20	3.40	3.6	81.82	0.82
43	6.02	10.60	3.10	1.40	4.20	3.3	78.24	0.78
58	6.40	7.40	3.00	1.50	2.00	1.1	85.61	0.86
94	6.45	6.89	3.00	1.40	0.80	1.1	93.38	0.93
117	6.50	7.30	3.30	1.40	1.60	0.9	88.24	0.88
119	6.60	6.98	3.20	1.30	1.00	0.9	91.99	0.92
160	6.50	4.71	2.00	0.80	1.60	0.7	82.55	0.83

* cmol. charge/kg. soil; extraction with ammonium acetate

Table 8. Soil macronutrients of sites in ppm*.

depth	P	K	Ca	Mg
Butte				
8 cm.	62	220	404	53
15 cm.	54	141	252	34
20 cm.	94	131	386	37
58 cm.	63	143	803	61
97 cm.	40	105	1377	68
97+ cm.	60	84	1088	86
Teton				
15 cm.	62	257	1502	111
36 cm.	56	230	1190	105
56 cm.	62	211	1032	97
84 cm.	113	222	1523	164
122 cm.	32	196	977	124
122+ cm.	28	162	708	91
Cinnabar				
8 cm.	78	256	2048	303
15 cm.	67	220	2128	312
20 cm.	64	198	2260	332
33 cm.	63	268	2163	352
43 cm.	78	371	2095	351
58 cm.	130	437	1487	312
94 cm.	119	462	1360	295
117 cm.	149	434	1383	316
119 cm.	163	389	1348	299
160 cm.	167	264	1134	209

*Figures include exchangeable and acid-soluble nutrients, resulting from double-acid extraction.

Table 9. Soil micronutrients in ppm*.

Depth	Zn	Mn	Fe	Al	Cu	B
Butte						
8 cm.	10.7	48.4	31.6	155	43	0.7
15 cm.	7.7	14.0	72.4	264	116	0.7
20 cm.	19.7	33.9	35.2	588	142	0.1
58 cm.	72.9	101.9	9.9	388	3.5	0.1
97 cm.	1.7	7.0	3.2	71	0.3	0.1
97+ cm.	0.6	9.6	5.0	58	0.3	0.1
Teton						
15 cm.	3.3	45.2	15.8	128	0.5	0.4
36 cm.	2.6	32.6	14.2	117	0.7	0.2
56 cm.	1.9	27.6	19.1	111	1.3	0.1
84 cm.	1.3	15.3	28.1	157	3.8	0.8
122 cm.	0.8	4.5	20.3	133	1.9	0.1
122+ cm.	3.7	5.7	21.4	124	13.8	0.03
Cinnabar						
8 cm.	4.7	29.9	12.3	135	0.2	0.4
15 cm.	3.6	21.4	10.2	138	0.2	0.4
20 cm.	3.5	18.6	9.3	138	0.2	0.4
33 cm.	1.3	14.8	14.0	150	0.2	0.3
43 cm.	1.3	12.8	14.0	141	0.2	0.3
58 cm.	0.6	9.8	22.3	131	0.4	0.1
94 cm.	0.6	10.6	25.2	122	0.6	0.1
117 cm.	0.6	11.3	26.3	118	0.6	0.1
119 cm.	0.4	11.4	24.9	116	0.5	0.1
160 cm.	0.7	10.1	21.8	103	0.5	0.1

*Figures include exchangeable and acid-extractable nutrients, resulting from double-acid extraction.

Chapter 2: A COMPARISON OF ECTOMYCORRHIZAL COMMUNITIES FOUND IN THREE POPULUS TREMULOIDES STANDS OF VARYING CONDITIONS AND SOIL TYPES

INTRODUCTION

Populus tremuloides is an adaptable species. It is widely distributed in North America (Little 1971) and is found in diverse habitats on many types of soil. P. tremuloides is predominantly ectomycorrhizal (McDougal and Jacobs 1927; Thomas 1943; Hackskaylo and Vozzo 1971) although it has been reported to be both ecto- and endomycorrhizal (McDougal and Jacobs 1927). Popular field guides and scientific papers (Mitchel and Smith 1978; Watling 1969; Godbout and Fortin 1985) seem to suggest that P. tremuloides may be associated with a plethora of mycosymbionts. Other species of Populus are apparently associated with a variety of symbionts (Trappe 1962; Heslin and Douglas 1986; Anselmi, Pirazzi, and Giorcelli 1990). However, none of these studies have addressed the ecology or community structure of ectomycorrhizal fungi, possibly because aspen is traditionally considered to be a short-lived pioneering species, quickly serel to conifers, and of little economic importance. Aspen trees are often found in marginal habitats where the fruiting period of ectomycorrhizal fungi is brief, and dense understories often preclude easy

observation of fungi.

Individual tree species are known to have their own characteristic mycorrhizal flora and these communities are quite variable (Trappe 1977; Wilkins, Ellis, and Harley 1937; Fleming, Deacon, and Last 1985). Shifts in the composition of the ectomycorrhizal community have been correlated with specific preferences by fungal species for certain habitat conditions such as soil factors (Tyler 1985; Jansen and Dighton 1990; Kelley 1941; Slankis 1974), amount of litter accumulation (Last, Dighton, and Mason 1987), tree age (Jansen and Dighton 1990), soil moisture (Boucher and Malajczuk 1990), and temperature (Marx, Bryan, and Davey 1970). Catastrophic events such as fire may also change the assemblage of mycorrhizal fungi (Singer 1971). In conifer sites in Western Montana, a majority of the ectomycorrhizae occur in the soil organic layer (Harvey, Larsen, and Jurgensen 1976 and 1979). However, not much is known about the influence of organic matter on individual fungal species occurrence. Tyler (1985) found a correlation between the presence of certain fungal species and the amount of organic matter and percent ion saturation (K + Mg + Ca + Mn / K + Mg + Ca + Mn + H).

Aspen occur on a wide array of soils, often in pure stands, and these circumstances facilitate studies of differences in the species composition of ectomycorrhizal fungal communities. This study recorded the ectomycorrhizal

fungal species found on three aspen-covered sites in the north central Rocky mountains at 45° latitude, 110-112° longitude and elevations ranging from 6300′ to 6700′ along with such soil characteristics as percent organic matter, pH, nutrient concentrations, and particle size distribution. This is the first report of a systematic study of aspen mycorrhizae on sites of variable age, conditions, and widely differing soil types. A knowledge of the fungal species on each of these soils is important in beginning to understand the effects of soil conditions on the composition of the ectomycorrhizal flora.

METHODS AND MATERIALS

ANALYSIS OF ECTOMYCORRHIZAL COMMUNITIES

Three sites containing nearly pure aspen stands and of different soil types were chosen for the study (see Chapt.1 for site descriptions). Sporocarps were collected every 14±6 days from May through August in 1990 and 1991, an average of 11 times per site. Collecting was done in a nonmethodical manner, covering as large an area as possible in order to maximize the number of species observed on the large sites. Sporocarps were photographed, tissue-cultured (Chapter 3: methods and materials), spore-printed, described, and dried.

References used for identification are starred in the bibliography. Selected voucher specimens reside in the VPI and SU herbarium. Collection numbers and dates are listed in the Appendix.

A phenetic analysis (Fig. 6a) was done to determine the relationship of the ectomycorrhizal communities on the three sites using the NT-SYS package of multivariate statistical program developed by Rohlf (1988). Characters used in the analysis are the ectomycorrhizal species listed in Table 10. The two possible character states are: 1 = presence on a site; 0 = absence from a site. An eigenvector analysis (Table 12) determined the relative importance of each character in distinguishing communities.

SOIL ANALYSIS

Soil samples taken from pits excavated on each site were analyzed (see Chap.1: Methods). Figure 7 is a synopsis of the soil analysis. Values for the synopsis were calculated by averaging parameter measurements from all soil samples collected on each of the sites and displaying them in ratio form.

The character state matrix shown in Table 13 was used to compare soil characteristics on the sites. Actual data are from the analysis of soil samples taken from pits on the three

sites. These results are given in Chapter 1. The physical characteristics of the three soils are given in Table 6, the pH, %OM and % base saturation measurements are listed in Table 7, macronutrients in Table 8, and micronutrients in Table 9. Information from soil samples taken at three comparable depths was used to determine the phenetic relationship of the three soils (Fig. 6b) using the NT-SYS package of multivariate statistical program developed by Rohlf (1988). An eigenvector analysis (Table 14) lists the importance of each character in determining the phenogram. An interval similarity matrix was used for the soil phenogram and both eigenvector analyses. A qualitative similarity matrix was used for the analysis of the ectomycorrhizal communities. An r value pertaining to the goodness-of-fit was determined for each phenogram.

RESULTS AND DISCUSSION

The putatively ectomycorrhizal species found on each of the aspen-covered sites are listed in Table 10. The frequencies shown in Table 10 reflect the number of times a species was collected, but does not represent a quantitative survey. A total of 39 ectomycorrhizal species were observed on the sites. Twenty-five species were recorded at Cinnabar, 18 at Butte, and 17 at Teton (part A, Table 11). Since the number of

Table 10. Species of ectomycorrhizal fungi associated with P. tremuloides on the three study sites*

	BUTTE	CINNABAR	TETON
AMANITACEAE			
Amanita constricta Theirs and Ammirati			+
Amanita fulva (Schaeff.)per Pers.			+
Amanita muscaria var. formosa (Pers. per Fr.)Bertillon	+	++++	++
Amanita pantherina (DC. per Fr.)Krombh.		++	
Amanita vaginata (Bull. per Fr.)Vitt.		+	++
RUSSULACEAE			
Lactarius controversus (Fr.)Fr.	+	+++	+
Lactarius pubescens Fr.			++
Russula aeruginea Lindbl. ex Fr.			+
Russula alutacea (Pers. ex Fr.)Fr.	+	++++	+
Russula claroflava Grove	+		
Russula xerampelina (Schaef. ex Secr.)Fries		+	
TRICHOLOMATACEAE			
Laccaria laccata (Scop.:Fr) B. & Br.	+++		
Laccaria tortilis (Bolt.) Cooke	++	++++	
Tricholoma terreum (Schff. ex Fr.)Kummer		+++	
CORTINARIACEAE			
Cortinarius trivialis Lge.	++	++	++++
Cortinarius sp 1			++
Cortinarius sp. 2		+	
Cortinarius sp. 3		++++	
Hebeloma mesophaeum (Fr.)Quel.	+++	+++	
Hebeloma sinapizans (Paulet ex Fr.)Gill.		+	
Hebeloma sp.1		+++	
Hebeloma sp.2		++	
Inocybe rimosa (Bull:Fr.)Kummer		+++	+
Inocybe geophylla (Fr.:Fr.)Kummer	+		
Inocybe lacera (Fr.:Fr.)Kummer	+++		
Inocybe sp.1	+++		
Inocybe sp.2	+		
Inocybe sp.3		++	+
Inocybe sp.4		+	
Inocybe sp.5			+
Inocybe sp.6	+		+
PAXILLACEAE			
Paxillus vernalis Watling	+++++	+++	+
BOLETACEAE			
Chalciporus piperatus (Bull. ex Fries)Singer		+	+
Leccinum aurantiacum (Bull. ex St.Amans) s.F.Gray	++++	+++++	+++++
Leccinum insigne Smith, Thiers & Watling		++	++
Phylloporus rhodoxanthus (Schw.)Bres.	+		
Xerocomus subtomentosus Fr.	+		
THELEPHORACEAE			
Thelephora terrestris Ehrh. ex Fries	++		
PEZIZACEAE			
Geopora cooperi Harkness (mycorr?)			++

species doubled from the first to the second collecting season the species list is probably not complete. Usually at least 4 collecting seasons are needed to record all or a high percent of all fungi present (Bills 1985). However, the fungi that occurred most frequently on each site were the same for both collecting seasons. The fact that 39 putative symbionts were found on such a small part of the extensive range of P.tremuloides suggests that aspen may potentially be associated with an extensive mycorrhizal flora. Godbout and Fortin (1985) found P. tremuloides was capable of forming mycorrhizae with a large number of symbionts, i.e. 29 of 54 fungal species tested . This contrasts with Alnus, a tree restricted in habitat and having a limited ectomycorrhizal flora (Molina 1979; Brunner, Brunner and Miller 1990; Miller and Koo 1991). The significance of a diverse ectomycorrhizal flora on the ability of a tree to occupy various habitats has not been well studied.

FUNGI FOUND ON ALL SITES

Of the 39 species collected, only Amanita muscaria v. formosa, Cortinarius trivialis, Lactarius controversus, Leccinum aurantiacum, Paxillus vernalis and Russula alutacea were found on all 3 sites (part B, Table 11). The five other

than R. alutacea, are common associates of aspen and birch as the following citations attest. P. vernalis is associated with aspen and birch in sandy soil in Michigan (Watling 1969). C. trivialis and L. controversus are found with P. tremuloides in Colorado (Mitchel and Smith 1978) and with both aspen and birch in Germany (Kreisel 1987). L. aurantiacum is associated with P. tremuloides in the Pacific Northwest (Trappe 1962) and with aspen in Michigan (Smith and Thiers 1971). A. muscaria has been found with P. tremuloides in Canada (Godbout and Fortin 1985) and with birch in Greenland (Elborne and Knudsen 1990). Leccinum aurantiacum and C. trivialis were found on three very different soil types in the present study and are reported to occur on many types of soil in Germany (Derbsch and Schmitt 1987). Another ectomycorrhizal fungus that was found on all three sites, but is not listed in Table 10, is Cenococcum sp. It was present in 88% of root samples collected randomly from all three sites.

Although these six species occurred on all three sites, their frequency differed greatly between sites. L. controversus and A. muscaria were observed more frequently at Cinnabar, C. trivialis was more frequent on the Teton site and P. vernalis on the Butte site. Only the ubiquitous L. aurantiacum fruited in quantity on all sites. It occurred in many areas of the Teton and Cinnabar sites but was restricted to the NW slopes and valley bottom in Butte. It did not occur

Table 11. Synopsis of ectomycorrhizal fungi associated with P. tremuloides on the study sites.

A. Number of species occurring on each site.

	Butte	Cinnabar	Teton
species collected in 1990	10	15	7
new species collected in 1991	8	10	10
total of species collected	18	25	17

B. Species found on all sites.

Amanita muscaria v. formosa
Cortinarius trivialis
Lactarius controversus
Leccinum aurantiacum
Paxillus vernalis
Russula alutacea

C. Characteristic species: species observed most frequently

Butte	Cinnabar	Teton
Inocybe lacera	Amanita muscaria	Cort. trivialis
Laccaria laccata	Leccinum aurantiacum	L. aurantiacum
L. aurantiacum	Hebeloma mesophaeum	
Paxillus vernalis	H. sp. # 1	
	Lactarius controversus	

D. Exclusive species: species restricted to one site.

Butte	Cinnabar	Teton
Inocybe geophylla	Amanita pantherina	Amanita constricta
I. lacera	Cortinarius sp. #2	A. fulva
I. sp. #1	C. sp. #3	Cortinarius sp. #1
I. sp. #2	Hebeloma sinapizans	Geopora cooperi
I. sp. #3	H. sp. #1	Inocybe sp. #5
Laccaria laccata	H. sp. #2	L. pubescens
Phylloporus rhodoxanthus	I. sp. #4	Russula aeruginea
Russula claroflava	Russula xerampelina	
Thelephora terrestris	Tricholoma terreum	
Xerocomus subtomentosus		

on Butte's SW-facing slopes.

ECTOMYCORRHIZAL FUNGI MOST COMMONLY OBSERVED ON EACH SITE

Of the ectomycorrhizal fungi commonly observed at Butte (part C, Table 11), the most acidic and least fertile site, only I. lacera and L. laccata were found on the dry, open, sw-facing slopes in the sandy washes with 40-year-old aspen clones (Fig. 5a). These were the youngest clones studied on the three sites (Table 2). L. laccata also occurred on the nw-facing slopes in areas free of litter (Fig. 5b). Both fruited regularly and in great abundance after the spring rains of May and June. Neither of these fungi occurred on the valley bottom where the soil is more developed and the aspens are up to 66 years old. Inocybe lacera has previously been observed with aspen on sandy soil (Smith 1979), with various trees in nutrient poor sand (Kuyper 1986), with aspen and birch on coalspoils (Schramm 1966), and with birch on acid sandy soil (Derbsch and Schmitt 1987). Neither species occurred on the valley floor. Paxillus vernalis was the most frequently observed fungus that occurred at Butte throughout the summer. It fruited on the valley floor and on the NW-facing slopes in gullies where the trees are older than those on the SW-facing slopes. Paxillus vernalis was rare on the other sites. It occurred only in the uplands on the Cinnabar site and only

one specimen was found in a sandy area on the Teton site. The species was described by Watling (1969) as occurring with aspen and birch in sandy soil as at Butte. The closely related P. involutus has been observed in many types of soil but seems to prefer somewhat acidic and xerothermic soils (Derbsch and Schmitt 1987; Kreisel 1987).

On the Teton site, which consists of uplands covered with a relatively fertile loess, Leccinum aurantiacum and Cortinarius trivialis, common associates of aspen, were observed more often than other species. L.aurantiacum fruited early in open areas at clone perimeters and later in the dense canopy beneath older trees. C. trivialis occurred singly or in small groups throughout the summer. Both L. aurantiacum and C. trivialis are reported to have no preference for soil type by Derbsch and Schmitt (1987), but Kreisel (1987) reports C. trivialis to occur especially on siliceous and limy soils. It was rare on the Butte and Cinnabar sites. Four species of Amanitas were found on the Teton site, which is more than were found on the other two sites. The Russulas and Lactarii present on the Teton site were observed in one of the older aspen stands on the site; the oldest tree in the vicinity was 69 years old. Amanitas, Cortinarii, Russulas and Lactarii are considered, in general, to be late colonizers (Jansen and Dighton 1990); this will be discussed further in the section on exclusive species. Chalciporus piperatus was found one

time in great profusion in moss near a sandy seep beneath pure aspen.

Even though it is the smallest site, Cinnabar is the most productive in terms of nutrients, OM, and moisture. A variety of species were common on the Cinnabar site (part C, Table 11). The two most common species were Leccinum aurantiacum and A. muscaria v. formosa (Table 10), which fruited together in great abundance on both years. Although A. muscaria has been reported to be associated with aspen (Godbout and Fortin 1985), this variety has not been mentioned in the literature in conjunction with aspen. Jenkins (1986) lists the habitat of A. muscaria v. formosa as mixed deciduous and coniferous forests. It was only observed once in Butte in the valley bottom where the soil is more developed and twice on the Teton site near a sandy seep. Lactarius controversus fruited regularly at Cinnabar in a moist area beneath dense canopy. It has been reported with old Populus in loamy soil in Europe (Derbsch and Schmitt 1987). It was observed once at Butte in the well-developed litter layer in the valley bottom and once on the Teton site. Hebeloma mesophaem fruited in the spring and fall in troops at Cinnabar. The variety H. mesophaeum v. aspenicola has been reported with aspen in Colorado (Smith, Everson, and Mitchel 1983). Several species of Hebelomas were found on the Cinnabar site, but they were noticeably sparse on the other sites (Table 10). Fruit body production was elevated

by the moist conditions on the Cinnabar site.

SPECIES EXCLUSIVE TO BUTTE

One of the most informative comparisons of the ectomycorrhizal communities is of fungal species restricted to one site, termed exclusive species (part D, Table 11). Russula Claroflava, X. subtomentosus, P. rhodoxanthus and L. laccata were observed on the NW-facing slopes of Butte at the clone perimeter where aspen trees are up to 56 years old (Fig. 5b). Xerocomus subtomentosus has previously been reported with aspen (Godbout and Fortin 1985), as has I. lacera (Smith 1979; Schramm 1966), and R. claroflava (Bills and Miller 1984). Four of the species found in the acid sandy soil of Butte, I. lacera, P. rhodoxanthus, R. claroflava and X. subtomentosus are found in acid sandy soils in Europe (Derbsch and Schmitt 1987; Kreisel 1987). Xerocomus subtomentosus has an "in vitro" pH optimum of 3.7-4.0. (Hung and Trappe 1983). Likewise, Thelephora terrestris, found only at Butte during this study, has been found on acidic coal spoils (Derbsch and Schmitt 1987; Schramm 1966). All of these species seem to have a requirement for acidic soil or are able to tolerate low pH. The pH of the Butte soil where these species were found ranged from 4.3 to 5.74.

A large body of evidence exists which shows that over time

a "succession" of mycorrhizal fungi associate with trees (Last, Dighton, and Mason 1987; Last, Mason, Wilson, and Deacon 1983; Last, Pelham, and Ingleby 1982; Mason, Wilson, and Last 1984). "Early-stage fungi" are considered to be pioneering R-strategists which colonize roots quickly and are often stress tolerant. "Late-stage fungi" appear in mature forests and are considered to be K-strategists (Jansen and Dighton 1990). Whatever the explanation, whether it be tree age or soil conditions, these two groups of fungi have different adaptive strategies. In the present study, Laccaria, Thelephora, Paxillus and Inocybe spp. were recorded at Butte; these genera are considered to be "early colonizers" on birch (Last et. al.1987). Succession studies on Betula, Pinus, and Picea are summarized as follows by Dighton, Poskitt, and Howard (1986): Thelephora, dominant in nurseries; Hebeloma spp., Laccaria spp., and Inocybe spp., dominant in trees 2-5 years old; Laccaria and Paxillus, dominant in 5-10 year old trees; Cortinarius, Inocybe, and Leccinum, dominant in 10-15 year old trees; Russula, Lactarius, and Amanita dominant in 15-20 year old trees. Most studies on mycorrhizal succession consider "early colonizers" to be those forming mycorrhizae within 1-6 years of tree planting and "late colonizers" to be those occurring after 6-10 years (Dighton, Poskitt, and Howard 1986; Jansen and Dighton 1990). The average maximum stand age on the sw-facing slopes of Butte where I. lacera and L.

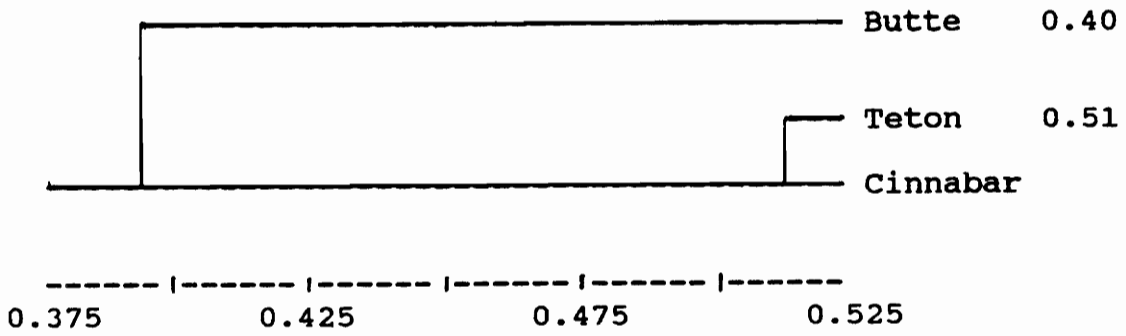
laccata were observed is 36 years, younger than clones on other areas of Butte and on other sites but significantly older than the 2-10 year old birch, pine and spruce stands that have been studied. Although "early colonizers" were found at Butte and "late colonizers" were found on the Teton and Cinnabar sites, the ages of the clones on the 3 sites in the present study are much older than those of other studies. Plants in the studies summarized by Jansen and Dighton (1990) are from outplants of nursery stock. The ectomycorrhizal fungi recorded in the present study were observed in naturally occurring forests. In additon, Butte is a stressed site recovering from the devastation of smelter fumes, and maturation of the soil may be delayed.

It is interesting to note that root systems of the young trees at Butte are much more extensive than those of young trees growing on the other two sites. At Teton and Cinnabar the young trees are attached to the large feeder roots of the older trees and are delayed in developing their own root systems.

PHENETIC ANALYSIS OF ECTOMYCORRHIZAL COMMUNITIES

A phenetic analysis (Fig. 6) indicated that the ectomycorrhizal community found at Butte is the most distinctive of the three sites. An eigenvector analysis (Table

a.



b.

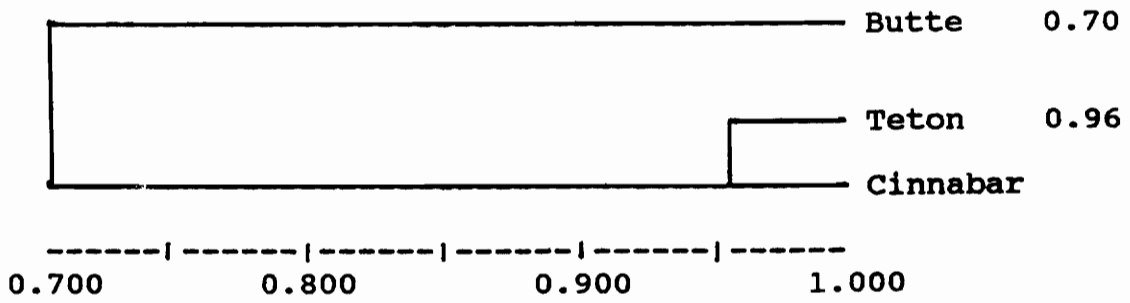


Figure 6. a) Phenogram showing the relationship between 3 ectomycorrhizal communities associated with P. tremuloides. r = 0.98. b) Phenogram showing the relationship between the soils of three aspen-covered sites. r = 0.99.

12) revealed that those species found exclusively on the Butte site plus absence of C. piperatus, A. vaginata, I. rimosa, and I. insigne are the characters which distinguished it from the other sites (high absolute vector values).

The distinction between the mycorrhizal communities of Cinnabar and Teton is less defined. More Amanitas and Cortinarii occurred on these two sites than at Butte, but species varied between sites (Table 10). Both groups, are considered to be "late stage fungi" by Last, Dighton, and Mason (1987), who hypothesized that "late stage colonizers" may be more host specific than "early colonizers". Last et.al. (1987) reported Amanitas likely to be found in soils with high recalcitrant litter and Wilkins, Ellis, and Harley (1937) mention a study done by Maire et.al (1901) reporting some Cortinarii to prefer calcareous soil. Hebelomas were common on the Cinnabar site but absent from the Teton site. Harley (1937) thought that the occurrence of the genus Hebeloma was strongly influenced by soil type. Hebelomas fruit commonly in nurseries and are sometimes listed as early colonizers (Dighton et al 1986): which contrasts with this study where Hebelomas were found predominantly on the Cinnabar site associated with 80 year old trees, some of the oldest trees found on all three sites. Further study might determine if Hebelomas in general prefer soils high in organic matter, a condition that nurseries and the Cinnabar site have in common.

Table 12. Eigenvector analysis of ectomycorrhizal fungi found on the three sites.

	A*	B**	
Amanita constricta	0.580	-0.815	-0.000
A. fulva	0.580	0.000	0.000
A. muscaria	0.000	0.000	0.000
A. pantherina	0.416	0.910	0.000
A. vaginata	0.995	0.095	0.000
Lactarius controversus	0.000	0.000	0.000
L. pubescens	0.580	-0.815	-0.000
Russula aeruginea	0.580	-0.815	-0.000
R. alutacea	0.000	0.000	0.000
R. claroflava	-0.995	-0.095	-0.000
R. xerampelina	0.416	0.910	0.000
Laccaria laccata	-0.995	-0.095	-0.000
L. tortilis	-0.580	0.815	0.000
Tricholoma terreum	0.416	0.910	0.000
Cortinarius trivialis	0.000	0.000	0.000
C. sp. #1	0.580	-0.815	-0.000
C. sp. #2	0.416	0.910	0.000
C. sp. #3	0.416	0.910	0.000
Hebeloma mesophaeum	-0.580	0.815	0.000
H. sinapizans	0.416	0.910	0.000
H. sp. #1	0.416	0.910	0.000
H. sp. #2	0.416	0.910	0.000
Inocybe rimosa	0.995	0.095	0.000
I. geophylla	-0.995	0.095	-0.000
I. lacera	-0.995	-0.095	-0.000
I. sp. #1	-0.995	-0.095	-0.000
I. sp. #2	-0.995	-0.095	-0.000
I. sp. #3	0.995	0.095	0.000
I. sp. #4	0.416	0.910	0.000
I. sp. #5	0.580	-0.815	-0.000
I. sp. #6	-0.580	0.815	0.000
Paxillus vernalis	0.000	0.000	0.000
Chalciporus piperatus	0.995	0.095	0.000
Leccinum aurantiacum	0.000	0.000	0.000
L. insigne	0.995	0.095	0.000
Phylloporus rhodoxanthus	-0.995	-0.095	-0.000
Xerocomus subtomentosus	-0.995	-0.095	-0.000
Thelephora terrestris	-0.995	-0.095	-0.000
Geopora cooperi	0.580	-0.815	-0.000

* factors important in distinguishing Butte from other sites
** factors important in distinguishing Teton from Cinnabar

A phenogram (Fig. 6) confirms that the Teton and Cinnabar sites have much in common floristically. An eigenvector analysis (Table 12) reveals that characters distinguishing the 2 sites are the species exclusive to each site (part D, Table 11) plus Inocybe sp. #6 found on the Teton site and L. tortilis and H. mesophaeum found on the Cinnabar site.

PHENETIC ANALYSIS OF THE SOILS FOUND ON THE THREE SITES

A synopsis of trends in soil characteristics (Fig. 7) shows soil fertility to be low on the Butte site, high on the Teton site, and highest on the Cinnabar site. Elevated levels of the micronutrients Cu, Zn, Fe and Al are found at Butte. These levels decrease on the Teton site and are lowest on the Cinnabar site (see Chapt. 1 for details of the soil analysis, Tables 7, 8, and 9). A phenetic analysis (Fig. 6b) of the chemical characteristics shown in the soil summary plus the physical characteristics of the soils (Table 6) shows that like the analysis of ectomycorrhizal communities, the Butte site is distinct from the other two sites. The character matrix (Table 13) gives the measurements of the parameters used in the phenetic analysis. An eigenvector analysis (Table 14) shows that the most important distinguishing characteristics of the Butte soil are low pH; organic matter that is distributed with depth (organic matter is measured

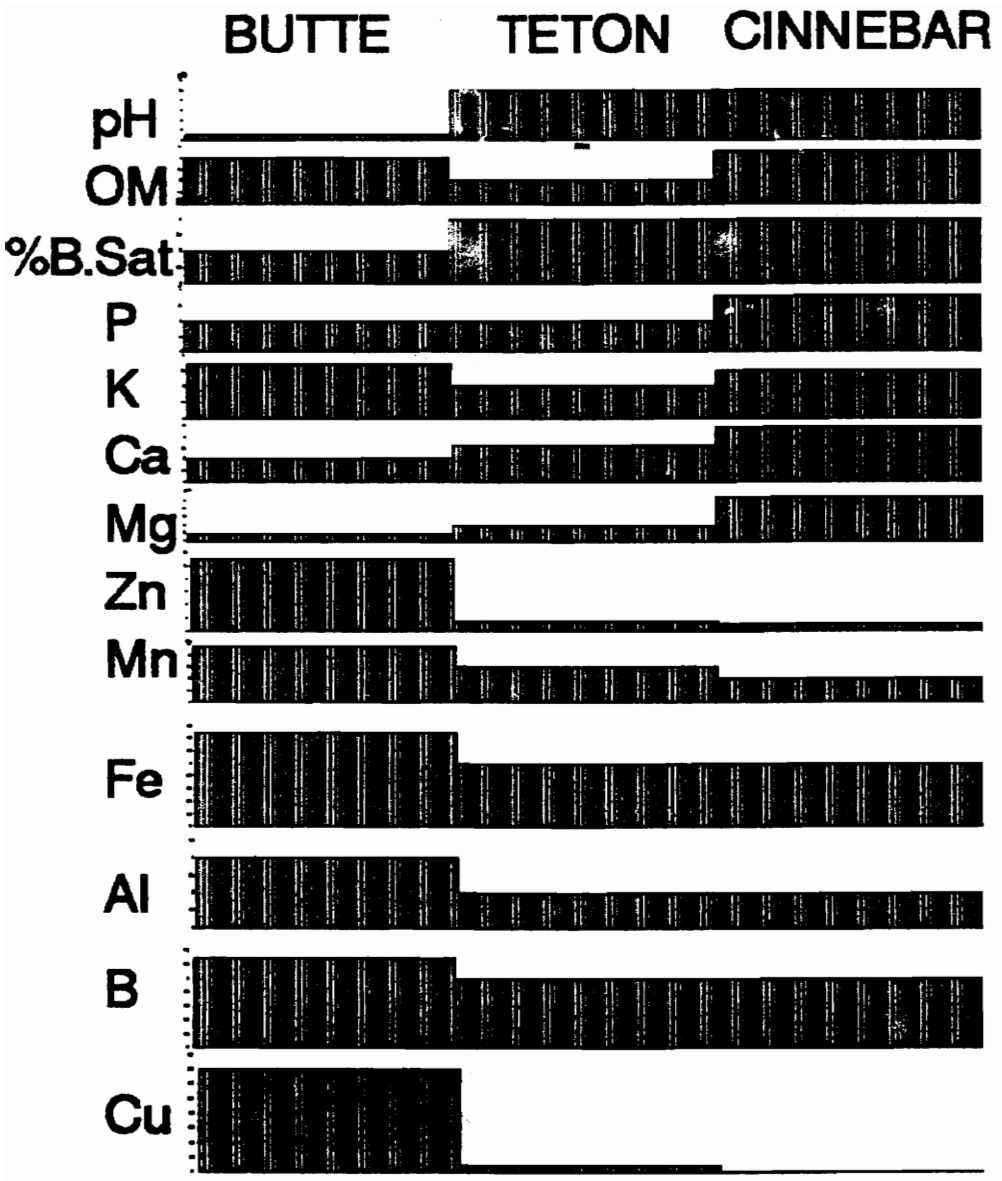


Figure 7. Macronutrient and micronutrient levels of soils found on the three sites, showing trends in relative amounts.

Table 13. Character state matrix used in phenetic analysis of soil variables by site.

	Butte	Teton	Cinnabar
%sand at depth 1*	81.0	14.6	63.0
%sand at depth 2**	74.2	11.7	69.5
%sand at depth 3***	84.1	11.4	67.4
%silt at depth 1	13.5	77.3	28.0
%silt at depth 2	17.6	79.1	24.3
%silt at depth 3	6.0	67.1	24.0
%clay at depth 1	5.2	8.0	9.0
%clay at depth 2	8.2	9.2	6.2
%clay at depth 3	9.8	21.4	8.5
pH at depth 1	4.30	5.50	5.70
pH at depth 2	4.95	6.06	6.40
pH at depth 3	5.58	6.28	6.45
%OM at depth 1	3.2	2.4	5.7
%OM at depth 2	2.7	1.2	1.1
%OM at depth 3	2.1	1.0	1.1
%base sat at depth 1	15.22	83.63	65.43
%base sat at depth 2	27.56	71.12	85.61
%base sat at depth 3	77.09	82.61	93.38
phosphorus at depth 1	54	62	67
phosphorus at depth 2	63	62	130
phosphorus at depth 3	40	113	119
potassium at depth 1	141	257	220
potassium at depth 2	143	211	437
potassium at depth 3	105	222	462
calcium at depth 1	252	1502	2128
calcium at depth 2	803	1032	1487
calcium at depth 3	1377	1523	1360
magnesium at depth 1	34	111	312
magnesium at depth 2	61	97	312
magnesium at depth 3	68	164	295
zinc at depth 1	7.7	3.3	3.6
zinc at depth 2	72.9	1.9	0.6
zinc at depth 3	1.7	1.3	0.6
manganese at depth 1	14.0	45.2	21.4
manganese at depth 2	101.9	27.6	9.8
manganese at depth 3	7.0	15.3	10.6
iron at depth 1	72.4	15.8	10.2
iron at depth 2	9.9	19.1	22.3
iron at depth 3	3.2	28.1	25.2
aluminum at depth 1	264	128	138
aluminum at depth 2	388	111	131
aluminum at depth 3	71	157	122
copper at depth 1	116	0.5	0.2
copper at depth 2	3.5	1.3	0.4
copper at depth 3	0.3	3.8	0.6
boron at depth 1	0.7	0.4	0.4
boron at depth 2	0.1	0.1	0.1

double-acid extractable nutrients in ppm

*depth 1: 15 cm on all sites
**depth 2: 58 cm at Butte and Cinnabar
56 cm at Teton
***depth 3: 97 cm at Butte
84 cm at Teton

Table 14. Eigenvector analysis of soil characteristics of 3 sites.

	A	B		
sand at depth 1*	0.694	0.720	0.000	
sand at depth 2**	0.537	0.844	0.000	
sand at depth 3***	0.659	0.752	0.000	
silt at depth 1	-0.657	-0.754	0.000	
silt at depth 2	-0.563	-0.826	0.000	A = factors important
silt at depth 3	-0.710	-0.704	0.000	in distinguishing Butte
clay at depth 1	-0.973	0.230	0.000	from other 2 sites
clay at depth 2	0.213	-0.977	0.000	
clay at depth 3	-0.267	-0.964	0.000	B = factors important
pH at depth 1	-0.994	0.108	0.000	in distinguishing the
pH at depth 2	-0.980	0.200	0.000	Teton and Cinnabar
pH at depth 3	-0.987	0.160	0.000	sites
OM at depth 1	-0.309	0.951	0.000	
OM at depth 2	1.000	-0.031	0.000	
OM at depth 3	0.994	0.107	0.000	
%base sat at depth 1	-0.961	-0.278	0.000	
%base sat at depth 2	-0.976	0.216	0.000	
%base sat at depth 3	-0.775	0.623	0.000	
phosphorus at depth 1	-0.934	0.358	0.000	
phosphorus at depth 2	-0.510	0.860	0.000	
phosphorus at depth 3	-0.999	0.044	0.000	
potassium at depth 1	-0.942	-0.336	0.000	
potassium at depth 2	-0.697	0.717	0.000	
potassium at depth 3	-0.768	0.641	0.000	
calcium at depth 1	-0.953	0.304	0.000	
calcium at depth 2	-0.773	0.635	0.000	
calcium at depth 3	-0.988	-0.156	0.000	
magnesium at depth 1	-0.731	0.682	0.000	
magnesium at depth 2	-0.630	0.777	0.000	
magnesium at depth 3	-0.823	0.554	0.000	
zinc at depth 1	-0.459	-0.889	0.000	
zinc at depth 2	1.000	-0.008	0.000	
zinc at depth 3	0.394	-0.919	0.000	
manganese at depth 1	-0.665	-0.747	0.000	
manganese at depth 2	0.987	-0.158	0.000	
manganese at depth 3	-0.811	-0.585	0.000	
iron at depth 1	0.998	-0.057	0.000	
iron at depth 2	-0.974	0.225	0.000	
iron at depth 3	-0.991	-0.131	0.000	
aluminum at depth 1	0.996	0.091	0.000	
aluminum at depth 2	0.996	0.089	0.000	
aluminum at depth 3	-0.904	-0.427	0.000	
copper at depth 1	1.000	0.022	0.000	
copper at depth 2	0.966	-0.258	0.000	*depth 1: 15 cm on all sites
copper at depth 3	-0.545	-0.839	0.000	**depth 2: 58 cm at Butte and Cinnabar
boron at depth 1	1.000	0.025	0.000	56 cm at Teton
boron at depth 2	0.000	0.000	-1.000	***depth 3: 97 cm at Butte
boron at depth 3	-0.478	-0.878	0.000	84 cm at Teton, 94 cm at Cinnabar

only in the fine fraction of soil and would be lower if the large coarse fraction is taken into consideration); lower percent base saturation; elevated levels of Al, Fe, Cu, Zn; and to some degree, lower Ca, P, and Mg levels.

The present study can give no direct evidence that differences in certain soil parameters influence species composition. This is due to the limited number of sites and because potential influences such as climate (Table 1), stand age (Table 2) and hydrology were only peripherally examined. However, it strongly suggests that soil factors influence the composition of an ectomycorrhizal community. Butte's exclusive species have been observed in Europe on acid, sandy soil; and all of these species were found in upland positions at Butte (except T. terrestris) where the soil is less developed and more acidic. Species such as A. muscaria and L. controversus, which were commonly observed in the productive Cinnabar soil, were rare at Butte and always occurred in the valley in the best soil conditions and with the older trees. Tree age is a potentially confounding factor. Researchers at the Institute of Terrestrial Ecology , UK are attempting to sort out the importance of tree age and soil factors.

Phenetic analysis (Fig. 6b) shows that the Cinnabar and Teton soils are closely allied (Fig.7). There are sporadic sample differences in some nutrients and OM, but the most consistent difference between the sites is particle size

distribution as shown by the eigenvector analysis (Table 14). The Teton soil contains a high percent silt and a fair amount of clay compared to Cinnabar's sandy soil. Landscape position was not considered in the analysis but may be an important difference between the two sites. The Teton site is situated in a well-drained upland position while the Cinnabar site is on the floor of a glaciated valley. The average age of the largest trees on the Cinnabar site is 81 years, 20 years older than the average of 61.5 years at Teton. Age, however, was determined by coring trees, and does not reveal the age of the clonal root system. It is possible that fires which swept the Teton area in 1910 and 1920 (see pg. 23) reached the Teton site and that the root system on this site is actually much older than that indicated by the age of stems.

FUNGI AS INDICATOR ORGANISMS

It has been historically difficult to link the presence of certain trees to soil type (Steele and Pfister 1991; Hironaka and Fosberg 1991). Besides broad climactic parameters, it is hypothesized that history and chance may most influence tree cover. Problems in soil classification may also complicate correlation. Attempts to correlate understory vegetation with soil type have been somewhat more successful. Some aspen understories in Michigan have been correlated with soil

characteristics (Roberts and Christensen 1987). After studying 70 aspen stands, they reported that the stands fell into 3 shrub-tree classifications and that each group was correlated with one of the following soil types: 1) sandy, low moisture, low nutrient availability; 2) sandy, wet, low nutrient availability; or 3) heavier-textured (clay to calcareous), wet, high nutrient availability. The understory plants in Roberts' study were not the same as those found on the three sites in the present study. The present study sites could be described in similar terms: 1) sandy, low moisture, low nutrient availability (plus elevated macronutrient levels and low pH), i.e. Butte; 2) sandy, wet, high nutrient availability, i.e. Cinnebar; and 3) silty, low moisture, high nutrient availability, i.e. Teton. Ectomycorrhizae are the direct link between trees and soil and show strong preferences for microhabitats and may be more sensitive to soil factors. Molina and Amaranthus (1991) state that recent studies have shown there is a strong correlation between microorganism activity in the soil and soil productivity. Tyler (1985) states that "the use of macrofungi as indicator organisms in environmental studies of soil acidification deserves future consideration."

Soil fertility is defined as the amount and proportion of nutrients available for plant growth. Productivity, a more comprehensive term, combines fertility with climate and

hydrology to assess the ability of a soil to produce a "crop". If "late stage" fungi such as Amanitas, Lactarii, Russulas, and Cortinarii are better adapted to soils with high organic matter and/or a high base saturation, then late stage colonizers could be considered indicative of a more productive site than early colonizers.

Percent base saturation (a measure of fertility) increases with decreasing acidity in acid to neutral soils. Tyler (1985) showed that percent OM and percent ion saturation accounted for most of the shifts in the composition of fungal communities on four plots in beech forests. He found a low correlation with percent clay, bulk-density, individual exchangeable cations, Al, and certain heavy metals. The present study found that the factors most consistently useful in distinguishing the Butte soil from that of other sites were pH and elevated levels of Fe, Cu, and Al. Acidity may be an important factor in fungal distribution. It is known that pH affects fungi preferentially (Slankis 1974; McAfee and Fortin 1987; Dighton and Skeffington 1987). Hung and Trappe (1983) showed that the optimum pH of ectomycorrhizal fungi varies between species "in vitro". Entry et al (1987) found that even when there was no change in Ca, N, K, and Mg, mycorrhizal formation by Hebeloma crustuliniforme was reduced when pH was lowered to 5 with increasing Al levels but mycorrhizal formation by C. geophilum and L. laccata were not.

In the present study, both Cenococcum sp. and L. laccata were found to be capable of tolerating the very acidic conditions on the sw-facing slopes of Butte where the pH dropped to 4.3. However, Cenococcum sp. was also found on the nearly neutral soils of the Teton and Cinnebar sites.

Jansen and Dighton (1990) state that evidence exists showing that the succession of mycorrhizal species is dependent on tree age but suggest that soil factors may ameliorate this. Blasius and Oberwinkler (1989) attempt to solve the tree age or soil dilemma by suggesting that stand age, not tree age is the crucial factor and that stand age changes simultaneously with substrate. Their paper states that Ricek (1981) studying stands of the same age, found early-stage mycorrhizal species in forested land that was previously meadow and late stage fungi on land that had been clearcut and returned to forest. These studies suggest that soil or other factors may override the normal succession. In these studies, below ground observation of mycorrhizae needs to be correlated with occurrence of fruit bodies on the sites to confirm that it is not fruiting conditions which are being correlated with environmental parameters. Cotter and Miller (1985) showed that there was a correlation between above ground fruit bodies and occurrence of below ground sclerotia. In the present study, occurrences of the same fungi over more than one field season helps to substantiate this.

SUMMARY AND CONCLUSIONS

1. Populus tremuloides is an adaptable species. It was found on diverse sites associated with at least 39 ectomycorrhizal species of fungi which varied by site.

2. Only Amanita muscaria v. formosa, Cenococcum sp., Cortinarius trivialis, Lactarius controversus, Leccinum aurantiacum, Paxillus vernalis, and Russula alutacea were found on all three sites although frequencies varied dramatically.

3. Each site had a unique assemblage of ectomycorrhizal species, and the same fungi were found dominant on each site in two field seasons.

4. Ectomycorrhizal species considered to be "early colonizers" such as Inocybe lacera and Laccaria laccata and acid-loving species such as Inocybe lacera, Phylloporus rhodoxanthus, Russula claroflava, Thelephora terrestris, and Xerocomus subtomentosus were only found in the acidic, relatively unfertile soil of the Butte site near the youngest trees on all three study sites.

5. More Amanitas and Cortinarii (and to some extent Russulas and Lactarii, and the only Tricholoma observed) occurred on the relatively fertile sites of Cinnabar and Teton in "older" Populus stands. These genera are considered to be "late

colonizers".

6. Hebelomas were common on the Cinnabar site but inexplicably absent from the Teton site.

7. "Late stage" ectomycorrhizal fungi not only occurred on the more fertile sites (Teton and Cinnabar), but their presence may possibly be of use as indicators of these kinds of sites.

8. Stress tolerant, acid-loving species and "early stage" ectomycorrhizal fungi not only occurred on the least fertile site (Butte), but may be of use in indicating this kind of site.

Chapter 3: ASEPTIC SYNTHESES OF SELECTED ECTOMYCORRHIZAL FUNGI AND P. TREMULOIDES

INTRODUCTION

Populus tremuloides, or quaking aspen, is found over a large part of North America and is predominantly ectomycorrhizal (Thomas 1943; Hackskaylo and Vozzo 1971; Malloch and Malloch 1981). Although anecdotal evidence such as field guides suggests a large number of ectomycorrhizal fungi may be associated with aspen, the ultimate proof that they are mycorrhizal with aspen is "in vitro" formation of mycorrhizae by the prospective partners.

Only one major study (Godbout and Fortin 1985) has attempted to clarify the mycorrhizal status of fungi associated with *P. tremuloides*. The 54 fungi used in their pouch study included 29 fungi actually associated with *P. tremuloides* in nature. The rest were pure cultures from fungi associated with six gymnosperms and eight other angiosperms. Some decomposers such as *Mutinus caninus*, *Lycoperdon perlatum*, and *Morchella conica* were also included. Mycorrhizae were formed by 19 of the fungi associated with aspen and 10 fungi associated with either birch, beech, alder or in which the host was unknown. The successful isolate of *Cenococcum*

geophilum was originally associated with Picea mariana, but other gymnosperm mycorrhizae were unsuccessful.

Synthesis experiments in Europe show that other species of Populus form mycorrhizae with Boletus rufus (Melin 1923), C. graniforme (Lihnell 1942), Hebeloma longicaudum and Hebeloma hiemale (Fontana 1961 and 1963), Tuber albidum (Fontana and Palalenza 1969), and Thelephora terrestris and P.involutus (Heslin and Douglas 1986). Anselmi, Pirazzi, and Giorcelli (1990) found 17 fungal species to be mycorrhizal with various species of Populus. These species are listed in the Literature Review.

The sterilization of aspen seed has always been a problem in synthesis experiments. Aspen seeds are tiny, fragile and prone to rapid desiccation. In nature, they are not dormant but germinate within a few days if a suitable habitat is found or they lose viability. Melin (1923) sterilized aspen seed with quicksilver (mercury), a substance now known to be toxic. Recent European workers surface sterilized minicuttings (Heslin and Douglas 1986), a lengthy and labor-intensive procedure. Godbout and Fortin (1985) germinated previously frozen aspen seeds on nutrient- enriched white sand for 30 days in nonsterile conditions before placing them in growth pouches. Seeds grown under nonaseptic conditions are a possible source of contamination if used in synthesis tubes. While the pouch method gives a rapid assessment of the ability

to form mycorrhizae, it is not amenable to long term growth or physiological studies.

One purpose of this study was to develop a method of sterilizing aspen seeds so that the ability of putative fungal symbionts to form mycorrhizae with seedlings might be easily tested in culture tubes under sterile conditions. The second goal of this study was to assess the ability of three broad-host range fungi, e.g. Cenococcum graniforme, Pisolithus tinctorius and Piloderma bicolor from the VT culture collection as well as the ability of six fungi isolated from sporocarps found in aspen stands in western Montana to form mycorrhizae with aspen in culture tubes.

METHODS AND MATERIALS

1. FUNGAL ISOLATES: Isolates of Pisolithus tinctorius (VT 1398), Cenococcum graniforme (VT 1009) and Piloderma croceum (VT 987) were obtained from the Virginia Tech culture collection. Amanita muscaria var. formosa (VT 2238), Amanita pantherina (VT 2239), Chalciporus piperatus (VT 2240), Inocybe lacera (VT 2241), Paxillus vernalis (VT 2242) and Tricholoma terreum (VT 2243) cultures were isolated from sporocarps collected in 1990 in nearly pure Populus tremuloides. The stands were located in southwestern Montana at elevations of 1900 to 2000 meters in the foothills of the northcentral Rocky Mountains (see Chap. 1 for complete site description).

Isolates are located in the Virginia Tech (VPI) culture collection and original sporocarps are located in the Virginia Tech Massey Herbarium (VPI) (see Appendix for voucher numbers and Table 10 for authorities).

Cultures were initiated by removing tissue from sporocarps according to the sterile technique described by Molina and Palmer (1982). Tissue isolates were grown on Hagem's media (Hagem 1910) modified by Cotter (1987, unpublished): 4 g malt extract, 1 g yeast extract, 5 g d-glucose, 0.5 g NH_4Cl_3, 0.5 g KH_2PO_4, 0.5 g $MgSO_4*7H_2O$, 0.5 ml $FeCl_3$ (1% aqueous), 100 ul biotin (50 mg/ml aqueous), 100 ul thiamine (1 mg/ml aqueous) and 15 g agar added to 1000 ml of distilled H_2O. Cultures were incubated in the dark at 20 C. until there was sufficient mycelial mass to inoculate liquid media. Twenty 5 mm squares of each kind of mycelium was transferred to a 250 ml. flask containing 75 ml. of liquid Cotter's modified Hagems using sterile technique. The flasks were incubated in the dark at room temperature until there was sufficient mycelium for tube inoculation.

2. SEED STERILIZATION AND GERMINATION: Seeds of Populus tremuloides Michx. seeds were obtained from the Northplan Seed Company in Moscow, Idaho. They had originally been collected on Red Mountain Pass in Ouray, Colorado and were used in all experiments. Seeds were stored in a dry container at 0° to 5° C in the dark. Although aspen seed is noted for rapid

decreases in viability over time (Zasada and Densmore 1977), this was not a problem for the duration of this experiment.

A new protocol was developed for the sterilization of aspen seed. Without sterilization an average of 99.2% of the seeds germinated but 98.3% of these were contaminated with fungi and bacteria. Attempts at sterilization with hydrogen peroxide were unsuccessful. High concentrations of H_2O_2 killed the fragile seeds or stunted radicle development, and low concentrations did not prevent contamination. Similarly, seeds soaked in H_2O_2 over seven minutes did not germinate properly and a high degree of contamination was present in seeds soaked less than seven minutes. The rapid development of seed crown hairs whose function it is to absorb water is critical to the development of aspen seeds, and H_2O_2 prevented proper root crown development. Seeds developed normally after sterilization with a 10-15% solution of sodium hypochlorite (commercial Chlorox™) for 3-15 minutes. After a 15-minute soaking, 86% of the seeds were uncontaminated and germinated normally, 3% were not viable and 11% were contaminated. The following protocol was used for the synthesis work:

1. the seeds were soaked in 15% clorox for 15 minutes
2. rinsed three times in distilled water, 10 min./rinse
3. and germinated on modified Hagems media for 23-30 days

Since aspen seeds are small, technique and handling is of prime importance. Two drops of the detergent "Tween" were

added to each beaker to reduce the surface tension so the seeds would sink. Seeds were gently agitated in the chlorox solution for 15 minutes and rinsed three times in double distilled H_2O. The seeds were placed in petri dishes containing Cotter's modified Hagems made with 11 g/L of agar instead of the usual 15 g/L. This allows seedling radicals to penetrate the agar more easily, and avoid desiccation.

Petri dishes were placed in a growth chamber under incandescent and fluorescent lights for 16 hours alternated with 8 hours of dark. Aspen is a shade intolerant species and adequate lighting is needed for proper development. Cotter's modified Hagems media was used not only as a nutrient source but to screen for contaminants. Contaminated seeds were removed as they appeared. Seedlings were planted in tubes after 23 days (see Appendix for complete seed protocol).

3.MYCORRHIZAL SYNTHESIS: Molina's tube method of synthesis (1979) was used in the experiments. Larger particles of peat were removed using a 1 mm mesh sieve and the remaining was autoclaved for 40 min at 20 psi. Smaller particles were sifted out of vermiculite using the same sieve and discarded. Ninety ml of vermiculite and 10 ml of peat were added to each 200 ml synthesis tube, which were shaken thoroughly. Seventy milliliters of Cotter's modified Hagems were pipetted onto the substrate and the tubes were autoclaved for 45 min at 20 psi.

Liquid cultures of each fungal isolate were homogenized in

a sterile Waring blender for 5 seconds, and 5 ml of the slurry pipetted onto the substrate of each tube under sterile conditions. Mycelium of four cultures (Amanita muscaria VT 2238, Amanita pantherina VT 2239, Inocybe lacera VT 2241, and Paxillus vernalis VT 2242) showed no signs of growth after blending: therefore, a wide mouth pipette was used to add 5 ml. of nonblended mycelium to each tube. Ten tubes were inoculated with each fungal isolate. After two weeks of fungal colonization sterile seedlings were introduced into the tubes. Tubes were placed in a growth chamber under the same light and temperature regime used for seedling germination.

4. SEEDLING HARVEST: Tubes were periodically checked for mycorrhizae and seedlings were harvested between 3 and 9 months. Seedlings were removed from the tubes and the roots gently washed with deionized water to remove adhering substrate. Morphology descriptions and chemical tests were performed on fresh material, and roots were preserved in FAA (formalin/acetic acid/70%ethanol in a ratio of 5:5:9). Roots were dehydrated and embedded in paraffin using the following protocol: 50%EtOH/20% H_2O/30%tertbutyl alcohol, 2 hours.; 50%EtOH/10%H_2O/40%TBA, 2 hrs; 50%EtOH/50%TBA, 2 hrs; 25%EtOH/75%TBA, 16 hrs; 100%TBA, 2 hrs; 100%TBA 16 hrs; 100%TBA, 16 hrs; 50%TBA/50%paraffin oil, 24 hrs; 100%paraffin oil, 24 hrs; 50%paraffin oil/50%paraplast, 24 hrs; 100% paraplast, 24 hrs, and 100% paraplast, 24 hrs. Mycorrhizae

were embedded in melted paraplast in plastic molds on a hotplate. The molds were cooled and removed 24 hours later.

Slides were thinly coated on one side with egg albumin (powder plus water) and allowed to dry. Excess paraffin was trimmed from the blocks and the embedded mycorrhizae sliced on a microtome into 5 um sections. Sections were relaxed in warm water and pulled onto semi-submerged slides. Slides were dehydrated for 24 hrs and stained in the following manner: xylene, 5 minutes; fresh xylene, 5 min; fresh xylene, 5 min; 100% EtOH, 5 min; 100% EtOH, 5 min; 95% EtOH, 5 min; Safranin O solution (4 g of safranin O stain in 200 ml ethoxyethanol, plus 100 ml 95% ethanol, 8 ml. formalin and 4 g sodium acetate and stored under the hood), 30 min; 95% EtOH 1, a few seconds; 95% EtOH 2, a few seconds; Fast Green solution (37.5 ml absolute ethanol, 37.5 ml clove oil, 25 ml ethoxyethanol, 500 mg fast green powder), 10 seconds; 95% EtOH, a few seconds; 50% clove oil/25%abs.EtOH/25% xylene, 5 min; 50%xylene/50%EtOH, 5 min; xylene, 15 min; fresh xylene, 20 min. Permount and a coverslip were added and the slides examined microscopically 24 hours later.

5. MYCORRHIZAL DESCRIPTION: Descriptions of mycorrhizae followed the checklists of Agerer (1990). The only deviation from Agerer's protocol is that in the present study ECt is defined as average tangential length of epidermal cells and ECq as average ratio between tangential length and radial

width of epidermal cells. These measurements are listed by Agerer as CCt and CCq for the cortical cells of conifers. Dark collapsed cells or residues external to the epidermal cells were described as "residues of calytra cells". Heslin and Douglas (1986) describe the same structures as tannin cells.

RESULTS

Four of the isolates, i.e. A. muscaria, A. pantherina, P. vernalis and I. lacera showed no signs of mycelial growth after two weeks in the culture tubes. New tubes were inoculated with unblended mycelium and colonization followed in all species except I. lacera. Boyle et.al. (1987) found that of nine fungal species used to test various commercial inoculation methods, P. involutus and P. tinctorius did not withstand blending. The culture of I. lacera contained a yeast apparently naturally associated with this fungus as it was found in independent isolations as well as in cultures of Laccaria laccata collected in the same area. Only scant mycelium was observed in the tubes inoculated with I. lacera, although pockets of yeast were apparent. All of the aspen seedlings in these tubes were stunted, the leaves turned black, and the trees eventually died. A plug of I. lacera mycelium was added to an already established six month old seedling, and mycorrhizae were observed 7 months later.

Pisolithus tinctorius, C. graniforme, Chalciporus piperatus, P. croceum and T. terreum grew well in the synthesis tubes, reaching the bottom in 2-3 months.

P. tinctorius formed mycorrhizae in less than a month: all other isolates took a minimum of two months. Results of the syntheses are shown in table 15. The mycelium of C. piperatus grew well, but failed to form mycorrhizae and in its vicinity many of the aspen roots turned dark brown. T. terreum, likewise, did not form mycorrhizae although the mycelium grew well. P. croceum (synonym = P. bicolor) formed a mantle, but no Hartig net was detected. I. lacera formed a mantle from which uncovered root tips protruded as if the root was surrounded by the fungus but not associated with it: no Hartig net was formed. No sclerotia were formed by any of the fungal isolates.

Results are shown in table 15 and the mycorrhizal descriptions follow.

Table 15.Isolates tested for the ability to form mycorrhizae with Populus tremuloides.

Isolate number*	Fungal species	mantle	Hartig net	host, origin and date
VT 2238	Amanita muscaria var. formosa	+	+	P. tremuloides Park co, Mt. 1990
VT 2239	Amanita pantherina var. pantherina	+	+	P. tremuloides Park co, Mt. 1990
VT 1009	Cenococcum graniforme	+	+	Loblolly Pine Georgia 1978
VT 2240	Chalciporus piperatus	-	-	P. tremuloides Park co, Mt. 1990
VT 2241	Inocybe lacera	+	-	P. tremuloides Silverbow co, Mt., 1990
VT 2242	Paxillus vernalis	+	+	P. tremuloides Silverbow co, Mt., 1990
VT 987	Piloderma croceum	+	-	unknown
VT 1398	Pisolithus tinctorius	+	+	unknown
VT 2243	Tricholoma terreum	-	-	P.tremuloides Park co, Mt. 1990

*Virginia Tech Culture collection (VPI)

Amanita muscaria var. formosa (Pers. per Fr.) Bertillon in DeChambre. 1866.

MORPHOLOGY (Fig. 31a; Fig.8)
Unramified (simple) or irregularly pinnate with straight to slightly bent unramified ends; tips commonly clavate to swollen; length of unramified systems 2-3 mm and ramified systems up to 8 mm; diameter of unramified ends 0.4 to 0.8 mm; mantle distinct and no epidermal cells visible but mantle often covers only the tip of the short root; mantle white, smooth to cottony (pubescent) and silvery due to trapped air; mantle velvety out of water due to short emanating hyphae dispersed over entire surface; rhizomorphs sparingly-branched and hairy, present on base of mantle or along main roots; rhizomorph diameter 0.10 to 0.25 mm and mantle attachment restricted.

ANATOMICAL CHARACTERS OF SURFACE (Fig.9)
OUTER SURFACE OF MANTLE: plectenchymatous, densely interwoven to sometimes parallel; cells hyphal-like, rectangular, a few swollen (terminal or intercalary); cell length varies greatly 10-45 µm; cell diameter 2.5 to 3.5 mm, swollen cells up to 7 µm(rarely 17 µm); cell walls thin, smooth and hyaline; clamps rare.
INNER SURFACE OF MANTLE: (Fig.10) transitional between plectenchymatous and pseudoparenchymatous, no pattern apparent; irregular cells 3 to 5 µm in diameter and 10 to 22 µm in length; cell walls thin, smooth and hyaline; no clamps.
TIP OF MYCORRHIZAE: same as above
RHIZOMORPHS: common, white, sparse branches similar to emanating hyphae found on mantle surface; up to 50 µm in diameter; hyphae loosely woven, anastomosing to parallel, undifferentiated; cells 3 to 3.5 µm in diam., up to 40 µm in length; cell walls thin, smooth and hyaline; connection with mantle restricted; clamps rare to common in localized areas.
EMANATING HYPHAE: abundant cystidia-shaped end cells over mantle surface and on rhizomorph margins; 2 to 3 septate, bluntly rounded, 3 to 3.5 mm in diameter and up to 36 µm (60 µm) long; unramified, rarely branched; cell walls thin and smooth.
CYSTIDIA: none
CLAMPS: rare on mantle, rare to common in rhizomorph
ANASTOMOSES: peg to peg

ANATOMICAL CHARACTERS, CROSS SECTION (Fig. 32a)
MANTLE: pseudoparenchymatous, 55-95 µm thick, homogeneous throughout; cells 3-9(14) µm tangentially, 2-4 µm radially; cell walls thin.
RESIDUES OF CALYPTRA CELLS: present

TANNIN CELLS: none present
EPIDERMAL CELLS: 1 row with Hartig net, square to rectangular, tangentially 14-28 µm, radially 25-40 µm; ECt = 17.8 µm, ECq = 0.5.
HARTIG NET: periepidermal, 1(2) rows of round to square fungal cells surround epidermal cells; fungal cells 2-5 µm in diameter.

ANATOMICAL CHARACTERS, LONGITUDINAL
MANTLE: cells tangentially 3-12 µm, radially 2-3(4) µm; cell walls thin; homogeneous throughout.
VERY TIP: mantle 45-65 µm thick, similar to rest of mantle
RESIDUES OF CALYPTRA CELLS: present
TANNIN CELLS: none present
EPIDERMAL CELLS: rectangular, arranged obliquely; tangentially 14-30 µm, radially 23-60 µm; ECt = 20.7 µm, ECq = 0.5.
HARTIG NET: palmetti-type, strongly developed; lobes 3-5 µm in diameter.
COLOR REACTION IN DIFFERENT REAGENTS:
Brilliant cresyl blue, -; cotton-blue lactic acid, cell contents blue and granular; ethanol 70%, -; $FeSO_4$, -; KOH 15%, -; lactic acid, -; Melzers, -; phenol, -; sulfo-vanillin, -; Sudan IV, -; Sudan Black, -.

AUTOFLOURESCENCE: 254 nm, -; 366 nm, -.

MATERIAL STUDIED:
Populus tremuloides seed from Ouray county, Colorado; fungal culture isolated from sporocarp found in Park County, Montana; the fungal culture VT 2238 is located in the Virginia Tech culture collection and voucher sporocarp is in the Virginia Tech Massey Herbarium (see Appendix).

Amanita pantherina var. pantherina (DC.per Fr)Krombh.

MORPHOLOGY (Fig. 31b; Fig.11)
Ramification either simple (unramified) with swollen tip and constricted base or irregularly pinnate with bent to slightly tortuous unramified ends; length of ramified system up to 10 mm; unramified ends with a maximum length of 8 mm and a diameter of 0.6 to 0.8 mm; diameter of axis 0.5 to 0.8 mm; mantle surface velvety out of water, smooth to slightly cottony and silvery in water due to trapped air; all parts white, no senescent mycorrhizae observed after nine months in culture tubes; white, sparingly-branched, sparsely-hairy thin rhizomorphs present particularly along the main roots or rarely emanating from the base of the mycorrhizae.

ANATOMICAL CHARACTERS OF SURFACE (Fig.12)
OUTER SURFACE OF MANTLE: plectenchymatous, a coarse loose net of branching hyphae 3 to 4(5)µm in diameter, cells 9-35 µm long; cell walls thin and smooth; cells hyaline; no clamps present.
INNER SURFACE OF MANTLE: (Fig.13) transitional between plectenchymatous and pseudoparenchymatous, rarely branching coarse net, more densely interwoven than mantle surface; irregular cells 5-25 µm in diameter, thin-walled, hyaline; 15 to 20 cells in a 20 µm square; no clamps present.
TIP OF MYCORRHIZAE: same as two layers described above.
RHIZOMORPHS: white, up to 25 µm in diameter and undifferentiated (or rarely with one larger vessel-like hyphae in center), with sparsely hairy margins; hyphae 3 to 4 µm in diameter, thin-walled, hyaline, smooth except for distinctive emanating hyphae which are ornamented or incrusted; no clamps.
EMANATING HYPHAE: distinctive cystidia-shaped end cells, abundant on mantle surface giving it a velvety texture, also present on rhizomorph margins; bluntly rounded, 2 to 3 septate, with slight ornamentation or incrustation; 3 to 4 µm in diameter, up to 35 µm long; no clamps present.

CYSTIDIA: none
CLAMPS: none

ANATOMICAL CHARACTERS, CROSS SECTION (Fig 32b)
MANTLE: pseudoparenchymatous, (10)56-70 µm thick; cells of outermost layer tangentially 7-15 µm, radially 2-4 µm; innermost layer of cells tangentially 2-6 µm, radially 2-4 µm, cell walls thin throughout.
RESIDUES OF CALYPTRA CELLS: present
TANNIN CELLS: none present.
EPIDERMAL CELLS: 1 row with Hartig net, square to rectangular, tangentially (12)18-27 µm, radially (14)18-25 µm; ECt= 19.6 µm, ECq= 1.0.
HARTIG NET: periepidermal, 1 to 2 (3) rows of fungal cells completely surround epidermal cells; fungal cells round in cross-section, rectangular in longitudinal section; thickness of Hartig net 3-5 µm.

ANATOMICAL CHARACTERS, LONGITUDINAL SECTION
MANTLE: outermost cells tangentially 3-12 µm, radially 2-5(8) µm; innermost cells 4-8(10) µm tangentially, (2)3-7 µm radially; cell walls thin throughout.
VERY TIP: mantle 80-100 µm thick, pseudoparenchymatous with irregularly-shaped cells; cell size similar to rest of mantle.
RESIDUES OF CALYPTRA CELLS: present
TANNIN CELLS: none present.
EPIDERMAL CELLS: rectangular, arranged obliquely; tangentially

15-20 µm, radially (20)25-45 µm; ECt= 18.3 µM, ECq= 0.6.
HARTIG NET: Palmetti-type, lobes 2-3 µm in diameter.

COLOR REACTION IN DIFFERENT REAGENTS
Cotton blue in lactic acid highlights ornamentation on emanating end cells; brilliant cresyl blue, -; Melzers, -; ethanol 70%, -; $FeSO_4$, -; KOH 15%, -; lactic acid, -; phenol, -; sulfo-vanillin, -; NH_4OH, -.

AUTOFLUORESCENCE OF WHOLE MYCORRHIZAE: 254 nm,-; 366 nm,-.

MATERIAL STUDIED
Populus tremuloides seed from Ouray county, Colorado; fungal culture isolated from sporocarp found in Park county, Montana; Fungal culture VT 2239 is located in the Virginia Tech culture collection (VPI) and a voucher sporocarp is in the Virginia Tech Massey Herbarium (see Appendix). Described after nine months in synthesis tube.

Cenococcum graniforme (Sow.)Ferd. et Winge

MORPHOLOGY (Fig.31c; Fig.14)
Ramification irregularly monopodial with bent to slightly tortuous ends; length of ramified system up to 10 mm; length of unramified ends 7 mm, diameter 0.3 to 0.5 mm; diameter of axes, 0.5 mm; few mantles well-formed, cortical cells as well as root hairs often visible; copious black mycelium present along main roots; mantle surface loosely woolly, covered by emanating bristle-like hyphae; no rhizomorphs or sclerotia present.

ANATOMICAL CHARACTERS OF SURFACE (Fig.15)
OUTER SURFACE OF MANTLE: pseudoparenchymatous, stellate pattern (an epidermoidal pattern is also present; it is not readily apparent if these cells are internal or adjacent to the stellar pattern; they are described as the inner mantle surface); cells which comprise the stellate pattern are rectangular, 5-50 µm long, 4-5 µm wide, thick-walled; 6-10 cells in a 20 x 20 µm square.
INNER SURFACE OF MANTLE: (Fig.15) pseudoparenchymatous; consisting of epidermoidal type cells which are thick-walled, 5-15 µm in diameter; 12-15 cells in a 20 x 20 µm square.
VERY TIP OF MYCORRHIZAE: same as above
RHIZOMORPHS: none present
EMANATING HYPHAE: (Fig.16) regularly septate, up to 1 mm in length, 4-5 µm wide, occasionally ramified; cell walls very thick, up to 0.5 µm, rough; distal ends rounded or tapering

slightly.
CYSTIDIA:none
CLAMPS:none

ANATOMICAL CHARACTERS, CROSS SECTION (Fig.32c)
MANTLE: loosely organized, hyphal-like or round in cross-section, 2-10 µm thick; cells jammed between epidermal cells giving exterior of cross-section an eroded appearance; mantle cells tangentially 2-14 µm, radially 2-4 µm; cell walls thickened.
RESIDUES OF CALYPTRA CELLS: present
TANNIN CELLS: none present
EPIDERMAL CELLS: 1 row with Hartig net, irregularly shaped and isodiametric, tangentially 11-18 µm, radially (7)12-25 µm; ECt = 13.8 µm, ECq = 0.9.
HARTIG NET: periepidermal, present as a chain of round to epidermoidally-shaped cells which surround each epidermal cell, having a beaded appearance; cells 2-4 µm across. Palmetti-type cells in plan view look epidermoidal enmasse; lobes 2-4 µm in diameter.

MATERIAL STUDIED:
Populus tremuloides seed from Ouray county, Colorado; fungal culture originally associated with Loblolly pine located in the VPI and SU culture collection, Vt 1009. Described 9 months after seedling inoculation.

Inocybe lacera (Fr.:Fr.)Kummer

MORPHOLOGY (Fig.31d; Fig.17)
Ramification simple (unramified), up to 4 mm in length, 0.5 mm in diameter; unramified ends somewhat flexuous, swollen in the middle tapering towards the distal end, often tuber-like with small roots protruding from mantle sides; a thick, distinct mantle surface present; epidermal cells not visible except for protruding roots; mantle surface smooth, silvery in patches, pale yellow-brown; no rhizomorphs or emanating hyphae.

ANATOMICAL CHARACTERS OF SURFACE (Fig.19)
OUTER SURFACE OF MANTLE: plectenchymatous; cells hyphal-like, short and highly ramified at 90° angles producing an interwoven to netlike hyphal arrangement; cells colorless, 14-55 µm in length, 3-5(7) µm in diameter, swollen cells to 7 µm; cell walls smooth and thin; medallion clamps rare in outer layer, but extremely common in extramatricular hyphae.
INNER SURFACE OF MANTLE: (Fig.18) pseudoparenchymatous; cells colorless, squarrose to rectangular, 5-10 µm long and 3-5(8)

µm in diameter producing no apparent pattern; clamps absent.
RHIZOMORPHS: none, some hyphae agglutinated in parallel fashion.
EMANATING HYPHAE: none
CYSTIDIA: none
ANASTOMOSES: frequent, tip to peg
REMARKS: extramatricular hyphae are highly septate and frequently branched at 90° angles; medallion clamps frequent (Fig.20).

COLOR REACTION IN DIFFERENT REAGENTS
Cotton blue, refractive cell contents (oil globules) apparent in some hyphae; other tests were not performed due to lack of fresh material.

MATERIAL STUDIED
Populus tremuloides seed from Ouray county, Colorado; fungal culture isolated from a sporocarp associated with P. tremuloides in Silverbow county, Montana; culture VT 2241 is located in the Virginia Tech culture collection (VPI) and the voucher sporocarp is in the Virginia Tech Massey Herbarium (see Appendix). Description done 7 months after 6 month old seedling was inoculated with unblended fungal culture.

Paxillus vernalis Watling

MORPHOLOGY (Fig.31e; Fig.21)
Ramification simple (unramified) with straight to rarely bent unramified ends; length of unramified ends up to 6 mm; diameter 0.17 to 0.25 mm, strikingly uniform up to bluntly rounded tips; mantle surface distinct but not always complete, epidermal cells visible in small areas especially at tip; mantle surface smooth to loosely cottony and silvery due to trapped air; hyphal strands oriented parallel to root axis; all parts light brown, senescent hyphae not present; hyphae form wefts which are repeatedly ramified and hairy and swell in water like balloons due to trapped air; no sclerotia present.

ANATOMICAL CHARACTERS OF SURFACE (Fig.22)
OUTER SURFACE OF MANTLE: plectenchymatous, of densely interwoven hyphae 3.5-5.0 µm in diameter (cells often swollen to 7 µm) and up to 30 µm in length; cell walls thin, smooth and hyaline; cell contents yellow in water; scattered intercalary and end cells with yellow refractive contents; clamps common, more frequent in hyphal wefts.
INNER SURFACE OF MANTLE: same as above.

TIP OF MYCORRHIZAE: (Fig. 23) transitional between plectenchymatous and pseudoparenchymatous, some cells square; cells 4.5 to 5.5 µm wide and up to 13 µm long; cell walls thin, contents highly refractive; 10 to 15 cells in a 20 µm square; no clamps.
RHIZOMORPHS: see emanating hyphae
EMANATING HYPHAE: sporadically abundant single hyphae 3 to 4 µm in diameter and loosely interwoven hyphal wefts up to 55 µm in diameter that resemble rhizomorphs under dissecting scope; clamps abundant.
CYSTIDIA: none
CLAMPS: common in hyphal wefts, rarer in mantle, none in tip.

ANATOMICAL CHARACTERS, CROSS SECTION (Fig.32d)
MANTLE: plectenchymatous interspersed with round hyphal cross-sections; inner layer of hyphae tightly woven, surface layer loosely interwoven; mantle 16-40 µm thick; outermost hyphae tangentially 3-40 µm, radially 3-4 µm; innermost hyphae tangentially 3-12 µm, radially 2-3 µm; cell walls thin; numerous cells with yellow refractive contents; clamps present in outer surface.
RESIDUES OF CALYPTRA CELLS: present
TANNIN CELLS: none present
EPIDERMAL CELLS: one row of square to rectangular cells; tangentially 13-20 µm, radially (17)22-30 µm; ECt= 17.5 µm, ECq= 0.7 µm.
HARTIG NET: paraepidermal, 1 to 2 rows of fungal cells found between epidermal cells; Hartig net cells round in cross section and rectangular longitudinally; thickness of Hartig net 2-3 µm.

ANATOMICAL CHARACTERS, LONGITUDINAL SECTION
MANTLE: plectenchymatous, tightly interwoven on inner surface to loosely interwoven on outer surface; hyphae of outermost layer tangentially 3-25 µm (round to hyphal-like), radially 2-4 µm; innermost hyphae tangentially 3-25 µm, radially 2-4 µm; tip of mantle 23-48 µm thick; cell size and arrangement similar to rest of mantle.
RESIDUES OF CALYPTRA CELLS: present
TANNIN CELLS: none present.
EPIDERMAL CELLS: 1 row, rectangular, arranged obliquely; tangentially 12-14 µm, radially 27-40 µm; ECt= 12.2 µm, ECq= 0.4.
HARTIG NET IN SURFACE VIEW: no Palmetti type lobes apparent, Hartig net may not be well-formed.

COLOR REACTION IN DIFFERENT REAGENTS
Lactic acid highlights cells with yellow refractive contents; brilliant cresyl blue, cell contents forest green; cotton blue

lactic acid,-; ethanol,-; $FeSO_4$, -; KOH 15%,- or highly refractive; Melzers, yellowish; phenol, -; sulfo-vanillin,-; Sudan Black,-.

AUTOFLUORESENCE: 254 nm,-; 366 nm, -.

MATERIAL STUDIED
Populus tremuloides seed from Ouray county, Colorado; fungal culture isolated from sporocarp found in Silverbow county, Montana; voucher specimen is located in the Virginia Tech Massey Herbarium (see Appendix) and the culture VT 2242 is in the Virginia Tech culture collection. Described nine months after seedling inoculation.

Piloderma croceum Erikss. & Hjortst.1981

MORPHOLOGY (Fig. 31f; Fig.24)
Ramification irregularly pinnate; length of ramified system up to 15 mm; unramified ends with a maximum length of 9 mm, diameter of axis up to 1 mm; mantle surface not distinct; epidermal cells visible especially at tip, silvery in restricted areas, densely cottony to woolly; all parts bright yellow; no rhizomorphs; copious emanating hyphae from mantle surface and along main roots up to 1 mm long.

ANATOMICAL CHARACTERS OF SURFACE
OUTER SURFACE OF MANTLE: (Fig.26) plectenchymatous to transitional between plectenchymatous and pseudoparenchymatous, no pattern although hyphae sometimes run parallel; irregular cells 4-20 µm in length and 2-3 µm in diameter; cell walls thin, smooth.
INNER SURFACE OF MANTLE: (Fig 25) transitional between plectenchymatous and pseudoparenchymatous; cells irregular or epidermoidal, 5-14 µm in diameter; cell walls thin; 25-35 cells in a 20 µm square.
VERY TIP OF MYCORRHIZAE: same as above
EMANATING HYPHAE: cells up to 90 µm long, 2-2.5 µm in diameter, dichotomously ramified; cell walls thickened and heavily incrusted with needle-shaped ornamentation which dissolves in KOH; some hyphal strands with slightly thickened, smooth cell walls 1.5-2 µm in diameter.
OLEIFEROUS HYPHAE: intercalary or end cells with yellow refractive contents.
ANASTOMOSES: peg to peg
CLAMPS: none

ANATOMICAL CHARACTERS, CROSS SECTION

MANTLE: cells hyphal-like or round in cross-section, 8-23 µm thick, tangentially 2-14 µm, radially 2-3 µm; cell walls thin except for emanating hyphae.
RESIDUES OF CALYPTRA CELLS: present
TANNIN CELLS: none present
HARTIG NET: none present

COLOR REACTION IN DIFFERENT REAGENTS
Brilliant cresyl-blue, highlights ornamentation; cotton-blue-lactic acid, -; ethanol,-; $FeSO_4$, -; guaiac, -; KOH, dissolves needle-like ornamentation; Melzers, dextrinoid; phenol, -; sulfo-vanillin, reddish-orange; Sudan Black, -; Sudan IV, -.

AUTOFLUORESENCE OF WHOLE MYCORRHIZAE: 254 nm, -; 366 nm, -

MATERIAL STUDIED
Populus tremuloides seed from Ouray county, Colorado; fungal culture from the Virginia Tech culture collection (VT 987). Described 9 months after seedling inoculation.

Pisolithus tinctorius (Pers.) Coker & Couch

MORPHOLOGY (Fig.31g; Fig.27)
Monopodially pinnate or irregularly pinnate, rarely monopodially pyramidal, often narrowing slightly at base; ramified system up to 20 mm long; axis, 0.6-0.7 mm in diameter; unramified ends up to 7 mm long, 0.4-0.6 mm in diameter, ends straight or bent; mantle thick, well-developed, cortical cells never visible; mantle surface silvery, somewhat smooth to loosely woolly; all parts yellow-brown to light brown, rarely with dark brown areas; rhizomorphs frequent, occurring near axes,rarely on tips, with restricted connection to mantle; rhizomorph filaments interconnected, highly ramified with sparsely hairy margins; emanating hyphae common; no sclerotia.

ANATOMICAL CHARACTERS OF MANTLE
OUTER SURFACE OF MANTLE: (Fig.28) plectenchymatous, a loose net of hyphal-like cells; cells 8-37 µm long, 3.5-5(7) µm wide; cell walls somewhat thickened, smooth; emanating hyphae with thick rough walls and rounded ends; clamps common.
INNER SURFACE OF MANTLE: (Fig.29) transitional between plectenchymatous and pseudoparenchymatous; cells irregular, 5-20 µm long, 3.7-4.5 µm; 10-12 cells in a 20 x 20 µm square; cell walls thinner than those of outer surface; no clamps.
VERY TIP OF MYCORRHIZAE: same as above, but more densely interwoven.

RHIZOMORPHS:(Fig.30) typically 6-9 µm, but up to 24 µm in diameter, differentiated with one thicker hyphae in center; occasionally ramified with a few hairs on margin, restricted mantle connection; vessel-like hyphae septate, up to 5 µm in diameter, 70 µm in length, unramified with thickened cell walls; remaining hyphae septate, 3-3.5 µm in diameter, up to 50 µm long, cell walls thickened and smooth; clamps abundant.
EMANATING HYPHAE: septate, 3.5-4.5 µm in diameter, up to 70 µm long, rarely ramified with rounded distal ends; cell walls thick, rough (finely granular); clamps frequent.
CYSTIDIA: none
CLAMPS: present in outer mantle, emanating hyphae and numerous in rhizomorphs.

ANATOMICAL CHARACTERS, CROSS SECTION (Fig.32e)
MANTLE: pseudoparenchymatous, 35-75 µm thick; outer layer more hyphal-like, tangentially cells 3-20 µm, radially 2-4 µm; innermost layer of cells tangentially 3-25 µm, radially 2-4 µm; cell walls somewhat thickened.
RESIDUES OF CALYPTRA CELLS: present
TANNINS CELLS: none present
EPIDERMAL CELLS: one row (or 1.5 rows) of epidermal cells with Hartig net; cells square to rectangular, tangentially 14-23(30) µm, radially 23-70(80)µm; ECt = 19.5 µm, ECq = 0.4.
HARTIG NET: well-developed periepidermal or deeper, 3-5 µm thick.

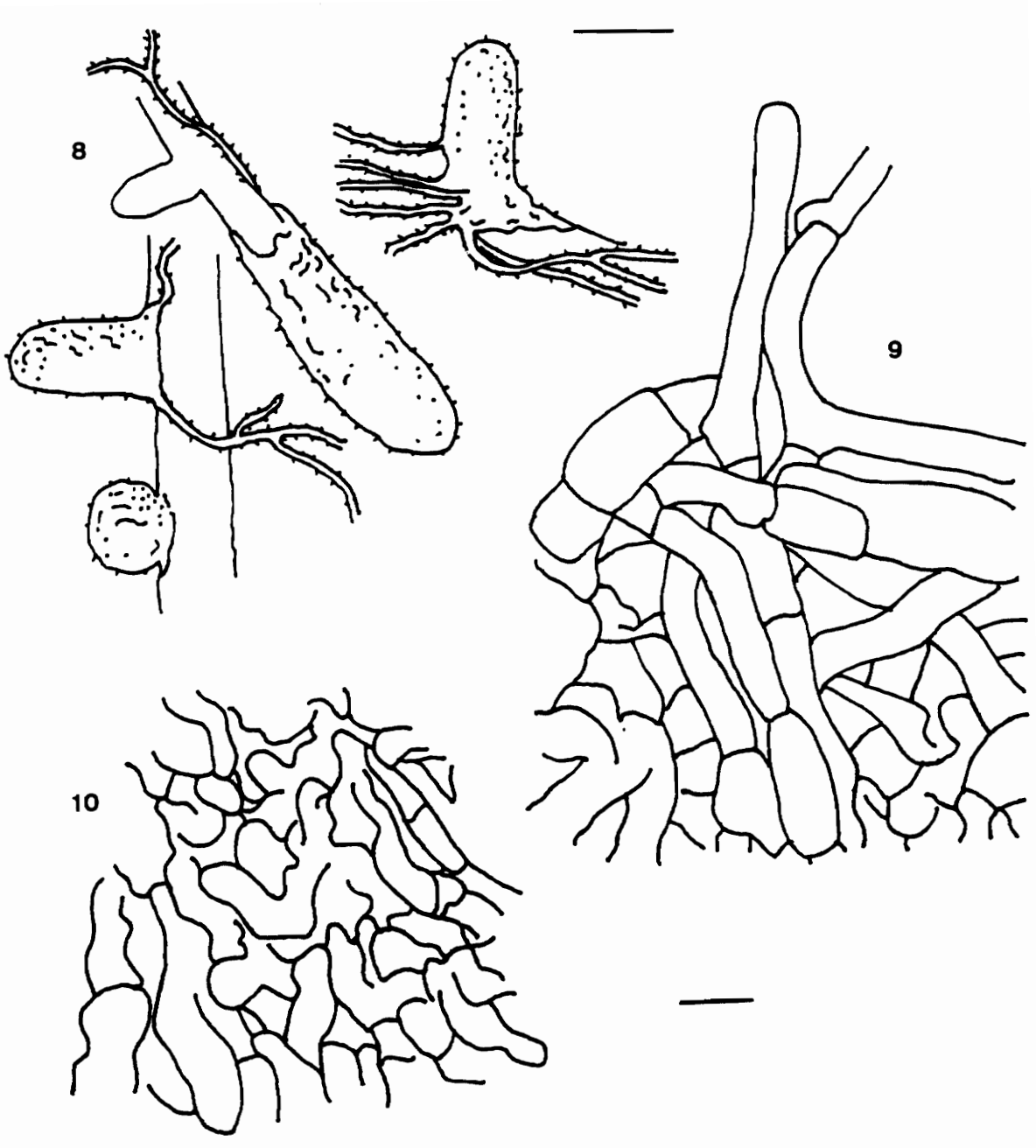
ANATOMICAL CHARACTERS, LONGITUDINAL
MANTLE: outermost cells 3-23 µm tangentially, 3-5 µm radially; innermost cells 3-15 µm tangentially, 3-5 µm radially; cell walls somewhat thickened.
VERY TIP OF MYCORRHIZAE: mantle 45-70 µm thick, similar to rest of mantle
RESIDUES OF CALYTRA CELLS: present
TANNIN CELLS: none present
EPIDERMAL CELLS: rectangular, arranged obliquely, tangentially (14)18-32 µm, radially 46-70 µm; ECt = 21.6 µm, ECq = 0.3.
HARTIG NET IN SURFACE VIEW: palmetti-type, well-developed, numerous lobes 2-5 µm in diameter.

COLOR REACTION IN DIFFERENT REAGENTS
Brilliant cresyl-blue, cells light green and emanating hyphae reddish-violet; cotton-blue-lactic acid, -; $FeSO_4$, -; guaiac, -; 15% KOH, -; lactic acid, -; Melzers, -; phenol,-; sulfo-vanillin, reddish-brown; Sudan Black, -; Sudan IV, -.

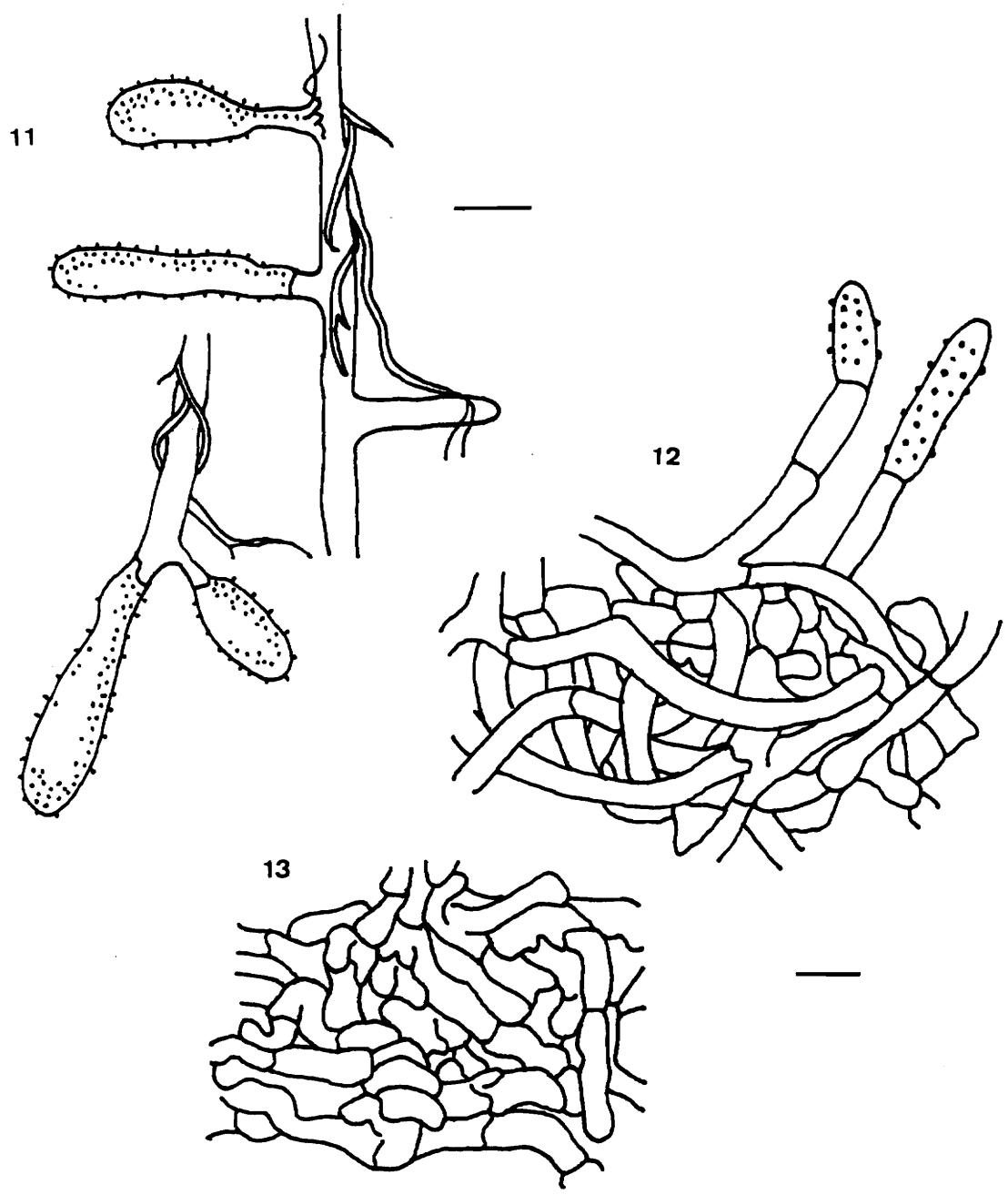
AUTOFLUORESCENCE OF WHOLE MYCORRHIZAE: 254 nm, -; 366 nm, -.

MATERIAL STUDIED

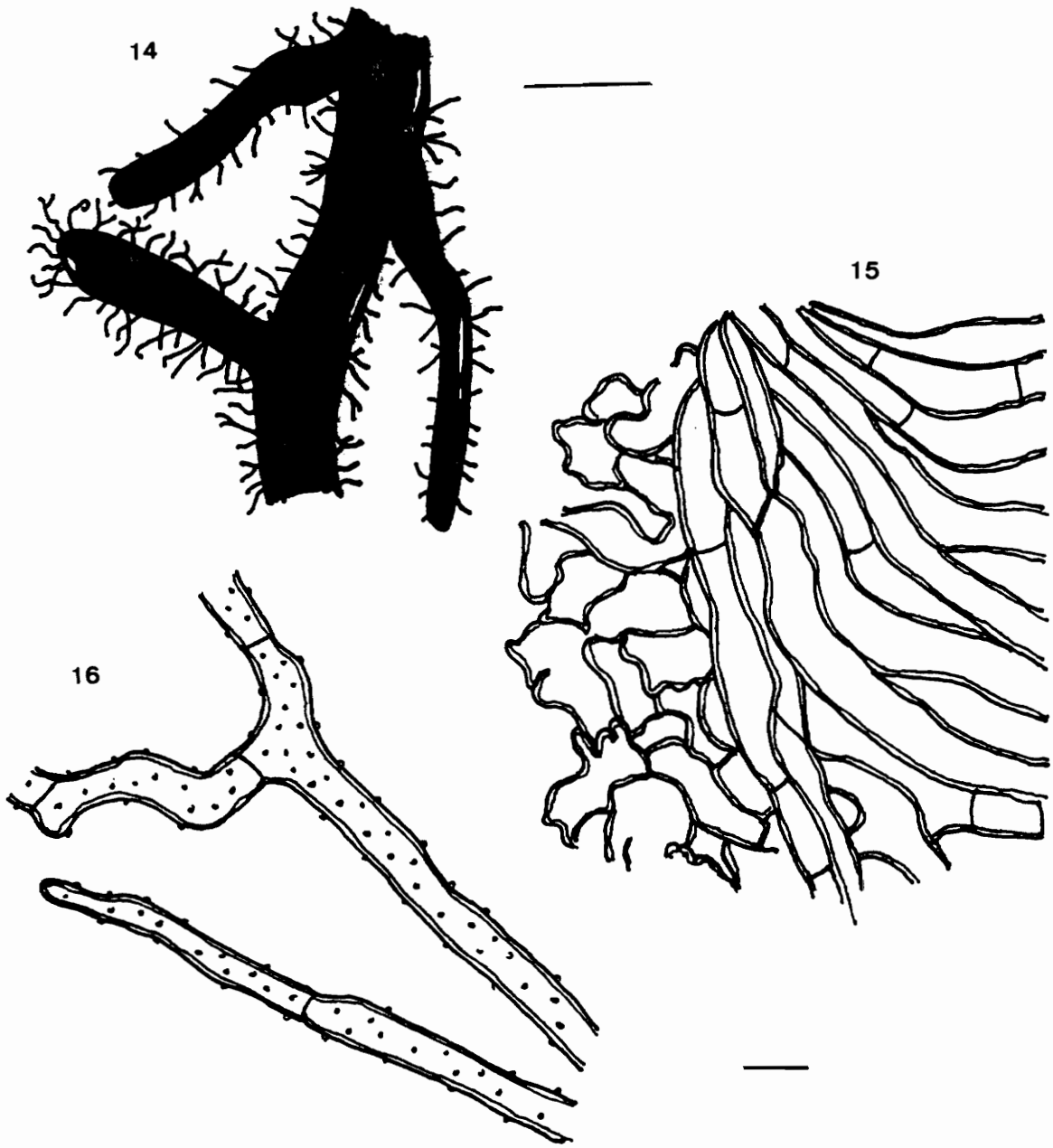
Populus tremuloides seed from Ouray county, Colorado; fungal culture VT 1398 is located in the VPI and SU culture collection. Described 9 months after seedling was inoculated.



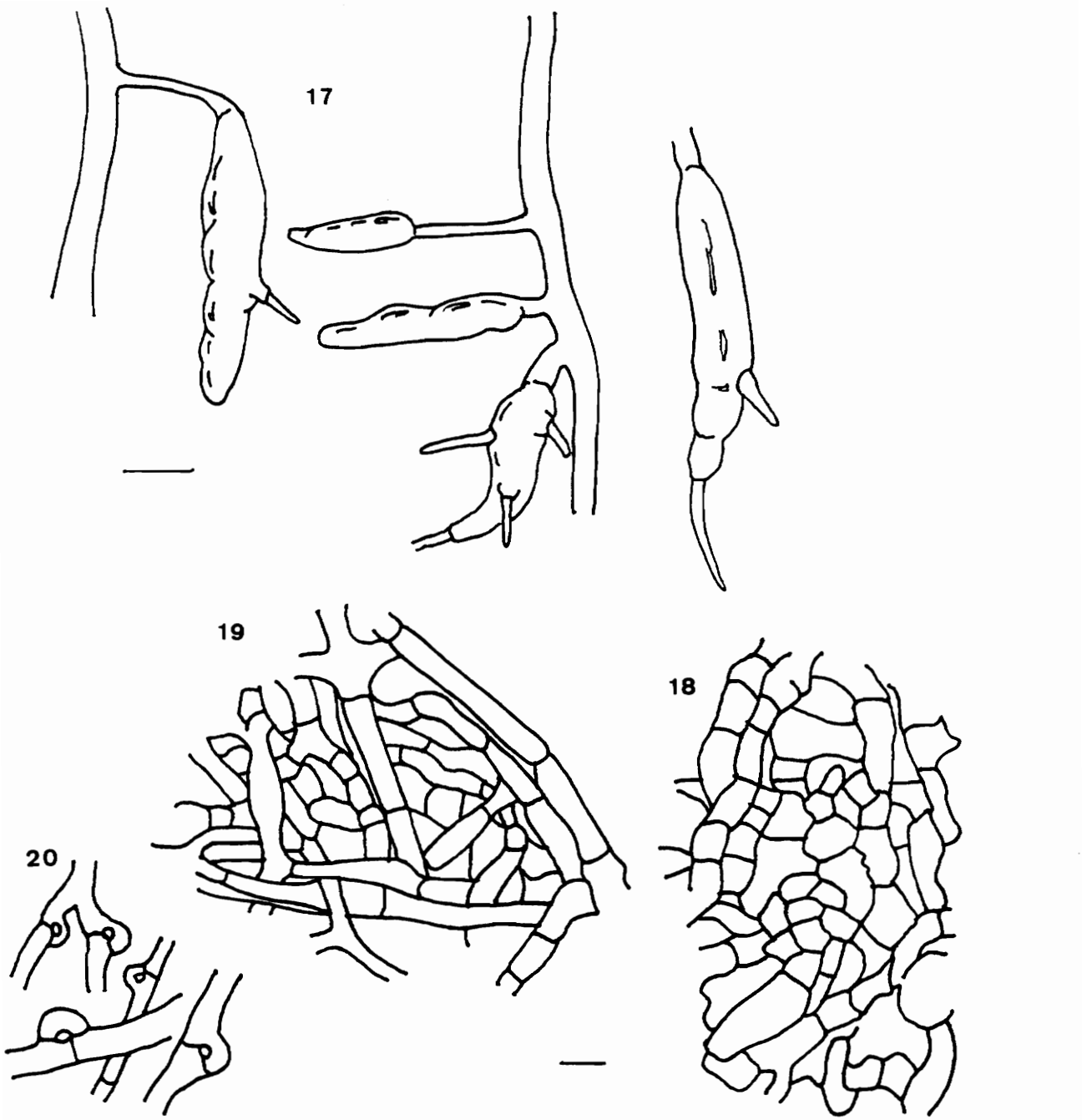
Figs.8-10. Mycorrhizae formed by Amanita muscaria var. formosa and Populus tremuloides. Fig.8, MORPHOLOGY, swollen and unswollen roottips and rhizomorphs. Fig.9, OUTER MANTLE, plectenchymatous, some cells swollen, clamps present. Fig.10, INNER MANTLE, transitional between plectenchymatous and pseudoparenchymatous, of irregularly shaped cells. Fig.8, upper bar scale = 1 mm. Figs.9-10, lower scale bar = 5 um.



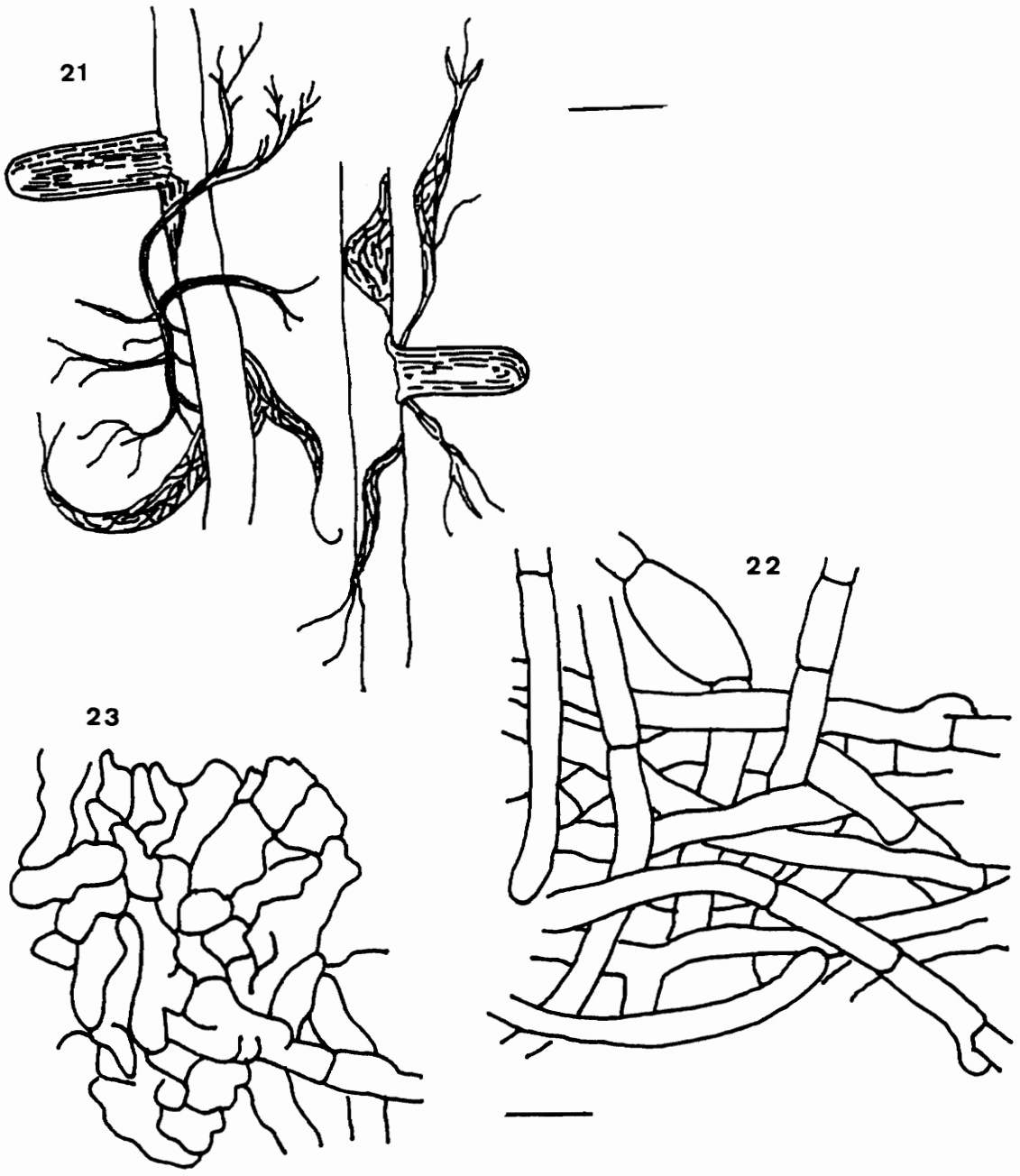
Figs.11-13. Mycorrhizae formed by Amanita pantherina and Populus tremuloides. Fig.11, MORPHOLOGY, swollen and unswollen unramified roottips. Fig.12, OUTER MANTLE, plectenchymatous with slightly roughened emanating hyphae. Fig.13, INNER MANTLE, transitional between plectenchymatous and pseudoparenchymatous, cells irregularly shaped. Fig.11, upper scale bar = 1 mm. Figs.12-13, lower scale bar = 5 um.



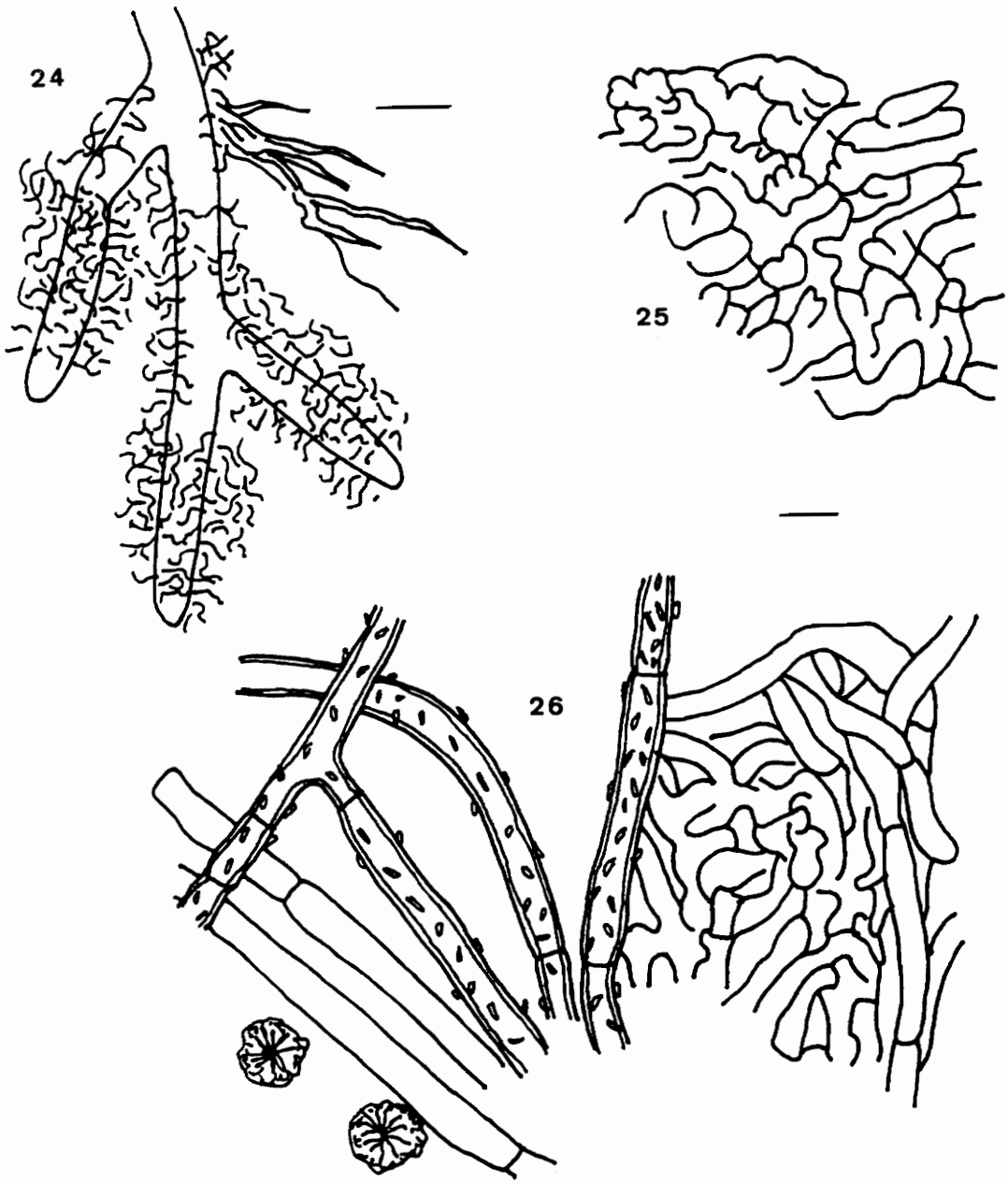
Figs.14-16. Mycorrhizae formed by Cenococcum graniforme and Populus tremuloides. Fig.14, MORPHOLOGY, black mantle and emanating hyphae. Fig.15, MANTLE, stellate pattern and epidermoidal cells. Fig.16, EMANATING HYPHAE, thick and rough cell walls. Fig.14, upper scale bar = 1 mm. Figs.15-16, lower scale bar = 5 um.



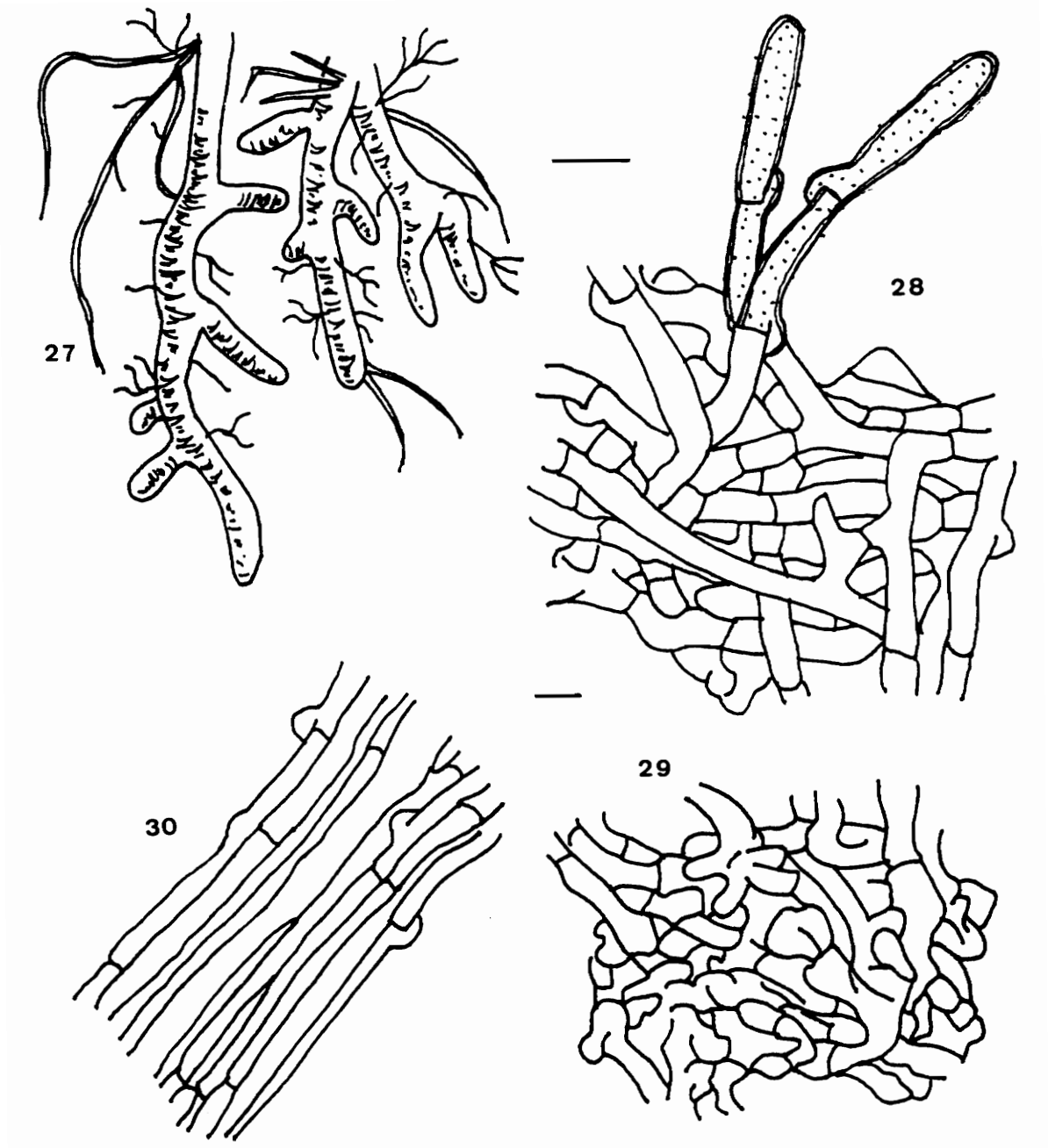
Figs.17-20. Mycorrhizae formed by Inocybe lacera and Populus tremuloides. Fig.17, MORPHOLOGY, smooth unramified mantle, sometimes with protruding roots. Fig.18, INNER MANTLE, pseudoparenchymatous, squarrose to rectangular cells. Fig.19, OUTER MANTLE, plectenchymatous, netlike arrangement. Fig 20, EXTRAMATRICULAR HYPHAE, medallion clamps. Fig 17, upper bar scale = 0.5 mm Figs. 18-20, lower bar scale = 5 um.



Figs.21-23. Mycorrhizae formed by Paxillus vernalis and Populus tremuloides. Fig.21, MORPHOLOGY, mantle and hyphal wefts. Fig.22, OUTER MANTLE, plectenchymatous, interwoven with a few swollen cells. Fig.23, TIP OF MYCORRHIZAE, transitional between plectenchymatous and pseudoparenchymatous. Fig.21, upper scale bar = 0.5 mm. Figs.22-23, lower scale bar=5 um.



Figs.24-26. Mycorrhizae formed by Piloderma croceum and Populus tremuloides. Fig.24, MORPHOLOGY, irregularly pinnate with copious emanating hyphae. Fig.25, INNER MANTLE, transitional between plectenchymatous and pseudoparenchymatous. Fig.26, OUTER MANTLE, plectenchymatous and pseudoparenchymatous, emanating hyphae with needle-like ornamentation, and crystalline bodies present. Fig.24, upper scale bar= 1 mm. Figs.25-26, lower scale bar= 5 um.



Figs.27-30. Mycorrhizae formed by Pisolithus tinctorius and Populus tremuloides. Fig.27, MORPHOLOGY, irregularly pinnate ramificaion and rhizomorphs. Fig.28, OUTER MANTLE, plectenchymatous with rough, thick-walled emanating hyphae. Fig.29, INNER MANTLE, transitional between plectenchymatous and pseudoparenchymatous. Fig.30, RHIZOMORPH, numerous clamps and central differentiated hyphae. Fig.27, upper scale bar = 1 mm. Figs.28-30, lower scale bar = 5 um.

Figure 31. Morphology of the mantles of mycorrhizae formed by Populus tremuloides seedlings and : a) Amanita muscaria v. formosa (x250), b) Amanita pantherina (x250), c) Cenococcum graniforme (x400), d) Inocybe lacera(x250), e) Paxillus vernalis (x250), f) Piloderma croceum (x250), and g) Pisolithus tinctorius (x250).

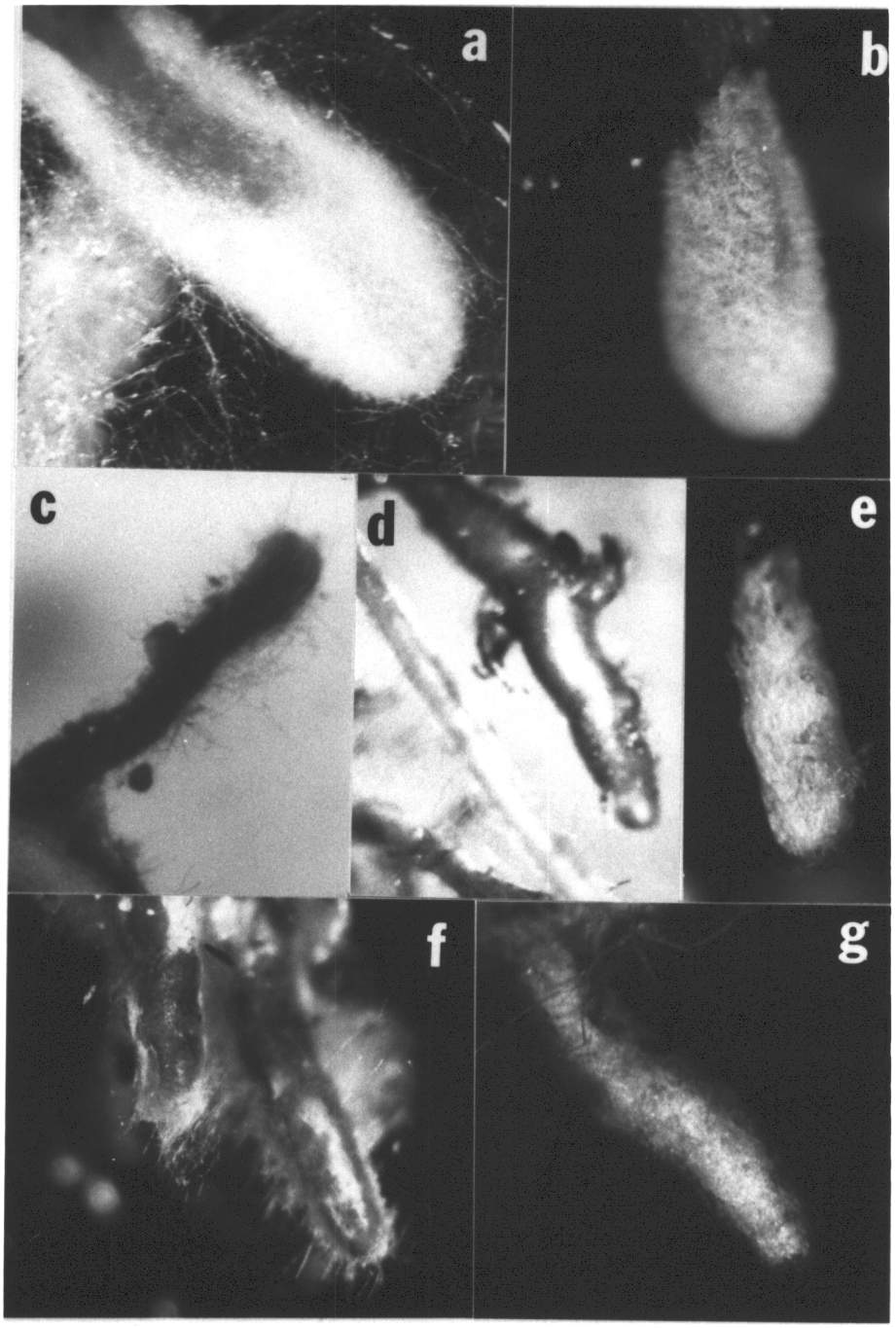
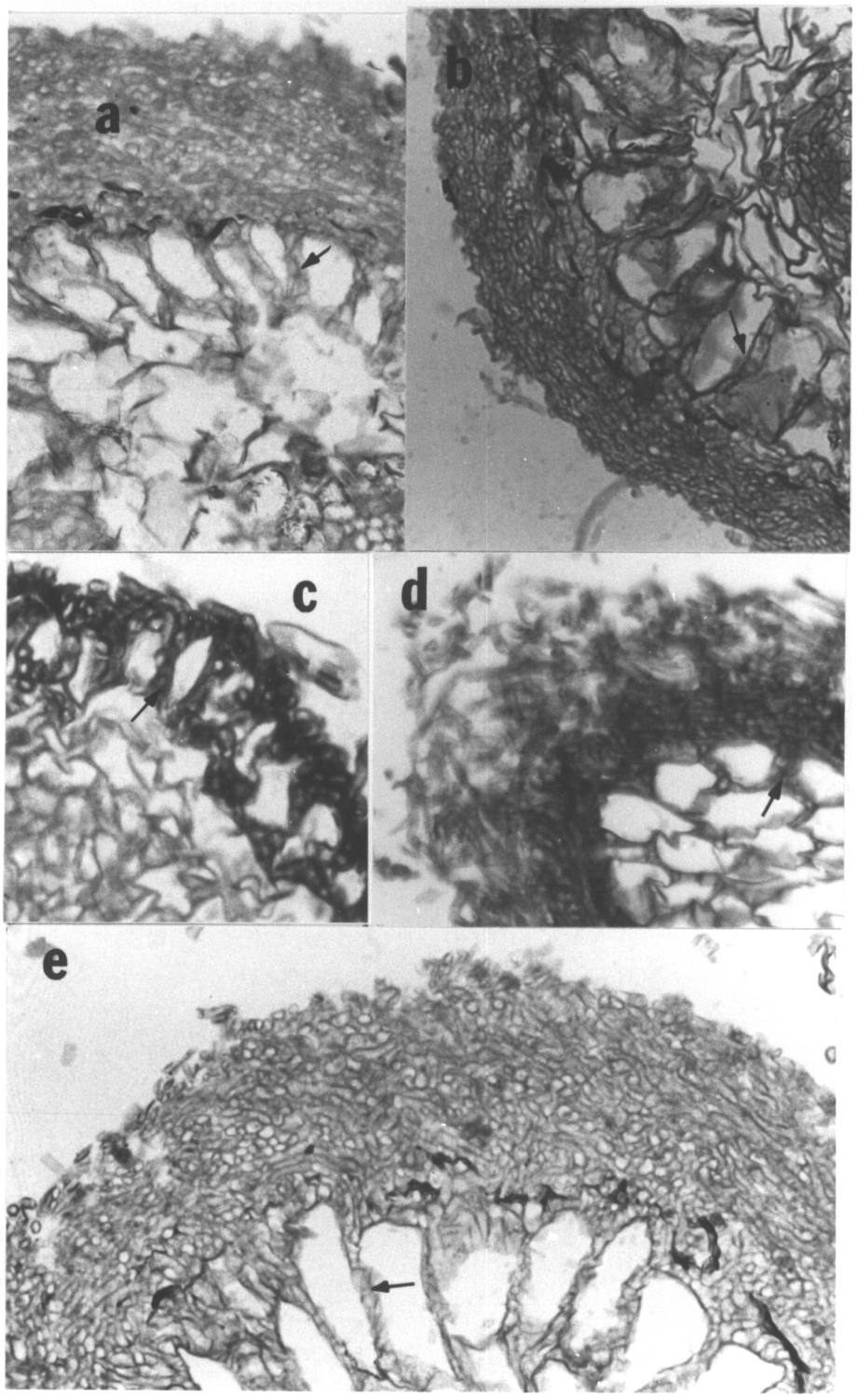

a
b
c
d
e
f
g

Figure 32. Cross-sections of mycorrhizae formed by Populus tremuloides seedlings and: a) Amanita muscaria v. formosa (x70), b) Amanita pantherina (x60), c) Cenococcum graniforme (x20), d) Paxillus vernalis (x50), and e) Pisolithus tinctorius (x40). Arrows point to Hartig net.


a
b
c
d
e

DISCUSSION

It is typical for angiosperms to form epidermal Hartig nets in contrast to gymnosperms where the Hartig net often penetrates into several rows of cortical cells (Godbout and Fortin 1983). The Hartig nets formed by five of the fungal isolates were epidermal, although P. tinctorius sometimes penetrated between the cortical cells. A. muscaria, A. pantherina, C. graniforme, and P. tinctorius formed periepidermal Hartig nets while P. vernalis formed a paraepidermal Hartig net. Godbout and Fortin (1985), using the pouch method, also found strictly epidermal Hartig nets both periepidermal (completely surrounding the epidermal cells) and paraepidermal (between the epidermal cells) in mycorrhizae formed by Populus tremuloides. With the pouch method, mycorrhizae formed in a minimum of 4 days and although no upper time limit is given, it can be assumed to be much shorter than the 3 to 9 months for the present study. Helsin and Douglas (1986) used a "jar" synthesis similar to the tube synthesis used in the present study. Of the 17 isolates they studied, a Hartig net was formed in 6 isolates, 4 of the successful isolates were P. involutus. In their study, aspen cuttings were established first, fungal plugs added 2 weeks later and mycorrhizae described 3-6 months later. All Hartig

nets were described as penetrating two cortical layers. The depth penetrated by the four Paxillus isolates in Heslin and Douglas' study varied but reached the cortical cells in contrast to Godbout and Fortins study in which P. involutus formed a paraepidermal Hartig net. These two studies show that the depth of the Hartig net formed by P. involutus varied in aspen with different isolates and/or by the synthesis conditions .

Epidermal cells in aspen elongate radially when an epidermal Hartig net is present. The ratio of tangential to radial cell length (ECq) in longitudinal sections of epidermal cells varied from 0.3 to 0.6 or a ratio of 3:1 to 1.5:1. Godbout and Fortin (1985) found the epidermal cell elongation ratio to vary from 4:1 to 1:1 in aspen. Because of this elongation it was often difficult to tell in transverse and longitudinal sections if 1 or 2 layers of cells were surrounded by Hartig net. This difficulty is mentioned by Godbout and Fortin (1983) in their study on alder mycorrhizae.

Both A. muscaria v. formosa and A. pantherina formed pure white mantles that were distinctive because of the presence of short emanating hyphae. These gave the mycorrhizae a velvety appearance out of water (Fig.31a,b; Figs.8,11). Godbout and Fortin (1985) described these hyphae as "cystidia-like, multiseptate, sometimes branched hyphae" in reference to A. muscaria/P. tremuloides mycorrhizae. A. muscaria has a broad

host range and has been found to form mycorrhizae with at least 7 conifers (Molina and Trappe 1982). The conifer mycorrhizae were "bright white with a finely tomentose to smooth-appressed mantle surface." The primitive rhizomorphs formed by both Amanitas in this study are described as thick hyphal strands in other studies. Neither of the above mentioned papers notes the variety or color of the A. muscaria used in the synthesis. In the present study, both Amanitas formed relatively thick mantles and well-developed periepidermal Hartig nets (Fig.32a,b). The two species could be distinguished by the presence of localized clamps in A. muscaria (Fig.9) which were absent in A. pantherina, and the presence of slightly ornamented end cells in A. pantherina (Fig.12). The presence of clamps only in localized areas may explain why Godbout and Fortin (1985) did not report them in A. muscaria mycorrhizae. This is the first report of a synthesis by A. pantherina. Jenkins (1986) lists its habitat as mixed deciduous and conifer woods. It is commonly encountered in the Montana Biological Station forest in mixed aspen-conifer stands (pers. comm. O. K. Miller).

The isolate labeled Cenococcum graniforme formed a thin mantle consisting not only of the typical stellate pattern formed by this species but also of epidermoidal cells (Fig.15). An unidentified mycorrhizae called "Picearhiza" which has a Cenococcum-like morphology and a mantle composed

strictly of epidermoidally-shaped cells has been described in Agerer (1990). Lobuglio et.al (1992) discovered variation in the ribosomal DNA of isolates of C. graniforme, which suggests that the species is heterogeneous or is a species complex. C. graniforme has been associated with at least 80 different hosts (Trappe 1962). Lihnell (1942) was the first to successfully synthesize C. graniforme with P. tremula. Two isolates formed mycorrhizae with P. tremuloides (Godbout and Fortin 1985): two other isolates failed to form mycorrhizae with hybrid poplar (Heslin and Douglas 1986). These results suggest variation with isolate and\or conditions. Fungal cells of C. graniforme in the present study were either compacted between the seedling epidermal cells or formed a beadlike periepidermal Hartig net (Fig.32c). Godbout and Fortin (1983) described Cenococcum hyphae as compacted between Alnus epidermal cells.

Changes in the method of synthesis regarding I. lacera are described in the methods and materials section of this chapter. Although I. lacera formed a mantle, cross sections and longitudinal sections did not reveal a Hartig net and no elongation of epidermal cells was observed. The mantle surface was smooth and somewhat shiny (Fig.31d). Medallion clamps were abundant in the extramatricular hyphae (Fig.20), but absent in the mantle itself. Naturally-formed Inocybe mycorrhizae may not often be recognized because microscopic

examination would not reveal the expected clamps. It is not known how much the presence of a yeast influenced mycorrhizal formation. Chu-Chou and Grace (1983) described mycorrhizae of I. lacera and Pinus radiata as smooth and without clamps.

Paxillus involutus is considered to be a broad-host range fungus although it may be a species complex or consist of many distinct types. The closely related P. vernalis, described by Watling (1969), has a darker spore color and associates with aspen and birch. Laiho (1970) found P.involutus to form mycorrhizae with Picea, Pseudotsuga, Alnus, Betula and Tsuga and listed Fagus, Pyrus, Quercus, Rhamnus, Salix and Populus as other probable associates. P. involutus has been shown to form mycorrhizae with P. tremuloides (Godbout and Fortin 1985), hybrid Poplar (Heslin and Douglas 1986) and P. tremula, P. deltoides, P. euramericana, and P. nigra (Anselmi, Pirazzi, and Giorcelli 1990). It should be noted that the fungus called P. involutus in these studies may be conspecific with P. vernalis. In the present study, the mycorrhizae formed by P. vernalis and P. tremuloides were extremely uniform in diameter up to the bluntly rounded tips, and the mantle was not always complete. This morphology is very similar to a description of the mycorrhizae formed by P. involutus and seven species of conifers (Molina and Trappe 1982). They were described as "strikingly narrow-cylindrical" and forming only fragmentary mantles.

In the present study, P. tinctorius (P.T.) formed a thick mantle and a periepidermal Hartig net with P. tremuloides. Godbout and Fortin (1985) found P.T. in aspen mycorrhizae to have a thick mantle and a paraepidermal Hartig net in pouch cultures whereas Heslin and Douglas (1986) reported a thick mantle but no Hartig net with hybrid poplar in jar culture. P.T. is known to have a broad host range and has been proven to be mycorrhizal with at least 48 tree species (Marx 1977). However, different isolates may vary in their ability to form mycorrhizae with various hosts.

In conclusion, A. muscaria v. formosa, A. pantherina, and P. vernalis mycelium isolated from sporocarps associated with P. tremuloides formed mycorrhizae with seedlings of this tree. C. graniforme and P. tinctorius, two broad-host range fungi, also formed mycorrhizae with P. tremuloides. The other broad-host range fungus, P. croceum formed a mantle but no Hartig net. Inocybe lacera, also formed a mantle but no Hartig net. C. piperatus and T. terreum did not form mycorrhizae. The formation of a mantle and Hartig net affirms the ability of a fungus to form mycorrhizae with a host, but a negative response may only indicate that mycorrhization does not take place in the given conditions.

Chapter 4: EARLY GROWTH RESPONSE IN ASPEN SEEDLINGS TO INOCULATION WITH NINE FUNGAL ISOLATES

INTRODUCTION

The enhancement of growth in host plants is considered to be one of the major effects of mycorrhization (Harley and Smith 1983) and is often used to assess mycorrhizal efficiency (Schenk 1982). A number of researchers including Hackskaylo (1967) and Trappe (1977) have shown that conifer growth improves under some conditions when inoculated with certain ectomycorrhizal fungi. The effects of fungal inoculation on broad-leaf trees are not so well-known. Studies have shown that the growth of broadleaf trees such as eucalyptus (Boucher and Malajczuk 1989) and alder (Helm and Carling 1990) can be enhanced by fungal inoculation. Lee and Koo (1985) found that the dry weight of *Populus alba* and *P. glandulosa* cuttings inoculated with *Pisolithus tinctorius* increased 49% over uninoculated controls under nursery conditions.

Most studies have been concerned with the effects of one fungal inoculant on one host and few have investigated the responses of one host to different fungi. Comparative studies include those on *Pinus ponderosa*, *Tsuga heterophylla*, *Pseudotsuga menziesii* (Trappe 1977), *Pinus strobus* (Doak

1936), Pinus mugo (Benecke 1974), and Pinus resinosa and Picea abies (Moser 1956). These studies show that host response varies with fungal inoculant.

Even fewer comparative studies have been done on broadleaf trees. Moser (1956) found that Ulmus americana inoculated with Tylopilus felleus, Amanita muscaria, or Suillus granulatus showed no improved growth over uninoculated controls under field conditions. Levisohn (1957) found that Betula pendula grew better with Leccinum scabrum but not with Amanita muscaria in pits filled with sterilized soil. Anselmi, Pirazzi, and Giorcelli (1990) studied the growth response of Populus nigra and P. alba cuttings inoculated with ectomycorrhizal fungi in greenhouse conditions. Stem diameter, height, and volume of each kind of cutting inoculated with six and 13 fungal symbionts respectively were recorded after one year. The volume of the cuttings increased significantly with inoculation with some fungal species but not others (see Literature Review, pg. 11) for details).

One reason that only a few studies have tested host responses to fungal symbionts may be that factors such as isolate age (Trappe 1977), strain variation (Fries 1989), seed source (Cline and Reid 1982), and different experimental conditions complicate the interpretation of such studies. However, if these kinds of studies are eventually compiled and correlated with ecological observations and physiological

knowledge, conclusions may be more meaningful. Information on the effects of various fungal symbionts may also be helpful in choosing the proper fungal inoculant for nurseries and for reforestation and reclamation work. However, it must be kept in mind that enhanced growth under experimental conditions does not necessarily result in increased fitness under natural conditions.

The first objective of this study was to determine which tree characters might be useful in measuring differential growth responses in aspen seedlings. Since few studies have investigated growth response in fungally inoculated broadleaf trees, leaf width, leaf length, petiole length, and leaf surface area were measured as well as parameters typically measured in conifers such as shoot height, shoot dry weight, root dry weight, plant dry weight, number of root tips, and percent mycorrhization. The second objective was to determine if inoculation with any of the nine fungal isolates stimulated the growth of young aspen seedlings "in vitro" as evaluated by the chosen parameters. A new method of seed sterilization (described in the Methods and Materials: Chapter 3) was used in this study. All of the isolates used in this study had been tested for their ability to form mycorrhizae in another study (see Table 15). Five of the isolates had been mycorrhizal with P. tremuloides, and two formed mantles but no Hartig net.

METHODS AND MATERIALS

1. FUNGAL ISOLATES. The isolates and tissue culturing protocols used in this study have been described in Chapter 3. The isolates of C. piperatus (VT 2240), T. terreum (VT 2243), A. pantherina (VT 2239), A.muscaria v. formosa (VT 2238), P.vernalis (VT 2242), and I.lacera (VT 2241) came from sporocarps found in pure aspen stands in Montana in 1990 (see Tables 19, 20, and 21). P. tinctorius, C. graniforme, and P. croceum cultures came from the Virginia Tech fungus culture collection. Ten replicate tubes were inoculated with each fungal isolate with the exception of that of I. lacera which was used to inoculate only six tubes due to lack of sufficient mycelium.

2. SEED STERILIZATION AND GERMINATION. The seeds used in this experiment came from the Northplan Seed Company in Moscow, Idaho, and were originally collected on Red mountain pass in Ouray, Colorado. The Methods and Materials section of Chapter 3 describes the protocol used for seed sterilization and germination. In this study, the largest and smallest seedlings were discarded as well as "bushy" seedlings which had numerous small leaves. After culling, 10% of the sterile seedlings were considered suitable for the growth study.

3. MYCORRHIZAL SYNTHESIS: TUBE METHOD: The synthesis protocol

is described in the Methods and Materials in Chapter 3. Mycelia of fungal cultures were blended except for A.muscaria, A. pantherina, P. vernalis and I. lacera which do not withstand blending.

4. SEEDLING HARVEST AND PARAMETER MEASUREMENT. Seedlings were removed from the tubes after 90 days. Roots were soaked and washed with deionized water to remove adhering substrate. A majority of the following measured parameters are those suggested by Schenk (1982):

STEM DIAMETER: a cross section of the stem was taken at the substrate level and measured in mm using the low power objective (100X) of the light microscope.

HEIGHT: measured 1 mm above the substrate to the apical meristem (mm).

DRY WEIGHTS: plants were dried at 65° C. (150° F) for 48 hours, cooled for 1 to 2 hours and then weighed.

LEAF LENGTH AND WIDTH: averaged for each plant (mm).

PETIOLE LENGTH: averaged for each plant (mm).

TOTAL LEAF SURFACE AREA: the surface area of each leaf was measured on a grid and totaled for all the leaves of a seedling in cm^2.

NUMBER OF ROOT TIPS: all root tips were counted, and separated into both mycorrhizal and nonmycorrhizal groups.

PERCENT MYCORRHIZATION: the number of mycorrhizal roots divided by the total number of root tips was expressed as a

percent. Root tips with well-formed mantles were considered to be mycorrhizal.

MORTALITY: trees without green leaves were considered dead.

LEAF COLOR: noted for each plant as a measure of seedling condition.

ROOT:SHOOT RATIO: root dry weight was divided by shoot dry-weight. Although an artificial measure, this denotes changes in the allocation of the plant's energy.

5. EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS. Space limitations required that four separate experiments be done each with its own control consisting of 10 replicate tubes. In the following four tests, each tube contained one aspen:

1. 10/4/1990
   10 uninoculated control tubes: control # 1
   10 tubes inoculated with Pisolithus tinctorius
   10 tubes inoculated with Cenococcum graniforme
2. 12/1/1990
   10 uninoculated control tubes: control # 2
   10 tubes inoculated with Piloderma croceum
   10 tubes inoculated with Chalciporus piperatus
3. 12/16/1990
   10 uninoculated control tubes: control #3
   10 tubes inoculated with Tricholoma terreum
4. 12/20/1990
   10 uninoculated control tubes: control # 4
   10 tubes inoculated with Amanita pantherina
   10 tubes inoculated with Amanita muscaria
   10 tubes inoculated with Paxillus vernalis
   10 tubes inoculated with Inocybe lacera (and a yeast)

Normality of parameter distribution within each set of replicate tubes and homogeneity of variance between inoculated and uninoculated tubes could not be assumed in all cases. Therefore a nonparametric t-test, which is more conservative

than the usual t-test, was used to compare responses in inoculated and uninoculated seedlings.

A preliminary comparison of the four controls using a nonparametric Kruskal-Wallis test followed by Duncan's multiple range was done to insure that all tests were run under uniform conditions and were therefore comparable. Among all control seedings, there were no statistical differences in stem diameter, height ($p=0.09$) leaf number, root dry weight ($p=0.10$), and root/shoot dry weight ratio. There were significant differences among controls in other parameters, significance being defined as $F \leq 0.05$. Significant F differences among the control parameters are starred: stem diameter, 0.5824; height, 0.0905; plant dry weight, 0.001*; average leaf width, 0.003*; average leaf length, 0.0001*; average petiole length, 0.0001*; leaf number, 0.2911; leaf surface area, 0.0216*; shoot dry weight, 0.0097*; root dry weight, 0.1091; number of root tips, 0.0023*; and root:shoot ratio, 0.8952.

Because there were differences among some control parameters, the four experiments were analyzed separately. Ten replicates inoculated with each fungus were compared to 10 replicates of each appropriate control using a nonparametric Wilcoxon rank sum test. P-values for the tests are given in Table 17. A p-value of 0.05 or less was considered significant for the mean of a parameter of inoculated compared

with uninoculated seedlings.

In the following Results and Discussion section, when p-values are close to 0.05, they may be described as either significant or not significant in order to simplify the text, but in these cases the p-values are included in parentheses. In all cases except those specifically noted in Table 17, the mean values of inoculated seedlings can be assumed to be larger than those of the controls.

RESULTS AND DISCUSSION

Aspen seedlings inoculated with *Inocybe lacera* were stunted and most of the leaves blackened. Very little mycelial growth was observed in these tubes. However, pockets of a yeast that appeared to occur naturally with *I. lacera* were apparent, and it is strongly suspected that the effects recorded for *I. lacera* are actually due to the yeast. In another experiment (see Chapter 3), the mycelium of *I. lacera* was added to a six-month old seedling. In that case, the mycelium grew well, the yeast did not, mycorrhizae formed, and there were no signs of necrosis.

All inoculated seedlings showed significant increases in stem diameter except those inoculated with *I. lacera* (Fig. 33, Table 17). The standard deviation for the mean stem diameter

was small and uniform in inoculated and uninoculated trees alike (Table 16). Anselmi, Pirazzi, and Giorcelli (1989) found the stem diameter of poplar clones to increase after inoculation of some fungal species, but not others.

Seedlings inoculated with A. muscaria v. formosa, P. croceum, C.graniforme, C.piperatus and A. pantherina ($p = 0.06$) showed significant increases in height (Fig. 33, Table 17). Height did not increase in seedlings inoculated with P. tinctorius, T. terreum, P. vernalis and I. lacera. The standard deviation of the height mean increased with inoculation, i.e. there was more variation in height with fungal inoculation (Table 16). Anselmi, Pirazzi, and Giorcelli (1990) also found that the height of poplar clones increased after inoculation with some fungal species but not others.

Plant dry weight (shoot plus root dry weight) increased significantly in all inoculated seedlings except those paired with I. lacera (Fig. 33, Table 17). In comparisons of inoculated and uninoculated aspen seedlings, the p-values for plant dry weight were consistent and extremely small (0.0002-0.0016) (Table 17). Thus plant dry weight, more than any other characteristic, showed a clear-cut difference in the growth of inoculated and uninoculated seedlings. In the present study, seedlings inoculated with C. piperatus, T. terreum and A. muscaria averaged four times more biomass than the controls (Table 16). Seedlings paired with P. tinctorius and P. croceum

increased 3.5 times in biomass; those paired with P. vernalis increased three times in biomass, those paired with C. graniforme increased 2.7 times in biomass; those paired with A. pantherina increased two times. Seedlings inoculated with I. lacera (and a yeast) were the same biomass as the controls and were in very poor condition, and most leaves were black. Anselmi, Pirazzi, and Giorcelli (1990) reported greater differences in the volume of clones compared with other parameters. Volume, like plant dry weight, combines the separate effects of changes in height, petiole length, stem diameter, leaf size, and leaf number.

Average leaf width increased significantly in young aspens inoculated with P. tinctorius, C. graniforme, C. piperatus, T. terreum, A. muscaria v. formosa, A. pantherina ($p=0.0885$), and P. vernalis ($p=0.0752$) (Fig. 34, Table 17). The average leaf width did not increase after inoculation with P. croceum and I. lacera. Standard deviation of leaf width increased with inoculation of some species but not others (Table 16).

The leaves of all inoculated seedlings were longer on the average than leaves of the control seedlings except for those inoculated with C. graniforme and I. lacera (Fig. 34, Table 17). Leaves of seedlings inoculated with C. graniforme were both noticeably long and narrow. Standard deviation increased after inoculation for most fungal species but declined in

others (Table 16).

The average petiole length increased in seedlings inoculated with P. tinctorius, C. graniforme, P. croceum (p=0.086), C. piperatus, T. terreum, A. muscaria, and P. vernalis (Fig. 34, Table 17). Seedlings inoculated with A. pantherina and I. lacera had no increase in average petiole length. The standard deviation for the the mean petiole lengths was larger in fungally inoculated seedlings than in the controls (Table 16).

The number of leaves did not increase in seedlings inoculated with P. croceum, T. terreum, A. pantherina, and C. piperatus (Fig. 35, Table 17). The average number of leaves per plant increased in aspen seedlings inoculated with P. tinctorius, C. graniforme, A. muscaria, and P. vernalis. The average number of leaves decreased in tubes with I. lacera (and yeast) to two per plant.

The total surface area of leaves increased significantly on seedlings inoculated with P. tinctorius, C. graniforme (p=0.068), C. piperatus, T. terreum, A. pantherina (p=0.063), A. muscaria v. formosa, and P. vernalis (Fig. 35, Table 17). This increase reflected variable increases in leaf length, width, and leaf number. Only the leaves of aspen inoculated with P. croceum showed no increase in surface area, while the leaves of seedlings inoculated with I. lacera (and yeast) were smaller than the control seedlings (Table 16).

The shoot dry weight of seedlings inoculated with P. croceum (p=0.10) and I. lacera (and yeast) did not increase. Seedlings inoculated with the other fungi showed a significant increase in shoot dry weight (Fig. 35, Table 17). The standard deviation, which was small for this parameter in uninoculated trees, increased with inoculation (Table 16). Shoot dry weight combines all the individual increases in petiole length, leaf width, leaf length (and therefore surface area) and number of leaves into one parameter.

Root dry weight increased significantly in all inoculated aspen with the exception of those inoculated with I. lacera (Fig. 36, Table 17). The standard deviation of the mean root dry weight of inoculated plants (Table 16) was larger than that of the controls. Increases in the standard deviation of many of the seedling parameters after inoculation may be partly explained by differences in the amount and spatial dispersion of the mycelium and seedling genotype. The thick mantles formed by P. tinctorius contributed somewhat to the increase in the root weight in aspen inoculated with this fungus. Percentwise, this parameter increased more than other parameters over their respective controls: C. piperatus, 546%; P. tinctorius, 521%; A. muscaria, 491%; P. croceum, 485%; T. terreum, 360%, C. graniforme, 316%; P. vernalis, 309%, and A. pantherina, 264%.

The average number of root tips doubled with inoculation

of all fungal species except P. croceum (Fig.36, Table 17). It is interesting to note that in tubes containing I. lacera, even though the shoot condition deteriorated, the number of root tips was nearly double that of the control (p=.10)(Table 16).

An evaluation of the general condition of seedlings inoculated with each of the fungal isolates in terms of leaf color, seedling mortality rate, and root dry weight/shoot dry weight is provided in Table 18. Root dry weight/shoot dry weight is a measure of changes in the allocation of a plant's energy.

The root/shoot dry weight ratios (1.2-1.5 in controls) were not altered significantly after the introduction of C. graniforme, C. piperatus, T. terreum, and P. vernalis. In these species both the root and shoot weight increased proportionally. The root to shoot weight ratio increased in seedlings inoculated with P. tinctorius, P. croceum, A. muscaria and A. pantherina. In the case of P. tinctorius some of the increase in root weight can be attributed to the weight of the thick mycorrhizal mantles.

For comparison, it should be noted that none of the control seedlings died or showed any signs of necrosis (Table 18). Leaves of control trees were a lighter green than those of many of the inoculated trees, especially with P. tinctorius, which had dark green leaves. None of the leaves of

the controls turned black, yellow, or red. Twenty percent of seedlings inoculated with P. tinctorius died; they were physically overwhelmed by the mycelium. Ten per cent of seedlings inoculated with C. graniforme died: shoots had turned black and become hirsute with fungal hyphae. Ten per cent of the trees inoculated with P. croceum and C. piperatus died, but the cause was not clear. Black leaf tips were noted on seedlings inoculated with all four species. Of these four fungi, only the sporocarp of C. piperatus was originally associated with P. tremuloides. The leaves of seedlings inoculated with P. vernalis turned red, yellow, and black but none of the trees inoculated with this fungus died. All the seedlings inoculated with I. lacera were stunted and the leaves blackened and abscissed. As mentioned before, this was probably due to presence of the yeast. During the present 3-month study, there was no sign of necrosis in seedlings inoculated with T. terreum, A. pantherina, and A. muscaria (Table 18). Species in Tricholoma and Amanita genera are considered to be "late stage colonizers" (Jansen and Dighton 1990) and were isolated from sporocarps associated with P. tremuloides. The implications of this may warrant further study. However, Heslin and Douglas (1986) found that, for the first 3 months of their study, aspen clones developed normally in jars but after this time the lower leaves became chlorotic and abscissed in both inoculated and uninoculated hybrid

poplar clones. However necrosis was more pronounced in inoculated plants. In the present 3-month study no necrosis was observed in control plants. Godbout and Fortin (1986) also noted random "reddening, yellowing and necrosis of the leaves" in inoculated P. tremuloides seedlings in pouches.

The roots of seedlings inoculated with P. tinctorius formed mycorrhizae within one month and were 86% mycorrhizal after three months (Fig. 36., Table 16). C. graniforme mycorrhizae formed slowly and only 5% of the root tips were mycorrhizal after three months. The Amanitas also formed mycorrhizae slowly. After three months, 15% of the seedling roots inoculated with A. muscaria and 11% of those inoculated with A. pantherina were mycorrhizal. A mycorrhizal mantle was formed when P. tremuloides was inoculated with P. croceum, but a Hartig net did not form (Table 15). Neither Chalciporus piperatus or Tricholoma terreum formed mycorrhizae with aspen seedlings in the present three-month study nor did they form mycorrhizae with aspen in another study after nine months.

Aspen seedlings inoculated with C. piperatus, P. croceum, and T. terreum increased in plant biomass but did not form mycorrhizae. However, the presence of the fungi did cause growth responses. The fact that growth enhancement is not always directly related to mycorrhization under a specific set of conditions has been observed by other researchers. Trappe (1962) states that "some fungi stimulate tree growth without

actually forming mycorrhizae". Levison (1956) found that Leccinum scabrum and Rhizopogon luteolus stimulated seedling growth before forming mycorrhizae. Shemakhanova (1957) found that the growth of oaks was stimulated with an extract from C. graniforme identified as pantothenic acid. In the same study other fungi were found to secrete other vitamins and to affect hosts differentially. In addition, growth response varied by host. Some fungal species are known to produce growth regulators which might also cause enhanced growth without mycorrhization (Zak 1973). Root tip formation is stimulated by the production of IAA by Boletinellus merulioides, a nonmycorrhizal fungus (Gruhn, et.al, in press).

CONCLUSIONS

Plant dry weight, shoot dry weight, and root dry weight of aspen seedlings increased after fungal inoculation. Inoculated and uninoculated plants were most easily distinguished by these three parameters than others that were measured. Stem diameter also increased in a majority of the inoculated aspen seedlings. Increases in mean stem diameter were not large, but a small, homogeneous standard deviation allowed distinctions to be made between inoculated and uninoculated seedlings.

Changes in height, leaf width, leaf length, petiole length, leaf surface area, leaf number, number of root tips,

and root/shoot dry weight varied by fungal species.

Seedling mortality appeared to be attributable to the fungus in tubes inoculated with P. tinctorius and C. graniforme. The unhealthy condition of trees inoculated with I. lacera appeared to be due to a yeast that was also present. Leaf necrosis occurred after inoculation with P. tinctorius, C. graniforme, P. croceum and C. piperatus. No necrosis was observed in the controls or in species inoculated with the "late stage fungi", i.e. A. muscaria, A. pantherina or T. terreum. Mycorrhizae were formed by P. tremuloides and isolates of P. tinctorius, C. graniforme, P. vernalis, A. muscaria, and A. pantherina results of which are described in Chap. 3. P. tinctorius colonized the aspen roots and associated in mycorrhizae rapidly, whereas mycorrhizae were formed slowly by the other species. Parameter increases were not necessarily correlated with percent mycorrhization. Aspen seedlings did not form mycorrhizae with P. croceum, C. piperatus, and T. terreum, but their growth was stimulated by the presence of these fungi. This response warrants further study. This study showed that the growth of young P. tremuloides seedlings is stimulated by the presence or association of ectomycorrhizal fungi but responses vary in each of the individual parameters measured and depended on the fungal species present.

Table 16. Means and standard deviations of growth parameters of fungally inoculated and uninoculated aspen seedlings. Means calculated from 10 replicates. Measurements are in mm., cm^2 and gms.

Fungal species	stem diam	height	plant dry wt	av.leaf width
P. tinctorius	1.3±0.1	13.5±4.9	.14±.05	8.3±2.3
C. graniforme	1.4±0.3	18.5±5.4	.11±.04	7.8±2.0
control # 1	0.9±0.1	10.2±2.5	.04±.02	6.1±0.9
P. croceum	1.3±0.2	12.8±3.1	.09±.03	4.8±1.3
C. piperatus	1.2±0.2	12.4±2.6	.13±.08	6.3±1.0
control # 2	1.0±0.2	8.7±2.4	.03±.01	4.0±1.1
T. terreum	1.6±0.2	16.3±9.2	.13±.04	8.6±1.6
control # 3	0.8±0.1	10.8±3.9	.03±.01	5.3±0.8
A. pantherina	1.0±0.2	14.1±2.9	.05±.01	5.7±1.7
A. muscaria	1.2±0.2	15.5±3.0	.08±.03	6.1±0.9
P. vernalis	1.3±0.3	14.2±4.4	.06±.03	5.7±1.6
I. lacera*	0.7±0.2	13.0±2.0	.02±.01	3.6±2.4
control # 4	0.8±0.2	11.5±2.1	.02±.01	4.4±1.2

Fungal species	av.leaf length	petiole length	leaf number	leaf sur.area
P. tinctorius	19.5±4.0	2.0±0.7	8±2	11.9±5.3
C. graniforme	17.6±4.4	1.6±0.7	8±2	9.8±4.9
control # 1	15.3±1.6	1.2±0.6	6±2	5.2±2.3
P. croceum	11.3±2.4	3.4±1.1	7±2	3.6±1.6
C. piperatus	14.7±2.3	1.6±1.5	9±2	7.7±2.3
control # 2	8.9±2.8	1.4±0.8	8±2	3.1±1.1
T. terreum	18.4±3.6	1.8±0.9	9±3	11.6±4.2
control # 3	11.9±1.1	1.5±0.7	8±2	4.7±1.7
A. pantherina	12.7±3.9	2.1±0.9	7±1	4.9±2.0
A. muscaria	14.2±2.5	2.3±1.1	8±1	6.8±1.8
P. vernalis	13.3±3.9	1.7±1.6	10±4	7.3±3.8
I. lacera*	7.3±4.5	3.3±1.9	2±2	1.1±0.9
control # 4	9.6±2.5	1.4±0.7	7±2	3.2±1.5

*yeast also present

Table 16 (continued). Means and standard deviations of growth parameters of fungally inoculated and uninoculated aspen seedlings. Means calculated from 10 replicate tubes.

Fungal species	shoot dry wt	root dry wt	number roottips	%mycorr. roottips
P. tinctorius	.04±.02	.10±.04	452±224	86±11
C. graniforme	.05±.02	.06±.03	421±131	5±10
control # 1	.02±.004	.02±.01	220±108	0
P. croceum	.02±.01	.06±.03	212±131	0
C. piperatus	.06±.06	.07±.04	317±75	0
control # 2	.01±.005	.01±.005	161±41	0
T. terreum	.04±.01	.07±.03	368±80	0
control # 3	.01±.003	.02±.02	152±33	0
A. pantherina	.02±.01	.03±.01	195±70	11±10
A. muscaria	.02±.01	.05±.02	249±132	15±9
P. vernalis	.03±.01	.03±.02	165±79	12±13
*I. lacera**	.01±.003	.02±.01	190±109	0
control # 4	.01±.003	.01±.01	102±39	0

*yeast present

Table 17. P-values for a comparison of growth parameters of fungally inoculated aspen seedings and appropriate uninoculated control seedlings using the Wilcoxon non-parametric t-test. P-values ≤ .05 are significant.

Fungal species	stem diam	height	plant dry wt	leaf width	leaf length	petiole length
P. tinctorius	.0004	.1517	.0009	.0407	.0366	.0503
C. graniforme	.0006	.0017	.0016	.0366	.3059	.0259
P. croceum	.0099	.0093	.0003	.2057	.0337	.0863
C. piperatus	.0125	.0009	.0003	.0022	.0017	.0017
T. terreum	.0002	.1594	.0002	.0019	.0004	.0013
A. pantherina	.0526	.0610	.0003	.0885	.0376	.2723
A. muscaria	.0007	.0034	.0002	.0058	.0028	.0006
P. vernalis	.0045	.1933	.0004	.0752	.0538	.0140
I. lacera*	.2939[1]	.3676	.2446	.4616[1]	.3579	.2695

Fungal species	leaf number	leaf sur.area	shoot dry wt	root dry wt	# of rttips	rt:st
P. tinctorius	.0228	.0097	.0004	.0009	.0088	.0561
C. graniforme	.0680	.0677	.0004	.0022	.0051	.3508
P. croceum	.3170	.3902	.1023	.0004	.3913	.0009
C. piperatus	.1556	.0004	.0004	.0005	.0003	.9674
T. terreum	.3778	.0002	.0002	.0002	.0002	.4727
A. pantherina	.2975	.0634	.0376	.0003	.0113	.0539
A. muscaria	.0413	.0015	.0010	.0010	.0073	.0312
P. vernalis	.0329	.0072	.0006	.0041	.0695	.9698
I. lacera	.0104[1]	.0199[1]	.0429	.1590	.0982	.0120

[1] mean less than control
*yeast present

Table 18. Condition of fungally inoculated and uninoculated seedlings of P. tremuloides after 3 months.

Fungal species	root:shoot dry wt.	mortal.	color of plants
P. tinctorius	2.0±.7	20%	dark green, black leaf tips
C. graniforme	1.6±.7	20%	yellow-green, black leaf tips
control # 1	1.2±.6	0%	green leaves, no necrosis
P. croceum	3.4±1.1	10%	yellow-green, black leaf tips
C. piperatus	1.6±1.5	10%	green leaves black leaf tips
control # 2	1.4±.8	0%	green leaves, no necrosis
T. terreum	1.8±.9	0%	green leaves no necrosis
control # 3	1.5±.7	0%	green leaves no necrosis
A. pantherina	2.1±.9	0%	green leaves no necrosis
A. muscaria	2.3±1.1	0%	green leaves no necrosis
P. vernalis	1.7±1.6	0%	red/yellow/green black leaf tips
I. lacera*	3.3±1.9	0%	a few green leaves most leaves black
control # 4	1.4±.4	0%	green leaves no necrosis

*yeast present

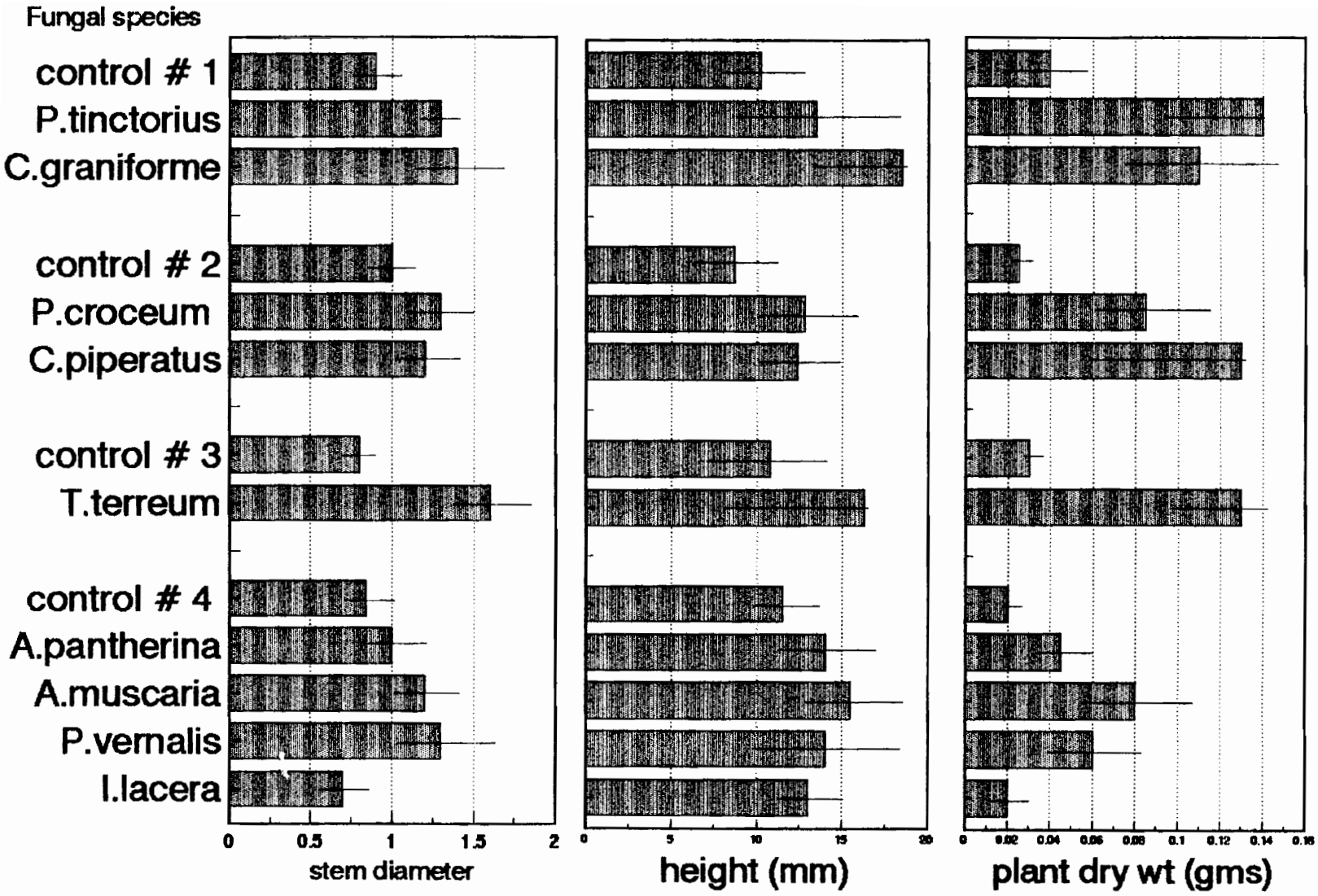


Figure 33. A comparison of the stem diameter, height and plant dry weight of P.tremuloides seedlings inoculated with 9 fungal isolates and uninoculated control seedlings after 3 months.

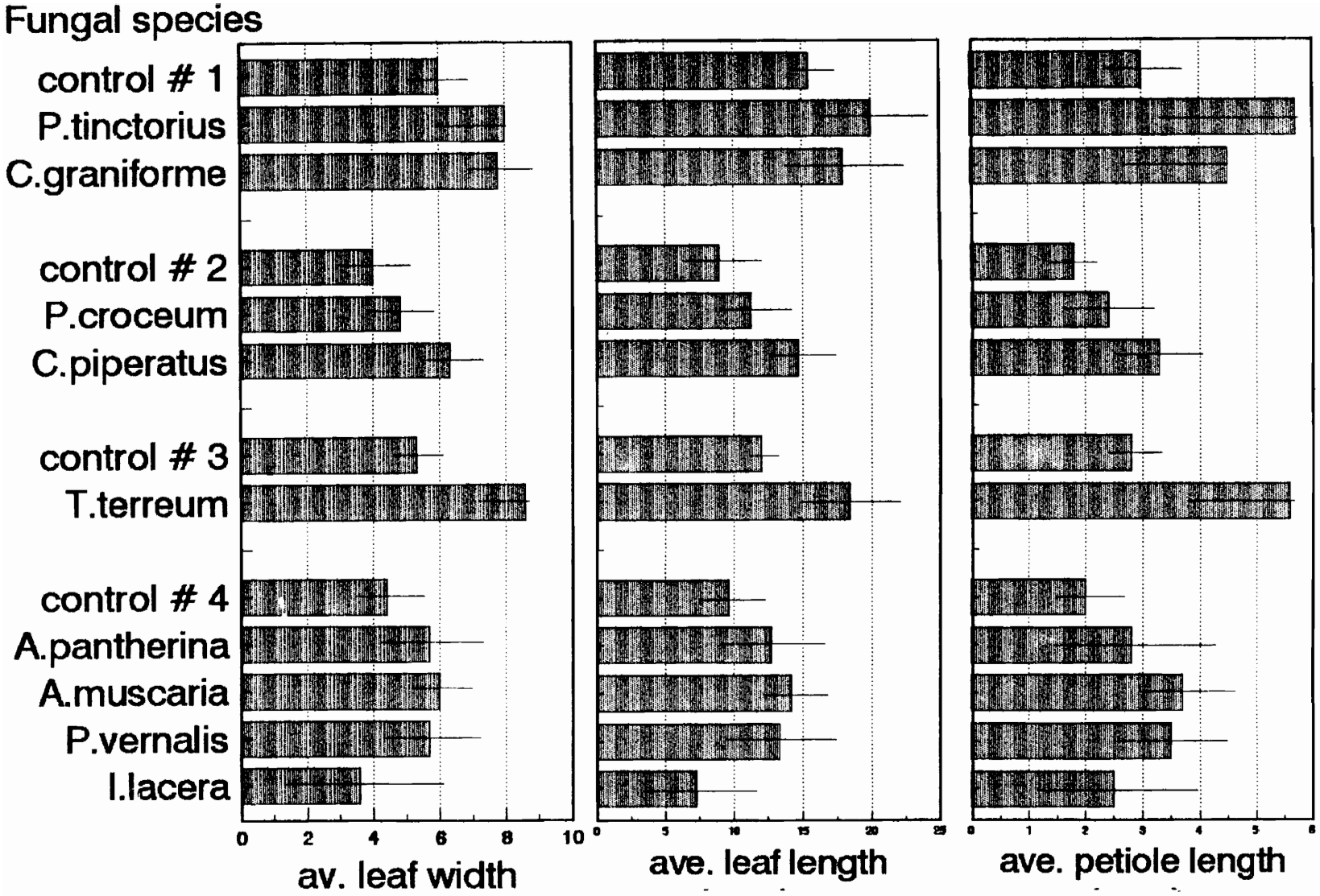


Figure 34. A comparison of the average leaf width, leaf length and petiole length of P.tremuloides seedlings inoculated with 9 fungal isolates and uninoculated control seedlings after 3 months.

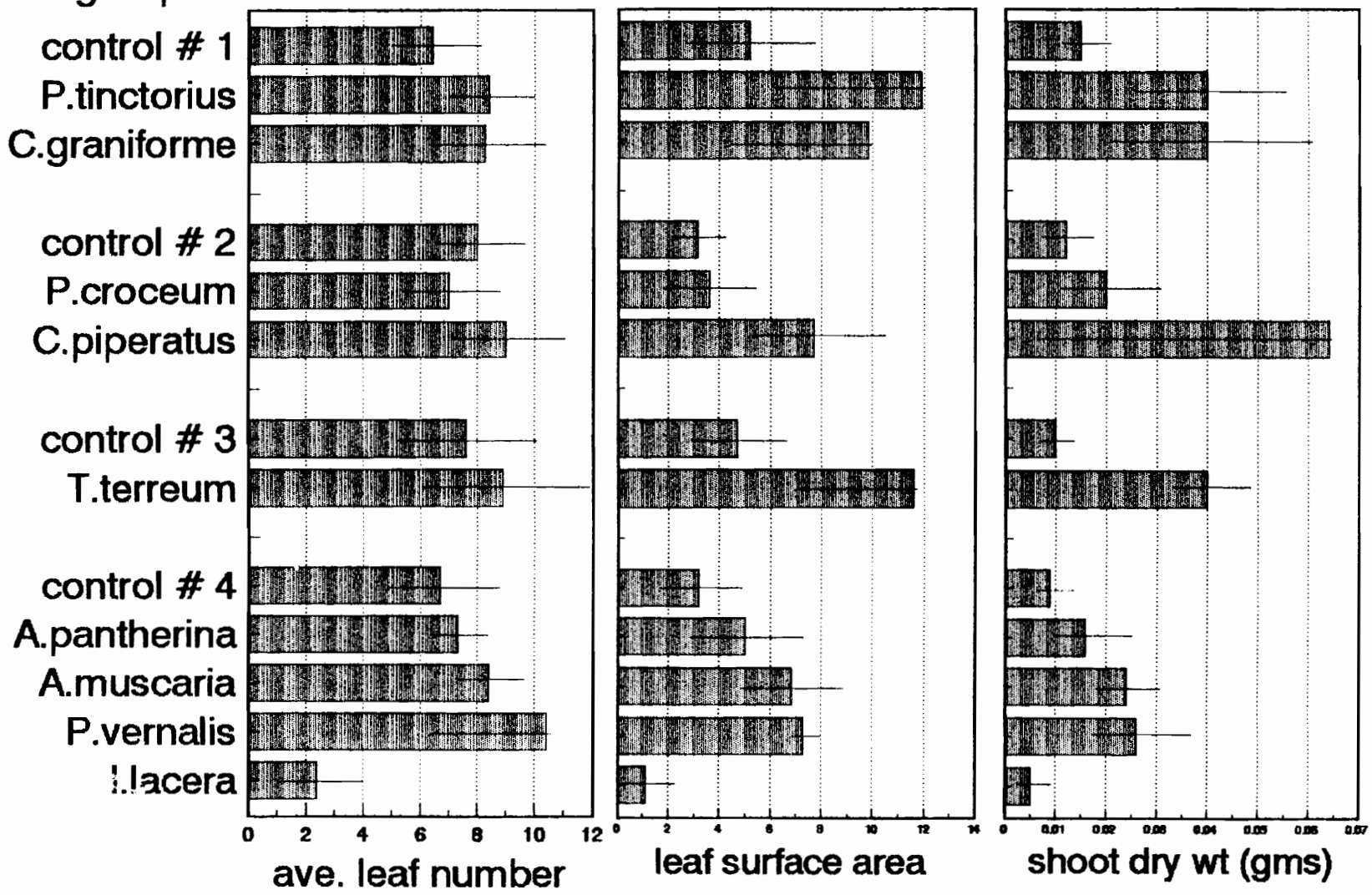


Figure 35. A comparison of the leaf number, leaf surface area and shoot dry weight of P.tremuloides seedlings inoculated with 9 fungal isolates and uninoculated control seedlings after 3 months.

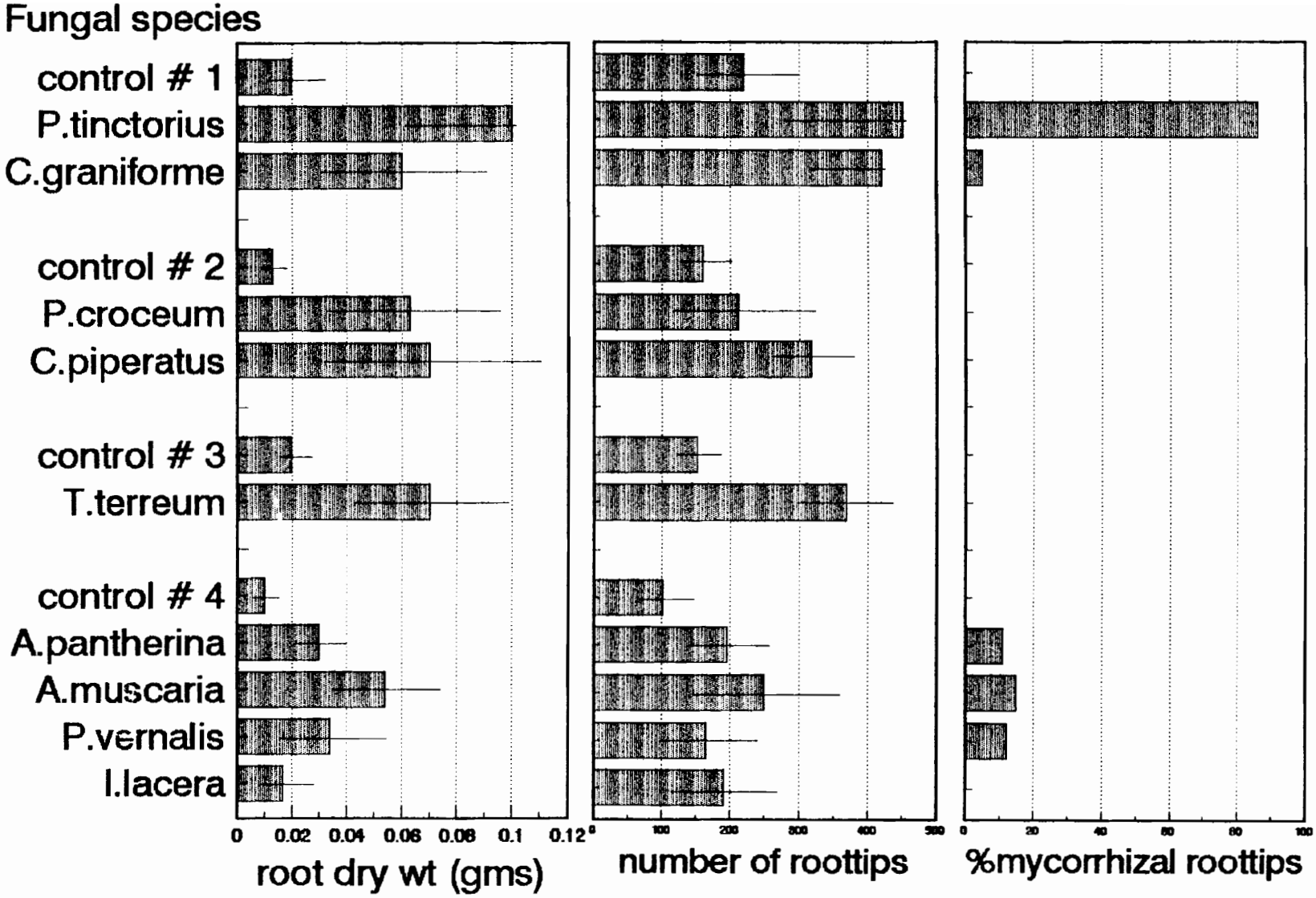


Figure 36. A comparison of root dry weight, number of roottips and % mycorrhizal root tips of P.tremuloides seedlings inoculated with 9 fungal isolates and uninoculated control seedlings after 3 months.

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*references used for identification of fungi

APPENDIX

1. Ectomycorrhizal Fungi collected on the three study sites in 1990 and 1991

2. Protocol for sterilization of aspen seed.

Table 19. Collections from Butte site.

coll #	species	date collected
CLC 076*	*Inocybe lacera*	6/21/90
CLC 077	*Laccaria laccata*	6/21/90
CLC 083	*Laccaria tortilis*	6/21/90
CLC 084	*Leccinum aurantiacum*	6/27/90
CLC 085	*Thelephora terrestris*	6/27/90
CLC 086	*Paxillus vernalis*	6/27/90
CLC 091	*Paxillus vernalis*	7/07/90
CLC 092	*Amanita muscaria* v. *formosa*	7/07/90
CLC 093	*Leccinum aurantiacum*	7/07/90
CLC 103*	*Paxillus vernalis*	7/07/90
CLC 120	*Inocybe* sp. # 1	8/08/90
CLC 158b	*Inocybe lacera*	6/07/91
CLC 160	*Hebeloma mesophaeum*	6/07/91
CLC 162b	*Inocybe lacera*	6/07/91
CLC 168	*Leccinum aurantiacum*	6/21/91
CLC 169	*Paxillus vernalis*	6/21/91
CLC 170a	*Hebeloma mesophaeum*	6/21/91
CLC 171	*Inocybe* sp. # 1	6/21/91
CLC 172	*Laccaria laccata*	6/21/91
CLC 173	*Inocybe lacera*	6/21/91
CLC 197	*Paxillus vernalis*	7/05/91
CLC 198	*Paxillus vernalis*	7/05/91
CLC 199	*Leccinum aurantiacum*	7/05/91
CLC 200	*Laccaria laccata*	7/05/91
CLC 201	*Inocybe* sp. # 6	7/05/91
CLC 202	*Inocybe* sp. # 1	7/05/91
CLC 205	*Phylloporus rhodoxanthus*	7/05/91
CLC 207	*Hebeloma mesophaeum*	7/05/91
CLC 208	*Laccaria tortilis*	7/05/91
CLC 225	*Russula claroflava*	7/17/91
CLC 226	*Thelephora terrestris*	7/17/91
CLC 227	*Leccinum aurantiacum*	7/17/91
CLC 228	*Paxillus vernalis*	7/17/91
CLC 229	*Cortinarius trivialis*	8/03/91
CLC 243	*Xerocomus subtomentosus*	8/03/91
CLC 244	*Lactarius controversus*	8/03/91
CLC 245	*Leccinum aurantiacum*	8/03/91
CLC 246	*Leccinum aurantiacum*	8/03/91
CLC 247	*Paxillus vernalis*	8/03/91
CLC 248	*Cortinarius trivialis*	8/03/91
CLC 249	*Russula alutacea*	8/03/91
CLC 252	*Inocybe geophylla*	8/03/91

*located in Virginia Tech Herbarium: CLC 076 = VTMH 673 (culture VT 2241); CLC 103A = VTMH 676 (culture VT 2242).

Table 20. Collections from Cinnabar site.

coll #	species	date collected
CLC 061*	Tricholoma terreum	6/17/90
CLC 063	Hebeloma mesophaeum	6/17/90
CLC 064	Cortinarius sp. # 2	6/17/90
CLC 068	Cortinarius sp. # 3	6/17/90
CLC 070	Laccaria tortilis	6/17/90
CLC 088	Leccinum aurantiacum	6/29/90
CLC 094	Amanita muscaria v. formosa	7/09/90
CLC 095	Paxillus vernalis	7/09/90
CLC 096	Leccinum aurantiacum	7/09/90
CLC 100*	Amanita pantherina	7/18/90
CLC 101*	Amanita muscaria v. formosa	7/18/90
CLC 102a	Leccinum aurantiacum	7/18/90
CLC 106	Lactarius controversus	7/27/90
CLC 107	Inocybe rimosa	7/27/90
CLC 108	Amanita muscaria v. formosa	7/27/90
CLC 109	Russula alutacea	7/27/90
CLC 115	Inocybe rimosa	7/02/90
CLC 116	Lactarius controversus	8/02/90
CLC 117	Russula alutacea	8/02/90
CLC 118	Russula xerampelina	8/02/90
CLC 124	Hebeloma sp. # 1	8/11/90
CLC 125	Hebeloma mesophaeum	8/11/90
CLC 126*	Chalciporus piperatus	8/11/90
CLC 153	Hebeloma mesophaeum	6/05/90
CLC 154	Hebeloma mesophaeum	6/05/91
CLC 155	Hebeloma sp. # 2	6/05/91
CLC 156	Hebeloma sp. # 2	6/05/91
CLC 175	Laccaria tortilis	6/24/91
CLC 177	Cortinarius trivialis	6/24/91
CLC 178	Leccinum aurantiacum	6/24/91
CLC 179	Inocybe sp. # 3	6/24/91
CLC 180	Cortinarius sp. # 3	6/24/91
CLC 181	Inocybe sp. # 4	6/24/91
CLC 190	Amanita muscaria v. formosa	7/03/91

*located in the Virginia Tech Herbarium: CLC 061 = VTMH 674 (culture VT 2243); CLC 100 = VTMH 671 (culture VT 2239); CLC 101 = VTMH 672 (culture VT 2238); CLC 126 = VTMH 675 (culture VT 2240).

Table 20(continued). Collections from Cinnabar site

coll #	species	date collected
CLC 191	Leccinum aurantiacum	7/03/91
CLC 192	Cortinarius trivialis	7/03/91
CLC 193	Cortinarius sp. # 3	7/03/91
CLC 194	Laccaria tortilis	7/03/91
CLC 195	Inocybe sp. # 6	7/03/91
CLC 211	Leccinum insigne	7/15/91
CLC 212	Leccinum aurantiacum	7/15/91
CLC 213	Leccinum aurantiacum	7/15/91
CLC 214	Leccinum aurantiacum	7/15/91
CLC 215	Leccimum aurantiacum	7/15/91
CLC 216	Amanita muscaria v. formosa	7/15/91
CLC 218	Paxillus vernalis	7/15/91
CLC 220	Cortinarius sp. # 3	7/15/91
CLC 221	Laccaria tortilis	7/15/91
CLC 232	Inocybe sp. # 3	7/15/91
CLC 257	Tricholoma terreum	8/11/91
CLC 258	Paxillus vernalis	8/11/91
CLC 260	Hebeloma sp. # 1 (Cort?)	8/11/91
CLC 262	Lactarius controversus	8/11/91
CLC 263	Russula alutacea	8/11/91
CLC 264	Cortinarius sp. # 3	8/11/91
CLC 266	Inocybe rimosa	8/11/91
CLC 267	Leccinum insigne	8/11/91
CLC 268	Leccinum aurantiacum	8/11/91
CLC 276	Cortinarius sp. # 3	8/11/91
CLC 310	Leccinum aurantiacum	8/28/91
CLC 311	Amanita pantherina	8/28/91
CLC 313	Hebeloma sinapizans	8/28/91
CLC 314	Floccularia straminea	8/28/91
CLC 316	Hebeloma sp. # 1 (Cort)	8/28/91
CLC 317	Russula alutacea	8/28/91
CLC 318	Russula alutacea	8/28/91
CLC 321	Inocybe rimosa	8/28/91
CLC 322	Tricholoma terreum	8/28/91
CLC 323	Amanita vaginata	8/28/91

Table 21. Collections from Teton site.

coll #	species	date collected
CLC 029	Cortinarius sp. # 1	6/02/90
CLC 030	Geopora cooperi	6/02/90
CLC 049	Cortinarius trivialis	6/12/90
CLC 054	Geopora cooperi	6/12/90
CLC 078	Leccinum aurantiacum	6/23/90
CLC 089	Leccinum aurantiacum	7/04/90
CLC 098	Leccinum aurantiacum	7/13/90
CLC 099	Amanita muscaria v. formosa	7/13/90
CLC 110	Amanita constricta	7/29/90
CLC 111	Amanita muscaria v. formosa	7/29/90
site visited, no mycorrhizal fungi		6/10/91
CLC 182	Amanita vaginata	7/01/91
CLC 184	Cortinarius trivialis	7/01/91
CLC 185	Leccinum aurantiacum	7/01/91
CLC 187	Inocybe sp. # 5	7/01/91
CLC 233	Lactarius pubescens	7/21/91
CLC 234	Russula alutacea	7/27/91
CLC 235	Leccinum aurantiacum	7/27/91
CLC 236	Leccinum insigne	7/27/91
CLC 237	Paxillus vernalis	7/27/91
CLC 238	Cortinarius trivialis	7/27/91
CLC 239	Amanita vaginata	7/27/91
CLC 283	Leccinum aurantiacum	8/24/91
CLC 284	Leccinum aurantiacum	8/24/91
CLC 285	Leccinum insigne	8/24/91
CLC 286	Leccinum aurantiacum	8/24/91
CLC 287	Inocybe rimosa	8/24/91
CLC 288	Chalciporus piperatus	8/24/91
CLC 290	Lactarius controversus	8/24/91
CLC 291	Amanita fulva	8/24/91
CLC 292	Cortinarius trivialis	8/24/91
CLC 293	Leccinum aurantiacum	8/24/91
CLC 296	Lactarius pubescens	8/24/91
CLC 299	Russula aeruginea	8/24/91
CLC 309	Inocybe sp. # 3	8/24/91

Protocol for the sterilization of Populus tremuloides seed

Synopsis:

1. soak seeds in 15% chlorox for 15 min.
2. 3 rinses in distilled water, 10 min. each
3. germinate seeds on modified Hagems agar
4. allow seeds to grow 23-30 days

Since aspen seeds are small, technique and handling seem to be of prime importance. A few hints are listed below.

1. Autoclave four 50 ml. beakers partially filled with distilled water and covered with aluminum foil for 20 min. at 20 psi.

2. Add enough Chlorox to the first beaker to produce a 15% solution, also add two drops of "tween" (a surfactant).

3. Add seeds and gently swirl the solution for 15 minutes.

4. Pour off the chlorox solution, pour seeds into the next beaker of water, quickly cover with foil, and swirl the seeds in the rinse water for 10 minutes.

5. Pour off rinse water, pour seeds into the next beaker and repeat the rinse procedure two more times for 10 minutes each.

6. Use sterile utensils to transfer the seeds to petri dishes of modified Hagems made with 11 grams of agar per liter.

7. Place in growth chamber with adequate lighting.

8. Periodically remove contaminanted seeds.

9. Wrap plates in parafilm after about 5 days to prevent drying.

10. Seedlings are ready to use in 23 to 30 days.

VITA

Cathy Lynn Cripps was born January 18, 1948 to Harold and Mary Cripps in Battle Creek, Michigan where she grew up with her sister Cindy (now a botanist for the Forest Service) and her brother Jeff (now a veterinarian). She attended Lakeview High School and received her B.S. in Science from the University of Michigan in 1971.

Cathy lived in a cabin in the Elk mountains north of the small mining town of Crested Butte, Colorado for several years. Here, she participated in typical Crested Butte activities such as hiking, camping, fishing, bicycling, canoeing, and skiing. She supported these avocations by fighting forest fires, doing construction work, caretaking the Y bar J ranch, and working at the ski area. An interest in edible mushrooms and fungal taxonomy led her to write a series of articles for the Crested Butte Chronicle on mushroom identification and collection. Cathy is a member of the Colorado Mycological Society, the North American Mycological Association, and the Mycological Society of America. She attended a course in mycology taught by Dr. Orson K. Miller, Jr., at the University of Montana Biological Station in 1985. Cathy attended classes at Western State college in Gunnison, Colorado and at the Rocky Mountain Biological Station in Gothic, Colorado from 1985 to 1989. She was supported in

these endeavors by several research grants which resulted in 1) a study of algal populations near a proposed dam site, 2) a study of the effects of sewage effluents on algal populations, and 3) a study of the vegetational response to sewage sludge dumping on sagebrush land. She assisted Dr. O.K. Miller, Jr. with his mycology course at the University of Montana Biological Station in the summer of 1989. In the fall of that year she headed "East" to attend Virginia Polytechnic Institute and State University as a Master's candidate which resulted in a thesis on aspen mycorrhizae which continues a long-standing interest in Populus tremuloides. She hopes to return to the Rocky Mountains in the future.