AN ABSTRACT OF THE THESIS OF

Bradley Ray Kropp for the degree of Doctor of Philosophy in Botany and Plant Pathology presented on March 19, 1981.

Title: THE MYCORRHIZAL ASSOCIATIONS OF WESTERN HEMLOCK

Abstract approved: _____
Dr. James M. Trappe

Two objectives of this research were to identify fungi which are mycorrhizal with western hemlock and to examine the ecology of hemlock mycorrhizal fungi indigenous to outplanting sites. Another objective was to evaluate the effectiveness of mycorrhizal inoculation in improving the performance of outplanted hemlock seedlings.

A list of over 100 mycorrhizal fungi associated with hemlock in the field was compiled. Many of these were tested in pure culture synthesis showing that 18 of them were indeed capable of mycorrhiza formation with hemlock. Due to the frequency with which mycorrhizal hemlock seedlings are found on rotten wood, an additional 18 wood inhabiting fungi were tested with hemlock in pure culture synthesis. Five of these proved to be capable of mycorrhiza formation. Field observations indicate that four more are mycorrhizal with hemlock growing in rotten wood.

Nonmycorrhizal western hemlock seedlings planted on two clearcuts in western Oregon were readily colonized by indigenous mycorrhizal fungi. Examination of excavated seedlings every two weeks showed a gradual increase in the proportion of short roots colonized by mycorrhizal fungi through the season. The first mycorrhizae appeared two

months after planting.

Another experiment showed that nonmycorrhizal containerized hemlock seedlings survived and grew well on both rotten wood and mineral soil. Mycorrhizal colonization took place at the same rate and in the same total numbers in rotten wood and mineral soil.

Finally, two outplanting studies were done to compare the performance of mycorrhizal and uninoculated hemlock seedlings. The first of these compared the survival and top growth of two year old containerized western hemlock seedlings outplanted on three plots in each of two areas. Mycorrhizal inoculation significantly improved seedling top growth for two years after planting in both of the areas. Survival was not improved in either area by mycorrhizal inoculation. The second study compared survival, root growth and top growth for outplanted mycorrhizal and uninoculated seedlings on both mineral soil and rotten wood. No growth or survival differences between mycorrhizal and uninoculated seedlings were seen on either substrate the first season after planting.

Two additional experiments testing rotten wood as mycorrhizal inoculum were done to support the above observations. They demonstrated that rotten wood can indeed serve as a habitat for mycorrhizal fungi.

THE MYCORRHIZAL ASSOCIATIONS OF WESTERN HEMLOCK

by

Bradley Ray Kropp

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed March 19, 1981

Commencement June 1981

APPROVED:

Redacted for Privacy

Professor of Botany and Plant Pathology in Charge of Major

Redacted for Privacy

Chairman of the Department of Botany and Plant Pathology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented March 19, 1981

Typed by Dianne Webster for Bradley Ray Kropp

ACKNOWLEDGEMENT

I would like to express my deep appreciation and gratitude to Dr. James M. Trappe for serving as my major professor and for placing confidence in my abilities.

Special thanks are extended to Crown Zellerbach Corporation for providing financial support and to the U. S. Forest Service Pacific Northwest Forest and Range Experiment Station, Forestry Science Laboratory for graciously allowing the use of their facilities.

I would like to thank John Hazard and Pat Williams of the U. S. Forest Service Pacific Northwest Forest and Range Experiment Station and also Arvydas Grybauskas of the Botany and Plant Pathology Department for their valuable statistical help.

The technical aid of John Chamard is gratefully acknowledged.

Additional gratitude is extended to the members of my committee, especially to Dr. Robert G. Linderman for providing particularly helpful advice on several occasions.

Finally, I would like to express appreciation to my friends and to members of the Botany and Plant Pathology Department for their friendship and support throughout my program.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE MYCORRHIZAL ASSOCIATIONS OF WESTERN HEMLOCK

CHAPTER I

INTRODUCTION

With increasing pressure being placed on our forest resources, managers are continually looking for methods of improving forest production and utilization. In the Pacific Northwest, much of the emphasis to date has been placed on management of Douglas fir. It is known that on many of the wetter sites in the northwest where Douglas fir has traditionally been planted, western hemlock can produce much more total wood fiber per acre in a given period of time. Thus, western hemlock, although once considered less valuable than Douglas fir, is now receiving considerable attention for use in reforestation.

Recent research has made it possible for nurseries to produce plantable containerized hemlock seedlings in a little less than a year. Although numerous experiments have been done to demonstrate the benefits of mycorrhizal fungi on the field performance of nursery grown tree seedlings, no work of this type has been done with western hemlock. Since most hemlock seedlings are commercially produced in containers, there is little opportunity for them to become mycorrhizal naturally. This situation provides an ideal opportunity to inoculate them artificially.

Thus, some rather exciting possibilities exist for applying mycorrhizal technology to hemlock regeneration. The research

presented in this thesis investigates the mycorrhizal associations of western hemlock and evaluates their potential to aid seedlings planted on reforestation sites.

CHAPTER II

LITERATURE REVIEW

A. Western hemlock management and ecology

Western hemlock (Tsuga heterophylla (Raf.) Sarg.) is one of the four major timber producing trees in the Pacific Northwest (Harlow and Harrar, 1969). It is distributed from northern California along the Pacific coast to the Kenai peninsula in Alaska and along the Cascade Mountains of Oregon and Washington. It is also found in the northern Rocky Mountains where it is usually limited to north facing slopes and moist creek bottoms (Fowells, 1965).

In general, the climatic regime in which hemlock occurs is characterized by high rainfall often in excess of 160 inches (Vierick and Little, 1975). In the northern Rocky Mountains, annual precipitation may be much lower with 25-50 inches falling (Fowells, 1965). Temperatures are generally moderate with the Rocky Mountain portion being relatively severe compared with the maritime areas. The coastal areas are characterized by high humidity, prolonged cloudy periods and frequent summer fog.

Western hemlock is generally considered to be less valuable for wood production than Douglas fir, but this attitude of management towards hemlock has been changing over the past several years (Richen, 1976).

Douglas fir is in many cases planted within the coastal western hemlock/Sitka spruce zone, thus extending its range westward. This

is in part justified by the higher value of Douglas fir as compared to hemlock. Hemlock values have recently increased relative to Douglas fir (Ruth and Harris, 1979).

Work done by Weyerhaeuser corporation (Steinbrenner, 1976) indicates that western hemlock has, on many soils, the potential to produce more wood fiber per acre than Douglas fir. This work indicates that hemlock on certain coastal soils has the potential for a 33% increase in wood fiber yield over Douglas fir even though the site index for Douglas fir on those soils is higher (130 as compared to 113 for western hemlock). On glaciated soils, where the site index for hemlock is closer to that for Douglas fir, hemlock has the potential for a 48% yield increase compared with Douglas fir. Much of the reason for this difference is that although hemlock site index is often lower on a given site than Douglas fir, hemlock can grow in denser stands and contains more volume than Douglas fir of the same diameter and height (Wiley, 1976). There appears to be a growing emphasis on planting western hemlock in many parts of its range.

Western hemlock is generally considered to be the climax species throughout most of its range (Ruth and Harris, 1973). It is very shade tolerant and, as a result, is able to reproduce under stands of Douglas fir or other species. Sitka spruce is less shade tolerant than hemlock. It is thought that even within the coastal hemlock/spruce type (which is often considered climax), hemlock is the true climax (Fowells, 1965).

Natural regeneration is in most instances prompt and produces

full or often over-stocked stands. Hemlock is a frequent and prolific seed producer. Heavy seed production occurs every 5-8 years with failures being unusual (Williamson, 1976). Seed dispersal can occur over distances as great as 3,900 feet. Amount of seedfall decreases sharply for the first 1300 feet and levels off for greater distances (Harris, 1967).

The seed germinates and grows well on a variety of seedbeds, including duff and rotten wood (Ruth and Harris, 1973; Williamson, 1976). Since hemlock is shade tolerant, seedlings often become established under old growth forests on rotten logs and stumps. After logging, these seedlings and other advance reproduction are often replaced by new regeneration from seed. This occurs over a period of about 10 years during which advance regeneration dies from exposure and the proportion of new regeneration reaches 80 percent (MacBean, 1941).

Hemlock can also germinate and grow on rotten wood in clearcuts. Berntsen (1955) found that on one clearcut in coastal Oregon, rotten wood (42% of the total plot area) was 97 percent stocked, whereas mineral soil (26 percent of the total area) was 83 percent stocked. The rotten wood had provided an excellent seedbed for trees but a poor one for competing vegetation.

Although planting conifer seedlings in organic matter, especially humus or rotten wood, is avoided by foresters, it may be a suitable practice for western hemlock. Berntsen (1960) showed that survival of Douglas fir and Sitka spruce planted on rotten wood was as good as on soil on one clearcut in western Oregon. Growth was also as good or

better on rotten wood than on soil. He concluded that planting on rotten wood is a sound practice in coastal areas with an average of 25 cm growing season precipitation. It is logical that the same thinking would also apply to western hemlock.

Rotten wood can cover a substantial portion of the forest floor. In an old growth Douglas fir ecosystem in the western Cascades, rotting logs from five different log decay classes covered a total of 13.2 percent of the forest floor (K. Cromack, personal communication). In the case of the plot examined by Berntsen (1955), nearly half (42%) of the plot area was rotten wood. Thus, if planting hemlock on decayed wood is a sound practice, wasteful openings can be avoided. Seedlings planted on rotten wood might also be relatively free from competing vegetation. Hemlock often colonizes brush covered clearcuts by becoming established on rooting logs and stumps (Ruth and Harris, 1979).

Examination of natural hemlock seedlings on rotten logs shows them to have mycorrhizal roots. Sporocarps of many fungi, some of which may be mycorrhizal, are found on rotten wood. Decayed wood has been shown to be an important substrate for ectomycorrhizal activity by the work of Harvey et al. (1976). This work showed that decayed wood supports a substantial portion of the total number of ectomycorrhizae in the Douglas fir/larch stands in Montana. They also found that rotten wood supports more mycorrhizal activity than other soil fractions, during the drier parts of the growing season in Montana (Harvey et al., 1979).

Since rotten wood is such an important factor in the natural regeneration of western hemlock, an evaluation of the mycorrhizal status of hemlock on wood is called for. If nursery seedlings can

potentially be planted into rotten wood, the mycorrhizal fungi in the wood may have an important effect on the performance of the seedlings.

The site preparation required for western hemlock varies with the types of seedlings to be planted. Seedlings are adversely affected by direct sunlight and by water stress. They are also limited by dense shade, but larger seedlings can benefit from partial shade. Litter from brushy species or from ferns may be inhibitory, especially if the seedlings are small (Newton, 1976). Large seedlings require minimal site preparation. Work by Newton (1978) has shown that large seedlings (24") perform relatively well under brush and require only one or two aerial herbicide applications.

Site preparation requirements for planting smaller seedlings or direct seeding are the most exacting. Direct seeding requires moist soil, protection from sun, and nearly total absence of broadleaf vegetation (hence, the common occurrence of natural hemlock regeneration on rotten logs under mature forests). Small seedlings also need protection from water stress but can do well with some herbaceous cover (Newton, 1976). The proper site requirements can be obtained by crushing slash along with light scarification, or in some cases burning the slash.

Production of bareroot western hemlock seedlings in outdoor nursery beds can be difficult because of the susceptibility of the germinants to environmental conditions. Containerized production of hemlock seedlings is often the preferred method. The techniques for raising hemlock in containers have been well worked out (Ruth and Harris, 1979).

Comparisons of field performance of containerized and bareroot hemlock seedlings have been made by Arnott (1975, 1976). Containerized hemlock consistently survive better in British Columbia than do bareroot seedlings. He has also found that growth of containerized and bareroot stock are equivalent. Although, in one area in British Columbia, bareroot seedlings grew substantially more than containerized stock (Arnott, 1975).

Other advantages to using containerized hemlock stock are: faster and more flexible production schedules, decreased planting shock because of less root disturbance, easier planting, higher quality seedlings and greater ability to manipulate the growing environment. Disadvantages would be: higher production and transportation costs, smaller seedlings, increased losses from frost heaving and a tendency towards abnormal root growth (though Arnott (1976) found that spiralling was not a problem) (Ruth and Harris, 1979). Another important aspect of containerized seedling production could be the relative ease with which mycorrhizal fungi can be added and manipulated.

There are several silvicultural problems in western hemlock regeneration. One problem is that it regenerates too abundantly under good conditions, resulting in overstocking and competition between the seedlings. This could be remedied by establishing optimum spacing through thinning (Ruth, 1964). According to Ruth (1964), the most important problem in hemlock regeneration is competition from brushy species.

This is supported by a study of natural regeneraion in a large clearcut in Alaska where Harris (1967) found the following types of

non-stocked plots: 1) disturbed soil, which provides good conditions for competing plants, especially alder (Ruth and Harris, 1979), 2) alluvial soils along creek bottoms which become covered with dense salmonberry and other brush, 3) some smaller areas with poor drainage. Ruth (1964) suggested that using herbicides or the use of a shelterwood cutting system could help favor hemlock establishment over other vegetation. Regeneration 17 years after a partial cutting in southeastern Alaska was good but the trees were much smaller than what would be expected of regeneration in a clearcut after that length of time (Farr and Harris, 1971). The use of large seedlings may also be of potential use in the reclamation of brush covered clearcuts (Newton, 1978).

It has been demonstrated many times that ectomycorrhizal fungi have the ability to stimulate the growth of many species of conifer seedlings in the field (Wright, 1957; Marx, 1977; Laiho, 1967; Briscoe, 1959; Vozzo and Hacskaylo, 1971; McComb, 1938; Clements, 1938). If mycorrhizal fungi also give western hemlock seedlings an initial boost in height growth, there could be potential for using mycorrhizal seedlings in reforesting brushy clearcuts. As with many tree species, western hemlock seedlings are subject to outplanting shock. In certain situations, they grow very slowly and are often somewhat chlorotic after planting. Although physiological factors may have an influence on the amount of transplanting shock experienced, the absence of mycorrhizal fungi might also increase transplanting shock on many sites (Marx and Barnett, 1974). Container grown seedlings are often mycorrhizal. They may become inoculated by means of airborne spores, but this is often erratic in its occurrence (Trappe, 1977).

In the introduction of seedlings to formerly treeless areas, mycorrhizal fungi have been shown to be essential to the survival and growth of transplanted seedlings (McComb, 1938; White, 1941). Trappe (1977) suggested that mycorrhizal fungi may not be as essential on sites where the soil contains abundant mycorrhizal inoculum and where seedlings are planted early enough to become mycorrhizal with indigenous fungi before they are under environmental stresses.

With western hemlock, most outplanting sites are expected to contain abundant mycorrhizal inoculum and are relatively mild climatically. In addition, seedlings are often planted as early as February, possibly allowing ample time for mycorrhizal colonization before much environmental stress is placed on the seedlings.

However, even on routine reforestation sites in the southeastern U.S., mycorrhizal fungi have been shown to stimulate seedling survival and growth (Marx, 1977).

Greenhouse studies by Heilman and Ekuan (1980a) showed that a phosphorus deficiency in two soil pedons (Vesta series) from western hemlock stands in coastal Washington was responsible for slow growth and poor mycorrhizal development of Douglas fir seedlings. This work was extended to show that poor growth of hemlock seedlings in the greenhouse could be attributed to a phosphorus deficiency in these soils (Heilman and Ekuan, 1980b). Mycorrhizal fungi have been shown to be important in aiding seedlings in the uptake of nutrients (Bowen, 1973).

Thus, in regenerating western hemlock, mycorrhizal fungi appear to have the most potential for improving seedling performance in areas where brush is a problem by giving seedlings an extra initial growth

boost, on specific soils (such as the ones described by Heilman and Ekuan, 1980a,b) where nutrient or mycorrhizal deficiencies occur, and by reducing outplanting shock on routine reforestation sites.

B. Mycorrhizae and forestry

At this point, it is necessary to discuss separately the application of mycorrhizae to forestry. By the late nineteenth century, Frank (1885) had proposed the hypothesis that certain soil fungi were symbiotic with trees and had coined the term "mycorrhiza" for this fungus-root interaction. He also distinguished between the two types of mycorrhizae: endotrophic and ectotrophic.

In an endotrophic association, the fungal partner actually penetrates into the cells of the host. This type of symbiosis is by far the most widely distributed and accounts for perhaps 95% of the mycorrhizae found in nature. The fungal associates are mostly members of the Endogonaceae. Many forest tree species are endotrophic as well as most herbaceous plants and grasses.

Ectotrophic mycorrhizae, on the other hand, account for only about 5% of the mycorrhizae found in nature. They occur primarily on members of the Pinaceae, Betulaceae, Fagaceae and Salicaceae. They are thus the mycorrhiza type of major importance to forestry in temperate regions. Ectomycorrhizae increase in importance in colder regions, becoming the primary mycorrhizae in subarctic or high elevation coniferous forests and gradually decreasing in relative abundance in tropical regions (Meyer, 1973).

Mycorrhizal fungi may function in several ways to aid trees.

Probably the most important is by increasing the surface area of the root system. This is done by: 1) increasing the branching and diameters, and thus the surface area of the short roots, 2) expanding the absorptive potential of the roots by the increased surface area of mycelial strands and hyphae from the mycorrhizal rootlets (Bowen, 1973). Schramm (1966) has shown that rhizomorphs of Pisolithus tinctorius can extend for substantial distances from mycorrhizal seedings. That mycelial strands of mycorrhizal fungi are able to transport nutrients was carefully demonstrated using P^{32} by Skinner and Bowen (1974). C^{14} has also been shown to be transported from one seedling to another via mycelial connections, although this varies with the mycorrhizal fungus in question (Reid and Woods, 1969).

Another function of mycorrhizal fungi is to aid the resistance of rootlets to invasion by pathogens. Zak (1964) suggested that the mycorrhizal fungus may stimulate protective microbial populations, utilize surplus carbohydrates produced by the roots and produce antibiotics which would contribute to the roots' resistance to infection. Ectomycorrhizal mantles may also act as a mechanical barrier to infection.

The production of growth regulators by mycorrhizal fungi has been reviewed by Slankis (1973). Culture filtrates from mycorrhizal fungi can stimulate the production of mycorrhiza-like dichotomous short roots as well as increase seedling growth (Shemakhanova, 1962). Byproducts of fungal metabolism or of the joint metabolism of the fungus-root union may also alter the environment around the roots. This could possibly contribute to the "tailoring" of seedlings to hostile

environments.

The importance of ectomycorrhizal associations to forestry first became recognized when various tree species were introduced into parts of the world where their mycorrhizal fungi did not exist. In his review of mycorrhizae in forestry practice, Mikola (1973) mentions a Russian named Vysotskii who, in 1902, was probably the first person to suggest mycorrhizal inoculation in afforestation of grasslands.

Roeloffs (1930) reported that in Asia, Pinus khasya and Pinus merkusii were stunted and yellow in several nurseries where they were being grown. He did notice that in two of the nurseries, certain seedlings began to green up and grow normally. This was attributed to the colonization of these seedlings by mycorrhizal fungi.

Introduced pines failed repeatedly in Nyasaland (Clements, 1938). The seedlings were chlorotic, stunted and eventually died. Again, the failure was attributed to the absence of mycorrhizal fungi. When soil from established pines was introduced into nurseries, seedlings developed normally.

Again, introduced pines in Puerto Rico failed to grow and survived poorly even though many types of treatments, including fertilization, were applied. When mycorrhizal inoculation was accomplished by either importing forest soil or mycorrhizal trees, the seedlings survived satisfactorily and grew rapidly (Briscoe, 1959). Vozzo and Hacskaylo (1971) found that inoculation of pines with pure cultures of mycorrhizal fungi in Puerto Rico improved their performance compared to fertilized nonmycorrhizal seedlings. In the United States, McComb (1938) found that pine seedlings in a prairie soil in Iowa were often stunted and

unhealthy in appearance. Comparison with healthy seedlings showed a mycorrhizal deficiency to be the cause. White (1941) supported this work by showing that a Wisconsin prairie soil produced small seedlings when compared to a forest soil. The prairie soil was not found to be nutrient deficient and the difference was attributed to the lack of mycorrhizae in this soil.

This general scenario has been repeated numerous times in afforestation attempts throughout the world (Mikola, 1970).

Mycorrhizal fungi can benefit seedlings on other types of sites which are, for one reason or another, deficient in mycorrhizal fungi. Strip mined areas are a good example, having surface material which is physically and chemically very harsh and is often a virtual biological desert (Marx, 1975). Schramm (1966) concluded that ectomycorrhizae were essential for the establishment of trees on mine spoils. Dramatic improvements in growth and survival of mycorrhizal pine seedlings on mine spoils as compared to controls have been obtained in experimental plantings (Marx, 1975; 1977).

Soils on routine reforestation sites can be expected to contain the fungi necessary to form mycorrhizae with outplanted seedlings. Even so, work has been done showing that mycorrhizal inoculation of nursery seedlings improves their survival and growth when planted on routine sites. Pisolithus tinctorius improved survival and growth of five pine species for two years on reforestation sites in the southeastern United States (Marx et al., 1977). In the Pacific Northwest, Wright (1957) showed that in field plantations of 1000 equal sized mycorrhizal and nonmycorrhizal ponderosa pine seedlings, mycorrhizal

seedlings survived the first season significantly better. This difference was not seen on a site with bunch-grass competition. Laiho (1970) found that Paxillus involutus was beneficial to out-planted pine seedlings but that it was a less efficient symbiont than other fungi. He also found that the response of mycorrhizal Scotch pines planted on several types of sites varied (Laiho, 1967). Shoot weights of mycorrhizal seedlings on some sites were much larger than the weights of the controls and about equivalent to the controls on others. In afforestation work done with pines in the Soviet Union, seedlings inoculated with Cenococcum geophilum showed a range in responses depending on the amount of humus in the soil. Seedling dry weight in sandy soil with a high humus content was 118% of the control but in sandy soil with very little humus, it was 231% of the control. For seedlings inoculated with Boletus luteus, the seedling response varied from 105% of the control to an actual depression in dry weight (Shemakhanova, 1962).

Nursery inoculation with ectomycorrhizal fungi has been shown to produce larger seedlings and fewer cull seedlings (Marx et al., 1979). The absence of mycorrhizal fungi in commercial nurseries has also been shown to result in stunted seedlings (Wright, 1957; Trappe and Strand, 1969). The fact that mycorrhizal seedlings may be larger upon out-planting may in itself bring about improved survival or growth in the field.

Thus, the inoculation of seedlings with mycorrhizal fungi has been shown to be of practical use in several aspects of forestry. Potential benefits from using mycorrhizal seedlings can be attained

in afforestation of previously treeless areas and reclamation of disturbed lands as well as on routine reforestation sites.

Routine reforestation can potentially benefit from mycorrhizal inoculation of seedlings in several ways:

a) increased seedling size and fewer culls in nurseries,

b) decreased outplanting shock (by providing a functioning mycorrhizal root system prior to planting),

c) stimulating seedling growth and survival, particularly on harsh or nutrient deficient sites.

The thrust of this thesis is to investigate the mycorrhizal associations of western hemlock and to evaluate their potential to aid seedlings planted on routine reforestation sites.

CHAPTER III

MYCORRHIZA FORMATION ON WESTERN HEMLOCK BY FUNGI INDIGENOUS IN SOIL AND ROTTEN WOOD

Bradley R. Kropp

A. Mycorrhiza formation and performance on nonmycorrhizal western hemlock outplanted on rotten wood and mineral soil.

ABSTRACT

Nonmycorrhizal western hemlock seedlings planted on two clearcuts in western Oregon were readily colonized by indigenous mycorrhizal fungi. The proportion of short roots colonized by mycorrhizal fungi gradually increased through the season from a few in the first 2-3 months to nearly total colonization by fall.

Hemlock seedlings survived and grew well on both rotten wood and mineral soil during the first growing season after outplanting. However, on the most recent clearcut, seedling growth at the end of the season was significantly greater on mineral soil than on rotten wood. No other differences were observed. Mycorrhizal colonization took place at the same time and in the same total numbers in rotten wood and mineral soil. Some mycorrhizal fungi occurred in both rotten wood and soil, but others occurred only in the one or the other substrate.

Western hemlock (Tsuga heterophylla (Raf.) Sarg.) is an important component of timber producting forests in coastal regions of the Pacific Northwest. It generally seeds successfully into clearcuts to produce full or overstocked stands (Ruth and Harris 1973). Hemlock does well

on a variety of natural seedbeds (Ruth and Harris 1973, Williamson 1976), including well decayed wood in coastal areas. Berntsen (1955) found that on one clearcut, rotten wood (42 percent of the total plot area) was 97 percent stocked, whereas mineral soil (26 percent of the total plot area) was 83 percent stocked. The rotten wood had provided an excellent seedbed for trees but a poor one for competing vegetation.

Berntsen (1960) found that survival of Douglas-fir and Sitka spruce planted on rotten wood was as good as on soil in a clearcut in a high rainfall area. Growth was also as good or better on rotten wood than on soil. He concluded that planting on either rotten wood or mineral soil is a sound practice in coastal areas with an average of 25 cm growing season precipitation. Wasteful openings created when planters avoid rotten wood are prevented. Rotting logs from 5 different log decay classes covered a total of 13.2 percent of an old growth Douglas-fir ecosystem in the western Cascades (K. Cromack, personal communication). Faster growth of seedlings on rotten wood might result from the relative freedom from competing vegetation.

Natural hemlock seedlings are mycorrhizal whether growing on soil or rotten wood. However, the manner and rate at which outplanted, nursery grown seedlings become mycorrhizal is largely unknown. In light of recent interest in inoculating nurseries with mycorrhizal fungi, it is useful to examine the mycorrhizal colonization of uninoculated nursery seedlings after outplanting.

This study was undertaken to (1) make observations on the general pattern and rate at which native fungi colonize outplanted nonmycorrhizal western hemlock seedlings on a 6-year-old clearcut, and (2) compare

survival, growth, and mycorrhizal colonization of seedlings planted in decayed wood vs. mineral soil.

MATERIALS AND METHODS

The study consists of two separate outplanting studies. The first, conducted in 1978, was purely observational and was directed towards objective number one. The second was done in 1979 and was directed towards objective number two.

Two clearcuts were used as planting sites in the following experiments. An old clearcut, harvested in 1972, was used in the 1978 outplanting and as part of the 1979 experiment. This clearcut is on a Klickitat gravelly clayloam (30-50% slope). This soil is deep and well drained, it is rocky and developed from basalt parent material. The site used in these outplantings has a northeast aspect. A recent clearcut, harvested in 1977, was used in addition to the above site in the 1979 experiments. This site has an eastern aspect and is on a deep, well drained gravelly loam (Slickrock gravelly loam - 3-25% slope). This soil is derived from sandstone. The study was conducted on Mary's Peak in the Suislaw National Forest in western Oregon. The sites are on the eastern edge of the coast range and receive an average of about 178 cm of precipitation with about 13 cm falling during the May-August growing season. They are at approximately 854 m in elevation.

1978 Outplanting: In mid-April, 109 one-year-old nonmycorrhizal containerized western hemlock obtained from the Crown Zellerbach Corporation nursery at Aurora, Oregon were planted at a 3' x 3' spacing in a 6-year-old clearcut. Beginning with the second week

after planting, five randomly selected seedlings were carefully excavated and examined every two weeks until the end of October. Observations were recorded for each seedling for a) presence or absence of mycorrhizae, b) types of mycorrhiza, c) general pattern of mycorrhizal colonization.

1979 Outplanting: Containerized hemlock seedlings were obtained from the Corwn Zellerbach Corporation nursery at Aurora, Oregon in mid-February 1979 and stored in plastic bags at about 1°C until being outplanted at the end of April 1979. Each tree was examined to insure that it lacked mycorrhizae before planting. The trees were planted on two different clearcuts in the vicinity of the 1978 outplanting. One had been harvested in Winter 1977 and the slash burned in Fall 1978. The other had been cut 7 years previously cut in 1972.

Five pairs of plots were planted on each of the clearcuts. Each plot contained 10 seedlings. One plot of each pair was on well decayed rotten wood which was being incorporated into the soil. The majority of these rotten wood plots were well defined; several consisted of the remains of entire logs into the full length of which trees could be planted. The other plot of each pair was planted in mineral soil adjacent to the rotten wood. The plots were mapped and the trees on each plot numbered from 1-10. Half of the trees were randomly selected for careful excavation and evaluation in July. The remainder were excavated at the end of November 1979.

The percentage of roots of each tree colonized by mycorrhizal fungi was estimated by stereomicroscopic scanning in a 5 class system:

1 = 0-10 percent, 2 = 10-25 percent, 3 = 25-50 percent, 4 = 50-75 percent, 5 = 75-100 percent. Root growth was evaluated as the mean length of the 5 longest roots newly grown from the root plug, and the current year's leader growth in cm was measured. Each mycorrhiza type was briefly described, and the types present on each seedling were recorded. An analysis of variance was used to evaluate differences between rotten wood and mineral soil.

RESULTS AND DISCUSSION

In the 1978 outplanting, the first mycorrhizae (a white type) appeared in June, 2 months after planting. At 3 months, the first black mycorrhizae formed with Cenococcum geophilum Fr. began to appear. Every seedling examined had formed some mycorrhizae by 3½ months, although many root systems were sparsely colonized. By 5½ months, mycorrhizal colonization was heavy on most trees.

On this site, mycorrhizal inoculum of several species (particularly Cenococcum) appears to have been abundant. Some of the fungi occurred in pockets: some trees were colonized by only one type of fungus while others were colonized only by another.

Trees that had been planted by chance in buried rotten wood quickly became heavily mycorrhizal as compared to those in mineral soil. This led to the hypothesis that rotten wood in clearcuts (some possibly being from old nurse-logs) could serve as a reservoir of mycorrhizal inoculum for western hemlock. This hypothesis is supported by the data of Harvey et al. (1976) showing that decayed wood supports a substantial portion of the total number of ectomycorrhizae in the Douglas-fir/larch

stands in Montana and that rotten wood supports more mycorrhizal activity than other soil fractions, during the drier parts of the growing season in Montana (Harvey et al. 1978). Harvey et al. (1979) found that mycorrhizae were concentrated in decayed wood through the growing season, on a site with a chronic moisture deficit.

The 1979 outplanting was designed to test the hypothesis that rotten wood is a reservoir of mycorrhizal inoculum for western hemlock seedlings and to compare first year survival and growth on rotten wood as compared to soil. In the 1979 planting, leader growth of the seedlings on mineral soil was significantly greater than on rotten wood for the recent clearcut. This difference was not observed at mid-season. No leader growth differences occurred on the old clearcut between substrates either mid-season or at season's end (Table 1). No root growth differences were found in the experiment. Survival did not differ significantly between the substrates or clearcuts when evaluated in November. Percent mycorrhiza formation at mid and end of season also did not differ significantly between substrates (Table 1). Mycorrhizae did not form more quickly on trees planted in rotten wood (as hypothesized from the 1978 observations).

Most types of mycorrhizae formed both in rotten wood and mineral soil, although some occurred only in one substrate or the other (Table 2). Types i and k occurred only in rotten wood, whereas type j occurred only in mineral soil. However, these types were found on only a small number of trees. Type c was more common, and occurred entirely in rotten wood except for two trees in soil. Type f occurred primarily on trees in soil although two trees in decayed wood did form

this type of mycorrhizae. Some trees planted in wood either came partially in contact with mineral soil or had roots which grew out into mineral soil, thus encountering both mineral soil and rotten wood fungi. The number of mycorrhiza types did not differ markedly between rotten wood or soil, although a number of types in both environments increased between mid and end of the season. Most mycorrhiza types occurred on both the young and the old clearcuts. Types f, j, and k were seen only on the old clearcut area.

Overall, type b, formed with Ceanococcum geophilum, predominated. At the time of the mid-season sample, it had formed mycorrhizae with only a few trees in rotten wood as compared to mineral soil on both the young and old clearcuts. However, by the end of the experiment, as many trees in wood had Cenococcum mycorrhizae as in soil.

In summary, during the first field season, containerized non-mycorrhizal western hemlock seedlings survived and grew well when planted on both rotten wood and mineral soil. Although there was a decrease in leader growth on one clearcut, those seedlings were healthy and not stunted. There appears to be little reason to avoid planting western hemlock on rotten wood. This concurs with Berntsen's (1960) results with Douglas-fir and Sitka spruce. The site on which this experiment was done receives considerably less growing season rainfall on the average than the site used by Berntsen (about 13 cm as opposed to 25 cm) indicating that rotten wood may be a suitable medium for planting on somewhat drier sites than was suggested in his paper. Because of the often cubical structure of rotten wood, extra care may be needed in planting seedlings in wood to avoid leaving air spaces

around the root plug which would increase mortality.

Mycorrhizae formed quite rapidly on nonmycorrhizal seedlings planted in these Coast Range sites and gradually increased through the season from a few in the first 2-months to nearly total colonization by fall. Colonization appears to occur at the same rate and in the same total numbers in rotten wood as in mineral soil.

LITERATURE CITED

Berntsen, C. M. 1955. Seedling distribution on a spruce-hemlock clearcut. USDA Forest Serv. Pac. Northwest Forest and Range Exp. Stn. Res. Note 119.

Berntsen, C. M. 1960. Planting Sitka spruce and Douglas-fir on decayed wood in Coastal Oregon. USDA Forest Serv. Pac. Northwest Forest and Range Exp. Stn. Res. Note 1973.

Harvey, A. E., M. J. Larsen, and M. F. Jurgensen. 1976. Distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. Forest Sci. 22:393-398.

Harvey, A. E., J. F. Jurgensen, and M. J. Larsen. 1978. Seasonal distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. Forest Sci. 24:203-208.

Harvey, A. E., M. J. Larsen, and M. F. Jurgensen. 1979. Comparative distribution of ectomycorrhizae in soils of three western Montana forest habitat-types. Forest Sci. 24:350-358.

Ruth, R. H., and A. S. Harris. 1973. Silvicultural systems for the major forest types of the United States. USDA Agric. Handbook 445, p. 5.

Williamson, R. L. 1976. Natural regeneration of western hemlock. In Proc. Western Hemlock Management Conference. Coll. Forest Resour., Univ. Wash., Seattle, pp. 166-169.

Table 1. Mean values for root growth, leader growth, survival and mycorrhiza class from the 1979 outplanting.[a/]

		Recent Clearcut		Old Clearcut	
		Rotten Wood	Mineral Soil	Rotten Wood	Mineral Soil
Root Growth:	July	11.23 cm	9.39 cm	11.75 cm	9.98 cm
	Nov.	16.33 cm	16.54 cm	13.37 cm	11.96 cm
Leader Growth:	July	5.20 cm	6.14 cm	6.66 cm	5.52 cm
	Nov.	12.55 cm	18.59 cm	9.49 cm	7.76 cm
Survival:[b/]	Nov.	4.6	4.8	4.4	4.4
Mycorrhiza class:[c/]	July	1.55	1.19	1.12	1.28
	Nov.	4.80	4.92	4.22	4.23

[a/] Analysis of variance shows leader growth in the November sample on the recent clearcut to be significantly greater for trees on mineral soil (.05 level). No other means differed significantly between rotten wood and mineral soil within either clearcut in July or November.

[b/] Mean survival figures are for the 5 trees left for the November sample on each of the 5 plots.

[c/] Mean mycorrhiza classes are based on a visual estimate of the percent colonization for each tree: 1 = 0-10%, 2 = 10-25%, 3 = 25-50%, 4 = 50-75%, 5 = 75-100%.

Table 2. Number of seedlings on which each mycorrhiza type was found.

	Mycorrhiza type:	a		b[a]		c		c		e		f		g[d]		h		i		j		k	
	Substrate:[b]	RW	MS	RW	MS	RW	MS	RW	MS	RW	MS	RW	MS	RW	MS	RW	MS	RW	MS	RW	MS	MW	MS
Recent Clearcut	July	7[c]	10	4	17	9	--	2	7	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Nov.	5	--	19	23	10	1	1	--	10	4	--	--	2	--	12	23	3	--	--	--	--	--
Old Clearcut	July	9	4	7	11	2	--	5	7	3	4	1	5	--	--	--	--	--	--	--	--	--	--
	Nov.	5	7	18	23	--	1	4	8	11	6	1	4	2	3	5	5	2	--	--	2	1	--

[a] Formed with *Cenococcum geophilum*.

[b] RW = rotten wood MS = mineral wood

[c] The number of trees out of a total of 25 (5 per plot in most cases except where trees had not survived) sampled at each time period and on each substrate for each of the two clearcuts.

[d] Types g through k colonized seedlings more slowly, appearing only after the July sample.

B. Rotten wood as mycorrhizal inoculum for containerized western hemlock.

ABSTRACT

Rotten wood served as mycorrhizal inoculum for containerized western hemlock seedlings. Rotten wood from a clearcut was less effective than that collected from within a forest. No pathogens appeared on seedlings grown on either source of wood. Mycorrhizal colonization was slow under the experimental conditions, probably because of the heavy fertilization typical of container operations.

Western hemlock (Tsuga heterophylla (Raf.) Sarg.) is an important timber species in the Pacific Northwest. Because of its good productivity on certain kinds of sites and its value for lumber and pulp, it has recently received increasing attention for use in planting coastal sites.

Hemlock seedlings commonly occur on rotting logs in mature forests (Minore 1972). Berntsen (1955) showed that hemlock seeds naturally and grows on rotten wood as well as mineral soil in one coastal clearcut. Studies in Montana (Harvey et al. 1976, 1978, 1979) have shown that rotten wood is an important substrate for ectomycorrhizal activity and that more active ectomycorrhizae are found in rotten wood than in other soil fractions during the drier period of the growing season. Natural hemlock seedlings on rotten wood are mycorrhizal, and nonmycorrhizal container-grown seedlings planted into rotten wood on clearcuts become mycorrhizal during the

first field growing season (Kropp 1980). In addition, isolates of certain fungi from rotten wood have been demonstrated to form mycorrhizae with hemlock and Douglas-fir (Larsen and Zak 1978, Zak 1969, Kropp unpublished data). Rotten wood on clearcuts may function as a reservoir for fungi that form mycorrhizae with western hemlock. Rotten wood also has potential as an inexpensive and readily available source of inoculum for hemlock nurseries.

This research was designed to determine for containerized western hemlock whether (1) rotten wood collected from recent clearcuts and forests serves as a source of mycorrhizal fungi, (2) addition of rotten wood to container mixes affects seedling top growth, and (3) rotten wood carries root pathogens that harm western hemlock seedlings.

MATERIALS AND METHODS

1978 Inoculation. Rotten wood containing abundant yellow mycelium of the mycorrhizal fungus, Piloderma bicolor (Peck) Julich, some Cenococcum geophilum Fr. and small numbers of other fungi was collected from the Mary's Peak area in the Siuslaw National Forest in western Oregon. It was fragmented by hand into small pieces for mixing in a growing medium of thoroughly mixed 50% peat and 50% vermiculite. Three treatments were then established: (1) growing medium alone, steam pasteurized for 3 hours; (2) growing medium plus rotten wood (3:1 ratio by volume), steam pasteurized for 3 hours; (3) growing medium alone steam pasteurized for 3 hours and amended after cooling by addition of rotten wood (3:1 ratio by volume). Medium from each of these treatments was then used to fill cells of two sterilized,

molded high density polyethylene blocks containing 100 cells each. The cells were sown with western hemlock in April and grown under semi-operational conditions in an experimental greenhouse at the Crown Zellerbach Corporation container nursery near Aurora, Oregon. Nutrients were applied at normal operational schedules. Seedlings were extracted and examined the following January.

Each seedling was examined for mycorrhizae. One mycorrhiza on a root system was taken as a positive result. Mycorrhizae were tallied by associated fungus: Piloderma bicolor, Cenococcum geophilum or others. Swollen root tips and presence of a mantle and hyphae around the rootlets were taken to indicate mycorrhizal colonization. In doubtful cases, rootlets were squash-mounted and examined under a compound microscope for the presence of a Hartig net. Differences between treatments in number of mycorrhizal trees were tested by analysis of variance.

1979 Inoculation. Rotten wood was collected from two sites in the Mary's Peak area, one a two year old clearcut, the other a stand of western hemlock. No Piloderma bicolor mycelium was observed, but some Cenococcum geophilum mycorrhizae were evident. The wood from both sites was fragmented by hand as in the 1978 inoculation. Obvious roots were removed from the wood, although some mycorrhizae doubtless remained.

The experiment was conducted in the greenhouse of the U.S.F.S. Forestry Sciences Laboratory, Corvallis, with three treatments: controls of 1:1 peat/vermiculite, 70% peat/vermiculite with 30% wood by volume from the clearcut, and 70% peat/vermiculite with 30% wood by volume from the hemlock stand. Each medium was used to fill 50

individual, surface sterilized, plastic Leach cells, 65 cc capacity, which were completely randomized in trays. Hemlock seeds were sown at the end of May and seedlings grown under greenhouse conditions until mid-January. Nutrients (Soluble Peters General Purpose Fertilizer 20-20-20) were manually supplied to the seedlings each week. For the first two weeks, the fertilizer solution was applied at 6.45 g/m^2. During the third and fourth weeks, the concentration was increased to 12.16 g/m^2. At the fifth week, 24.22 g/m^2 were applied and continued weekly until fertilization was stopped in the Fall. Watering was done with an automatic mist sprinkler system. In January, seedlings were extracted and examined for mycorrhiza formation as in the first experiment. Seedling heights were also recorded. Differences between treatments in number of mycorrhizal seedlings were tested by Chi-square tests and in seedling height by analysis of variance.

RESULTS AND DISCUSSION

Table 3 summarizes data from both experiments. In the 1978 inoculation, non-pasteurized rotten wood functioned effectively as mycorrhizal inoculum: only 6 out of 175 seedlings remained non-mycorrhizal. In steam pasteurized treatments all but 2 or 3 seedlings remained nonmycorrhizal. Although Piloderma bicolor was the fungus most evident in the wood at the outset, it formed mycorrhizae with only a small proportion of the inoculated trees. Cenococcum geophilum seemed to be most able to colonize seedlings under these conditions. Only one seedling had another type of

mycorrhiza formed with an unidentified fungus.

Considerable mortality occurred soon after seedling emergence in the first experiment. No pathogenic fungi could be cultured from the seedlings or from the wood, and the problem was attributed to sunscald. Isolation attempts from the rotten wood yielded mostly Trichoderma spp. and bacteria but no pathogens.

In the 1979 inoculation, all seedlings inoculated with rotten wood from a hemlock stand became mycorrhizal. No mycorrhizae of Piloderma bicolor were observed and Cenococcum geophilum again predominated. Only about 1/3 of the seedlings inoculated with wood from the clearcut formed mycorrhizae, but this was still significantly more than the control. The presence of live mycorrhizae in wood from the forest could account for its effectiveness relative to wood from a 2 year old clearcut.

Only 3 seedlings inoculated with wood from the clearcut formed mycorrhizae with Cenococcum geophilum. This contrasts with both inoculations with wood from a forest. In the 1979 experiment, no heat or pathogen damage was observed.

Height of trees inoculated with rotten wood from the forest was significantly less than that of trees inoculated with wood from a clear cut (Table 3). But height growth of trees inoculated with rotten wood (both wood treatments combined) did not differ significantly from controls which received no wood. Mycorrhizal inoculation rarely increases growth of containerized seedlings over uninoculated controls (Marx and Barnett 1975, Molina 1979).

Mycorrhizal colonization was slow in both the 1978 and 1979 inoculations, a phenomenon common in container systems fertilized to

produce maximum top growth (Marx and Barnett 1974). At the end of 9 months (1978 experiment) and 7½ months (1979 experiment), mycorrhizae were sparse with some seedlings having only 2 or 3. However, several wood-inoculated seedlings not harvested in 1978, became heavily colonized with Cenococcum geophilum mixed in some cases with Piloderma bicolor by September of 1979. Extra uninoculated seedlings had also become heavily colonized by Thelephora terrestris by this time, presumably from airborne spores.

Total colonization by mycorrhizal fungi from rotten wood under these conditions was much slower than on nonmycorrhizal seedlings planted in the field on rotten wood, in which root systems were totally colonized in the first growing season (Kropp 1980).

Along the West Coast, rotten wood is abundant and readily available as an inexpensive source of inoculum compared to laboratory produced mycelial inoculum. Further work is needed to determine if rotten wood is an effective inoculum for tree species that don't commonly regenerate on decaying logs and how wood compares to soil or humus as inoculum (Trappe 1977).

Hemlock seedlings planted in decayed wood on clearcuts become mycorrhizal to the same degree and at the same rate as those in mineral soil (Kropp 1980). Rotten wood, especially that collected in standing forests, now has also proven to be a source of inoculum for containerized western hemlock seedlings. Further studies are needed to determine if growing season length, proportions of rotten wood, or fertilizer schedules can be manipulated to increase the proportions of mycorrhizae on roots of individual seedlings. To date, rotten wood has not been a source of root pathogens on western hemlock.

LITERATURE CITED

Berntsen, C. M. 1955. Seedling distribution on a spruce-hemlock clearcut. USDA For. Serv. Pac. Northwest For. and Range Exp. Stn. Res. Note 119. 7 p.

Harvey, A. E., M. J. Larsen, and M. F. Jurgensen. 1976. Distribution of ectomycorrhizae in a mature Douglas-fir/Larch forest soil in western Montana. For. Sci. 22:393-398.

Harvey, A. E., M. F. Jurgensen, and M. J. Larsen. 1978. Seasonal distribution of ectomycorrhizae in a mature Douglas-fir/Larch soil in western Montana. For. Sci. 24:203-208.

Harvey, A. E., M. J. Larsen, and M. F. Jurgensen. 1979. Comparative distribution of ectomycorrhizae in soils of three western Montana forest habitat types. For. Sci. 25:350-358.

Kropp, B. R. 1980. Mycorrhiza formation and performance of nonmycorrhizal western hemlock outplanted on rotten wood and mineral soil. In review.

Larsen, M. J., and B. Zak. 1978. Byssoporia gen. nov.: taxonomy of the mycorrhizal fungus Poria terrestris. Can. J. Bot. 56:1122-1129.

Marx, D. H., and J. P. Barnett. 1974. Mycorrhizae and containerized forest tree seedlings. In Proc. North Amer. Containerized Forest Tree Seedling Symp. (R. W. Tinus, W. I. Stein, and W. E. Balmer, eds.) p. 85-92. Great Plains Agr. Counc. Pub. 68, 458 p.

Minore, Don. 1972. Germination and early growth of coastal tree species on organic seed beds. USDA Forest Service. USDA Res. Pap. PNW-135, 18 p. Pac. Northwest For. and Range Exp. Stn., Portland, Oregon.

Molina, R. 1979. Ectomycorrhizal inoculation of containerized Douglas-fir and lodgepole pine seedlings with six isolates of Pisolithus tinctorius. For. Sci. 25:585-590.

Trappe, J. M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annu. Rev. Phytopathol. 15:203-222.

Zak, B. 1969. Characterization and classification of mycorrhizae of Douglas-fir. I. Pseudotsuga menziesii + Poria terrestris (blue- and orange-staining strains). Can. J. Bot. 47:1833-1840.

Table 3. Results of inoculations with rotten wood on western hemlock.[a/]

Treatment		Total no. of trees	Number of trees colonized by each type of fungus[b/] Cenococcum	Piloderma	Other	Uncolonized	% trees Colonized	Mean Top Growth (cm)
1978 Inoculation:								
Unpasteurized rotten wood	a	175	168	23	1	6	97	---
Pasteurized rotten wood	b	188	0	0	2	186	1	---
No wood	b	142	0	0	3	139	2	---
1979 Inoculation:								
Wood from forest	a	43	41	0	20	0	100	16.04 a
Wood from clearcut	b	47	3	0	13	32	32	18.19 b
No wood	c	39	0	0	5	34	13	17.85 a b

[a/] Within each year, treatments with different letters differ significantly (P 0.5).

[b/] Many trees had more than one type of mycorrhiza.

CHAPTER IV

FIELD COMPARISON OF MYCORRHIZAL AND UNINOCULATED WESTERN HEMLOCK SEEDLINGS

Bradley R. Kropp

ABSTRACT

In the first of two separate experiments, two year old containerized western hemlock seedlings, mycorrhizal with Cenococcum geophilum, produced significantly greater top growth for two years after outplanting compared to uninoculated controls. Seedling survival was unaffected by mycorrhizal inoculation.

In the second experiment, western hemlock seedlings planted in rotten wood and mineral soil on a clearcut in western Oregon grew and survived equally well during the first growing season. There were no growth or survival differences between mycorrhizal and uninoculated seedlings in either substrate. The fungus Cenococcum geophilum, which was symbiotic with the outplanted mycorrhizal seedlings, survived and appeared vigorous at the end of the season.

INTRODUCTION

The beneficial effects of mycorrhizal fungi on tree seedlings have been demonstrated numerous times. It is generally accepted that seedling growth and survival are, under most conditions, improved with the presence of mycorrhizal fungi. But variations in seedling response

to mycorrhizal inoculation are to be expected with variation in site characteristics and the species involved. Striking improvements in growth and survival of pines on coal spoils have been obtained with the use of Pisolithus tinctorius (Pers.) Coker and Couch (Marx 1975). Less striking responses are sometimes obtained with other fungi, as shown by Laiho with Paxillus involutus on pine seedlings (Laiho, 1970). Occasionally, inoculation with mycorrhizal fungi can even cause an actual decrease in seedling growth. In certain soil types, the mycorrhizal fungus Boletus scaber Bull. has been shown to decrease growth of birch (Levisohn 1957). Authors in the Soviet Union have reported differing responses of pine and oak seedlings to mycorrhizal inoculation (Shemakhanova 1962). In some cases, the seedlings grew (to different degrees) more than controls while in other cases they grew less than the controls. These differences in response varied with soil characteristics and with the mycorrhizal fungi used.

Information on the response of Western hemlock (Tsuga heterophylla (Raf.) Sarg.) seedlings to inoculation with ectomycorrhizal fungi is scarce. Work done by Trappe et al. (1977) showed that in tube containers, seedling dry weight increased for hemlock with several mycorrhizal fungi. To date, no work has been published comparing the performance of mycorrhizal and nonmycorrhizal Western hemlock seedlings in the field.

Along the Pacific coast, Western hemlock commonly reproduces on logs and rotten wood residue. This is particularly evident in heavy to moderate shade under mature forests where most of the hemlock reproduction occurs on rotten logs or stumps rather than the forest floor

(Minore 1972). Western hemlock often colonizes brush covered clearcuts by establishing itself on rotten wood, which is relatively free of brush (Ruth and Harris 1979). Berntsen (1955) showed that, on one coastal clearcut, hemlock was able to seed and grow on rotten wood as well as mineral soil. However, MacBean (1941) suggested that good survival of germinants on rotten wood or thick humus required a mild moist summer climate. Kropp (1981a) showed that nonmycorrhizal containerized hemlock planted in rotten wood and mineral soil readily become mycorrhizal on both substrates during the first growing season. The seedlings on rotten wood and soil survived equally well and although the seedlings on wood grew less, they appeared vigorous.

The objectives of the experiments presented here were to: 1) compare the top growth and survival of two year old containerized seedlings mycorrhizal with Cenococcum to uninoculated seedlings for two years after planting and 2) because of the particular importance of rotten wood to hemlock regeneration to compare top growth, survival and root growth of mycorrhizal and uninoculated hemlock seedlings planted on rotten wood and mineral soil after the first field season.

METHODS AND MATERIALS

Two separate outplanting experiments were performed. These are described separately as follows:

In the first experiment, Cenococcum geophilum Fr. was isolated on agar from surface sterilized sclerotia using the method of Trappe (1969). The mycelium was cultured in capped bottles containing modified Melin-Norkrans nutrient solution. These cultures were grown in an incubator

at 20°C for six weeks, being shaken periodically to break up the mycelium (Molina, 1979).

The method described by Molina (1979) was used to culture vegetative mycelial inoculum. 1500 ml of vermiculite and 150 ml of peat moss were mixed in a two liter flask with 1100 ml modified Melin-Norkrans nutrient solution. The flask was capped and autoclaved for 60 minutes. Each flask was aseptically inoculated with 10 ml of mycelial slurry of Cenococcum from the liquid cultures and grown for 12 weeks at room temperature. The harvested inoculum was leached of nutrient solution with about ten volumes of cold tap water. Excess moisture was removed from the inoculum by suction filtration. The inoculum was placed in plastic bags and kept at 2°C until used the following three days.

Nonmycorrhizal one year old containerized western hemlock seedlings were obtained from an operational Crown Zellerbach nursery in January. They were kept dormant at 3-4°C until they were inoculated in early March. The seedlings had been originally grown in 98 cm^3 plastic containers. During inoculation they were transplanted into 656 cm^3 plastic containers of the "Deepot" variety filled with a pasteurized 1:1 mix of peat moss and vermiculite.

Seedling plugs were extracted from the original containers and placed along with the inoculum into a hole that had been pressed into the medium in the larger tubes. Approximately 16 cm^3 of inoculum was used per seedling. The inoculum was placed directly against the root plug. Uninoculated controls were transplanted in the same way. The seedlings were then grown in a random arrangement in a slat house under

natural light and temperature conditions until the following January when they were placed in storage at 2-3°C. The seedlings were watered regularly throughout the growing season. They were fertilized with 1.3 tablespoon Peters 20-20-20 soluble fertilizer per square meter twice during the growing season. The residual fertilizer left in the original seedling plug maintained healthy seedling growth.

In early April 1978, the seedlings were outplanted in six separate plots. Ninety seedlings were planted on each plot; these were divided into three treatments of 30 trees each. The treatments were completely randomized within each plot. The treatments were: uninoculated seedlings, well-colonized mycorrhizal seedlings (about 67 cm^2 of the plug area colonized) and moderately colonized mycorrhizal seedlings (about 32 cm^2 of the plug area colonized). The seedlings in the two mycorrhizal treatments had been sorted before planting to ensure reasonable uniformity of mycorrhizal colonization of the root plugs within each treatment. Each seedling was numbered, labeled and covered with vexar tubing to prevent animal damage.

Three of the plots were located in the moist, coastal zone near Seaside, Oregon at about 183 m elevation and receiving an average annual precipitation of 200-250 cm. The remaining three were located on Mary's Peak in the Siusal National Forest in western Oregon. These plots are at about 853 m elevation and receive about 178 cm of precipitation annually. The aspect, slope and vegetation varied on each plot.

In July of the first season, and at the ends of the first and second seasons after outplanting, current year's leader growth and survival data were taken for each seedling. Analysis of variance

was used to test differences between treatments.

In the second experiment, the Western hemlock seedlings were grown in 98 cm^3 molded polyethylene containers. All containers were surface sterilized before use by being submerged for about five minutes in a 10% chlorox solution. They were then filled with a 3:1 mixture of a half and half peat/vermiculite medium and fragmented rotten wood inoculum (Kropp 1981b). Uninoculated control seedlings were grown in peat/vermiculite medium alone.

The seedlings were grown for 10 months at the Crown Zellerbach Corporation container nursery near Aurora, Oregon and for another year in a lath house under natural light and temperature conditions at Oregon State University. Nutrients were applied at normal operational rates. The seedlings were kept through the winter under natural light and temperature conditions until planted.

The site chosen for the study was a clearcut which had been harvested in winter 1977 and the slash burned in fall 1978. The site is on Mary's Peak in the Siuslaw National Forest at an elevation of about 2850 feet. It receives an average annual precipitation of about 178 cm with about 13 cm falling from May to August. The soil is a deep, well drained gravelly loam in the Slickrock series. The area is relatively dry for Western hemlock, being located on the eastern edge of the coast range. Hemlock stands occur primarily in moist pockets along streams. There is abundant natural hemlock regeneration along roads and in openings near the experiment site but no natural hemlock regeneration was present on the clearcut at the time this experiment was done.

Five pairs of plots were planted in mid-April 1980. One plot of

each pair was on mineral soil, the other was on well decayed wood which was being incorporated into the soil. Each plot contained 20 seedlings, 10 of these were mycorrhizal and 10 were uninoculated.

Before planting, all seedlings in the experiment were selected to obtain reasonable uniformity of size. Inoculated seedlings were sorted for uniformity of mycorrhizal colonization. Most of the seedlings were nearly 100% colonized by Cenococcum geophilum Fr.

At the beginning of October, the plot pairs were mapped and the trees numbered. Five mycorrhizal and five uninoculated seedlings from the rotten wood and soil plots of each pair were selected at random. These seedlings were measured for current year's leader growth in cm and were excavated in order to evaluate root growth. Root growth was taken as the average of the five longest roots coming from each plug. Survival counts were made for all seedlings in the experiment. Other general observations on the mycorrhizal status of the roots were noted. Differences between treatments were tested by analysis of variance.

RESULTS AND DISCUSSION

In the first experiment, there was a significant increase in top growth for mycorrhizal as compared to uninoculated seedlings on both the Mary's Peak and Seaside areas (Table 4). This increase was apparent for both the 1978 and 1979 growth increments. Thus, the cumulative growth differences at the end of the second year from outplanting show a widening height gap between treatments (Fig. 1). These cumulative growth differences are largest between the well-colonized seedlings and controls for the Mary's Peak plots and

between the moderately colonized seedlings and controls for the Seaside plots. The largest cumulative growth increases due to mycorrhizal inoculation were in the range of 5.5 cm for both sites. Over time, the seedlings on the Seaside plots grew significantly more than those on the Marys Peak plots regardless of their mycorrhizal status. This result is likely due to the fact that the Seaside plots receive more moisture and would have a longer growing season due to their low elevation.

Of particular interest in the relatively large difference between the well colonized seedlings and the other two treatments for the Marys Peak location. It is possible that the drier climate of the Marys Peak sites contributed to this response. On a relatively stressful site such as this, the dependence on a symbiont would be much stronger than at the mild Seaside location.

On Marys Peak, the moderately colonized seedlings and the uninoculated seedlings differed very little from one another. This could imply that the mycorrhizae present on these seedlings were sufficient to provide enough additional nutrients or moisture to the seedlings to stimulate growth beyond that of the controls.

Work published by Marx (1977) shows that, two years after outplanting, plot volume indices for five pine species inoculated with Pisolithus tinctorius (Pers.) Coker and Couch produced growth curves similar to Figure 1. Mycorrhizal seedlings produced a larger plot volume index during the first season after outplanting. This difference in plot volume index was much larger after the second season.

Although the data in Figure 1 were presented as height growth

rather than plot volume index, the same general comparisons can be made for western hemlock. If this acceleration in growth can continue for 5-6 years after planting, mycorrhizal inoculation could be of considerable importance in the establishment of western hemlock seedlings. This would be particularly true in areas where competition from brush is an important factor in reforestation. It can be expected that after a few years, this difference between controls and mycorrhizal seedlings will disappear but the initial boost to their growth could provide an important edge on competing brushy species.

Survival over the two year period was not significantly affected by inoculation with Cenococcum geophilum on either the Seaside or Marys Peak plots (Table 4, Figure 2). Regardless of treatment, there were significantly more seedlings surviving in fall 1979 on the Seaside plots than the Marys Peak plots. This is again likely to be due to the greater precipitation and longer growing season of the Seaside location.

In the second experiment, there were no significant differences for first year survival of the seedlings (Table 5). They survived equally well on decayed wood and mineral soil and there were no differences between mycorrhizal and uninoculated seedlings as a whole or separately on either substrate.

At the end of the first season, no significant growth differences could be detected. Root growth was not significantly different between seedlings on wood and soil or between mycorrhizal and uninoculated seedlings as a whole or separately on either substrate. No leader growth differences were detected due to planting on wood versus

soil nor were any detected as a result of mycorrhizal inoculation. Some of the seedlings on rotten wood did appear slightly more chlorotic than those on soil.

One possible reason for the lack of first year differences between mycorrhizal and uninoculated seedlings in the second experiment could be that residual nutrients in the root plugs were available for use by the outplanted seedlings. Thus, a difference may not be seen the first season but could become apparent in the following years as these residual nutrients are depleted.

Another possible reason for a lack of response to mycorrhizal inoculation during the first year in the field is the buds on both mycorrhizal and uninoculated seedlings developed in the nursery under identical conditions. Under such conditions the buds from both treatments would be likely to have the same growth potential. Buds developing in the field under more stressful conditions would be more likely to show growth potential differences due to the presence or absence of mycorrhizal fungi on the outplanted seedlings. This difference may become apparent in subsequent growing seasons.

Earlier work on Western hemlock (Kropp 1981a) indicated that nonmycorrhizal containerized seedlings planted on clearcuts in rotten wood survived as well in wood as in soil. There were no differences in seedling root growth for soil or wood. However, on one clearcut, seedling leader growth decreased for seedlings planted on rotten wood. The present research agrees with this work except that no leader growth differences were found between rotten wood and soil.

Berntsen (1960) concluded that for Douglas-fir or Sitka spruce,

planting on rotten wood in high rainfall areas was a sound practice. These results indicate that the same is true for Western hemlock.

The inoculated seedlings used in this experiment were almost totally mycorrhizal with Cenococcum geophilum at the time they were outplanted. Observations of mycorrhizae on the roots of excavated seedlings showed that this fungus survived and appeared vigorous after the first season on the plugs of seedlings in both wood and soil. New roots growing from the plugs were colonized by Cenococcum and other indigenous mycorrhizal fungi. New roots of uninoculated seedlings were colonized by indigenous fungi.

In summary, in the first experiment, inoculation of Western hemlock seedlings with Cenococcum geophilum increased seedling top growth for two years after outplanting. Further experimentation is needed to determine how long this positive growth response to mycorrhical inoculation is continued.

For the second experiment, there was no response during the first field season to mycorrhizal inoculation. However, seedlings survived and grew equally well on rotten wood and mineral soil. Further work should be done to determine if planting hemlock on rotten wood can be used successfully in reforestation, especially on brush covered sites where patches of decayed wood are often relatively brush free.

Survival of mycorrhizal seedlings was not significantly improved compared to controls in either experiment. Seedling responses to other species of mycorrhizal fungi on various types of sites should be evaluated.

LITERATURE CITED

Berntsen, C. M. 1955. Seedling distribution on a spruce-hemlock clearcut. USDA For. Serv. Pac. Northwest For. and Range Exp. Stn. Res. Note 119. 7 p.

Berntsen, C. M. 1960. Planting Sitka spruce and Douglas-fir on decayed wood in coastal Oregon. USDA For. Serv. Pac. Northwest For. and Range Exp. Stn. Res. Note 197. 5 p.

Kropp, B. R. 1981a. Mycorrhiza formation and performance of non-mycorrhizal western hemlock outplanted on rotten wood and mineral soil. In press.

Kropp, B. R. 1981b. Rotten wood as mycorrhizal inoculum for containerized western hemlock. In press.

Laiho, O. 1970. Paxillus involutus as a mycorrhizal symbiont of forest trees. Acta Forestalia Fennica Vol. 106, 1970.

Levisohn, I. 1957. Researches on mycorrhiza. Gt. Brit. Forestry Commission Report on Forest Research for the year ended March 1957. London.

MacBean, A. P. 1941. A study of the factors affecting the reproduction of western hemlock and its associates in the Quatsino region, Vancouver Island. Brit. Columbia Forest Serv. Tech. Publ. 25. 38 p.

Marx, D. H. 1975. Mycorrhizae and establishment of trees on stripe-mined land. The Ohio J. of Sci. 75:288-297.

Marx, D. H. 1977. The role of mycorrhizae in forest production. TAPPI Conference papers, Annual Meeting, Feb. 14-16, Atlanta, GA. pp. 151-161.

Molina, R. 1979. Ectomycorrhizal inoculation of containerized Douglas-fir and lodgepole pine seedlings with six isolates of Pisolithus tinctorius. Forest Sci. 25:585-590.

Ruth, R. H., and A. S. Harris. 1979. Management of western hemlock-Sitka spruce forests for timber production. USDA For. Serv. Pac. Northwest For. and Range Exp. Stn. Gen. Tech. Report PNW-88. 197 p.

Shemakhanova, N. M. 1962. Mycotrophy of woody plants. U.S.D.A. and N.S.F., Washington, D.C. transl. by the Israel Program for Scientific Translations. 329 p.

Trappe, J. M. 1969. Studies on Cenococcum graniforme. I. An efficient method for isolation from sclerotia. Can. J. Bot. 47:1389-1390.

Trappe, J. M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Ann. Rev. Phytopathol. 15:203-222.

Table 4. Top growth increments and survival for mycorrhizal and uninoculated western hemlock seedlings for two years after outplanting.

Location	Top growth (cm)[a/]								
	July 1978			Fall 1978			Fall 1979		
	Control	Moderately Colonized	Well Colonized	Control	Moderately Colonized	Well Colonized	Control	Moderately Colonized	Well Colonized
Marys Peak	7.90	8.77	9.37	12.55	14.03	14.42	11.91	9.57	15.69
Seaside	9.89	11.42	10.48	23.16	24.46	22.88	29.30	33.68	32.51
$\bar{x}$	8.90	10.10	9.93	17.86	19.25	18.65	20.61	21.63	24.10
	Survival (%)[b/]								
Marys Peak	100	100	100	100	97.6	98.9	77.8	82.4	78.9
Seaside	100	100	100	100	100	100	96.6	97.8	92.2
$\bar{x}$	100	100	100	100	98.8	99.5	87.2	90.1	85.6

[a/] Analysis of variance show significant differences (.05 level) between the top growth increments of mycorrhizal seedlings and controls at each of the three sampling dates. The Seaside plots produced significantly greater growth (.05 level) than the Marys Peak plots over time.

[b/] Analysis of variance shows no significant difference in survival for mycorrhizal versus uninoculated seedlings. There was a significantly greater percent survival for the Seaside location in fall 1979 (.05 level).

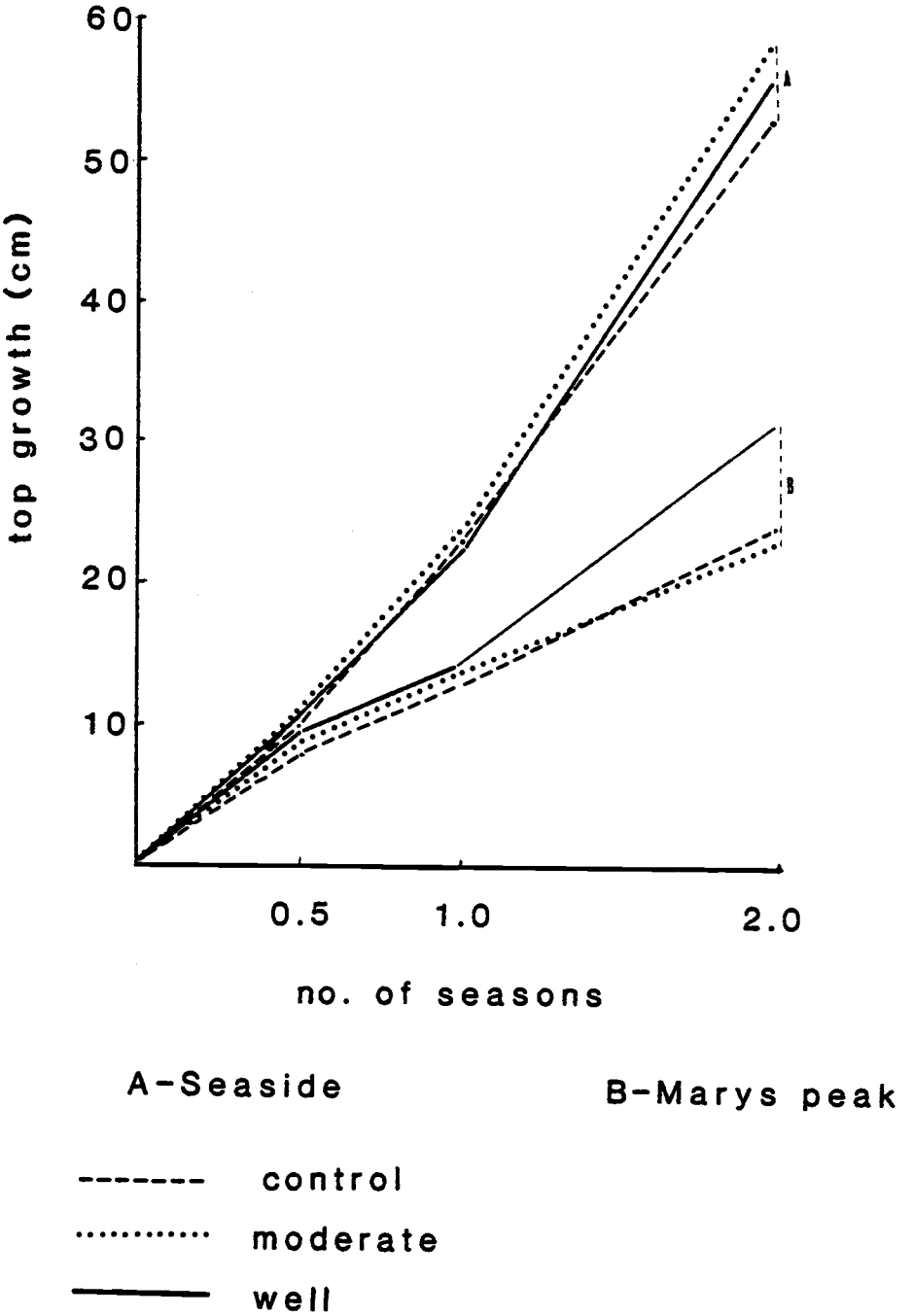
Table 5. Mean values[a,b/] for survival, leader growth, and root growth of mycorrhizal and uninoculated hemlock seedlings planted on rotten wood and mineral soil.

	Rotten Wood		Mineral Soil	
	Mycorrhizal	Uninoculated	Mycorrhizal	Uninoculated
Survival	92%	96%	94%	90%
Leader Growth	7.54	8.73	7.95	8.37
Root Growth	10.51	11.65	9.24	9.41

a/ Measurements of roots and leaders are in cm.

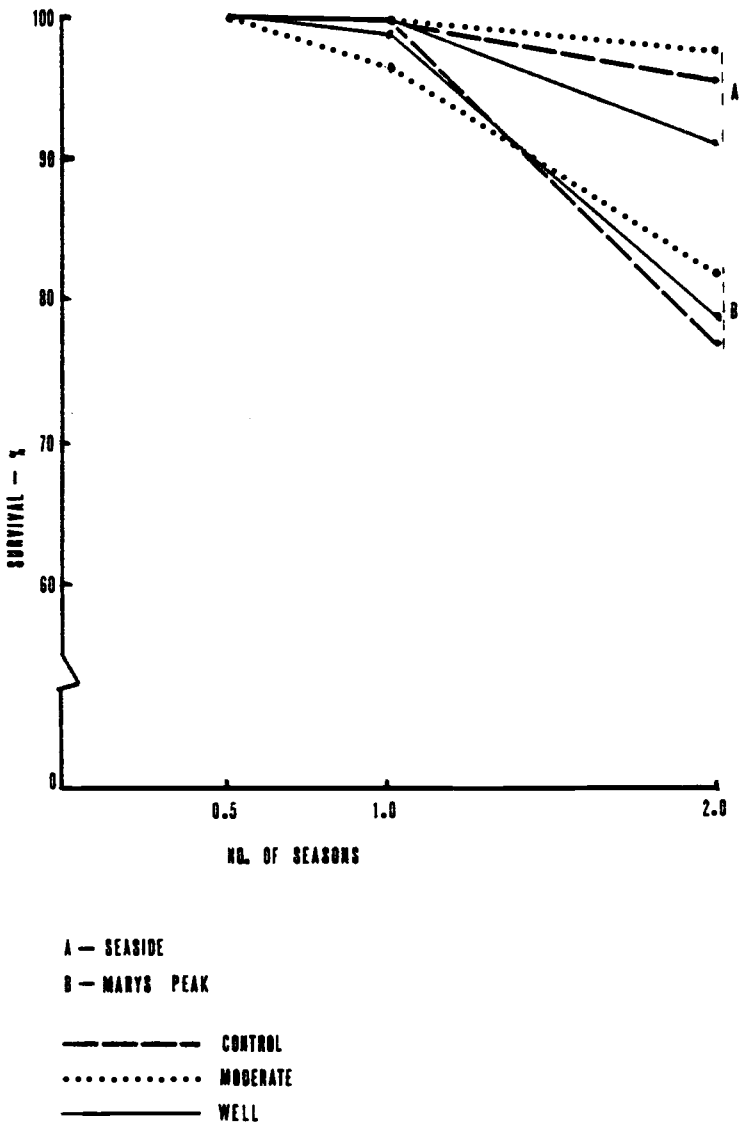
b/ No significant differences at the .05 level between any treatments.

fig. 1



Cumulative top growth of mycorrhizal and uninoculated western hemlock seedlings.

FIG. 2



Survival of mycorrhizal and uninoculated western hemlock seedlings.

CHAPTER V

THE FUNGI WHICH FORM MYCORRHIZAE WITH HEMLOCK

Bradley R. Kropp

and

James M. Trappe

A. The mycorrhizal fungi of Tsuga heterophylla.

SUMMARY

Fifty fungi have been demonstrated to form ectomycorrhizae in synthesis with western hemlock. More than a hundred additional fungi are reported to be probable mycorrhiza formers with various hemlock species. At present, few fungi seem to be host specific to hemlock. It is hypothesized that little selection pressure towards evolution of fungal host specificity exists with hosts such as hemlocks, which commonly establish in the understory of established stands of other species.

Western hemlock (Tsuga heterophylla (Raf.) Sarg.) ranges as a major timber species along the Pacific Coast from northern California to southwestern Alaska and inland to western Montana and eastern British Columbia. It occasionally forms pure stands but more often mixes with other conifers. Once regarded as a species of relatively low value, it is now recognized as a tree with useful wood quality and high productive potential on many kinds of sites. Consequently, increasing numbers of hemlock seedlings are being grown in western

forest nurseries for reforestation purposes. Much of this stock is produced in container nurseries on peat-vermiculate potting mixes. Seedlings thus grown often lack mycorrhizae unless the potting mixes are inoculated with appropriate mycorrhizal fungi.

Past literature contains little information on the mycorrhizal fungi of hemlocks. The pure culture syntheses and field studies reported here were designed to broaden the data base on hemlock mycorrhizal associates for potential use in nursery inoculations. The data also lead to some hypotheses about the mycorrhizal ecology of hemlocks.

MATERIALS AND METHODS

Ectomycorrhizal fungi prospectively associated with western hemlock were isolated by us, B. Zak, and R. Molina with tissue explants from sporocarps except for Cenococcum geophilum, isolated by A. Todd from surface-sterilized sclerotia by the method of Trappe (1969). The fungi were collected from a wide variety of habitats in the Pacific Northwest, mostly in association with western hemlock. Specimens from which isolates were obtained were assigned collection numbers and deposited in the Cryptogamic Herbarium of Oregon State University. Original isolation was on modified Melin-Norkrans agar (Marx 1969); transfers have since been maintained on that medium at the Forestry Sciences Laboratory.

For pure culture syntheses, western hemlock seeds were (1) rinsed 1 hr in running tap water, (2) shaken 30 min in tap water with a surfactant (Tween 20), (3) rinsed 1 hr in running tap water, (4) shaken

30 min in 30% H_2O_2, (5) rinsed aseptically in 2 l sterile, double distilled water, and placed on modified Melin-Norkrans agar in screwcap vials. After a week's incubation at room temperature, vials showing no contamination were refrigerated 3-4 weeks at 4°C, then removed from the refrigerator for seed germination at room temperature. Aseptically germinated seeds were then transferred to test tubes containing sterilized peat-vermiculite with nutrient solution (Molina 1979). The fungal inoculum was then added, with each isolate replicated in 2 tubes. Seedlings were then grown 4-5 months in the lighted water bath described by Molina (1979).

Root systems of all seedlings were examined by stereomicroscope for mycorrhizae. Rootlets from each seedling were sampled for compound-microscopic examination of squash mounts in 5% KOH to detect Hartig net formation. Results were recorded as +++ (50-100% feeder roots ectomycorrhizal), ++ (25-50% feeder roots ectomycorrhizal), + (25% feeder roots mycorrhizal), ? (only a few feeder roots colonized with, Hartig's nets erratic and shallow and mantles poorly formed or lacking), or nonmycorrhizal (Hartig net absent, mantle absent or occasionally present).

Field data on additional probable mycorrhizal associates of Tsuga spp. were obtained by collection and identification of sporocarps. The fungi were mostly collected in pure hemlock stands with other ectomycorrhizal hosts absent from both the overstory and understory. In some cases the sporocarps were associated with hemlocks in mixed stands, but then care was taken to insure that mycelium growing from the sporocarp was connected directly to hemlock mycorrhizae.

RESULTS

Of 22 fungi inoculated on western hemlock seedlings in pure culture syntheses, 15 formed typical ectomycorrhizae (Table 6). Three formed only questionable colonizations, i.e., mantles were lacking and Hartig nets penetrated erratically. Other successful pure culture syntheses of hemlock mycorrhizae as reported in the literature are included in Table 6 to provide a complete compilation of such data to date.

Four species produced no mycorrhizae under the experimental conditions: Boletus piperatus Fr. (isolate S-286), B. subtomentosus Fr. (S-316), Gastroboletus subalpinus Trappe and Thiers (S-395), and Tricholoma ponderosum (Peck) Sing.

Our field observations of probable mycorrhizal associates of Tsuga spp. plus other reports in the literature, as presented in Table 7, total more than a hundred fungal species. Hypogeous fungi figure prominently in the hemlock mycorrhizal flora.

DISCUSSION

Positive results of pure culture syntheses demonstrates the capability of particular fungal isolates to form mycorrhizae with particular hosts. Beyond that, considerable caution is needed in interpretation. In our experiments, for example, Pisolithus tinctorius proved to be a strong mycorrhiza former with western hemlock. In many years of extensive field work with both western and mountain hemlock (Tsuga mertensiana (Bong.) Sarg.), we have seen neither sporocarps of P. tinctorius associated with either

host nor mycorrhizae that might have been formed by P. tinctorius on field-grown roots. Both species tend to occupy relatively cool, moist sites with high levels of soil organic matter, whereas in the Pacific Northwest P. tinctorius appears to occur on warm to hot, relatively dry sites with low soil organic matter. The fungus is physiologically capable of forming mycorrhizae with western hemlock but apparently cannot compete with other fungi on most, if not all, hemlock sites. Consequently, it is not a likely prospect for inoculation of hemlock nursery stock intended for cool, moist habitats.

Formation of only a few mycorrhizae by a given fungus with a given host in pure culture synthesis suggests that under the experimental conditions the fungus is a less vigorous mycorrhiza former than others which form abundant mycorrhizae. Such information is useful in selecting fungi for nursery inoculation with cultured mycelium, because adequate amounts of inoculum cannot be produced with slow growing fungi by present technology. It cannot be inferred, however, that such a fungus is necessarily of low vigor or value to the host under field conditions.

Negative results in pure culture syntheses show that the experimental conditions were inappropriate for mycorrhiza formation. This information is useful for eliminating such fungi as candidates for nursery inoculation by present technology, but it does not definitively preclude mycorrhizal association between that fungus and host in nature. Three fungi strongly associated with hemlocks under natural conditions have not formed mycorrhizae with western hemlock in pure culture syntheses: Tricholoma ponderosum of this study and Boletus mirabilis

and Paxillus atrotomentosus studied by Kropp (1981a). All three grow extremely slowly in pure culture. In our experience, slow growth of an isolate in culture is a good indicator that it is mycorrhizal rather than saprophytic or pathogenic. B. mirabilis and P. atrotomentosus typically fruit from logs with advanced brown cubical rot, a habit suggestive of cellulose-decomposing saprophytism. In the many cases we have examined, however, the rotten logs which produce sporocarps of these fungi always contain abundant hemlock roots. B. mirabilis fruits only in the presence of hemlocks, and mycelium of P. atrotomentosus in rotten logs has been traced from sporocarps to nearby hemlock mycorrhizae mantled with identical mycelium. Tricholoma ponderosum is a demonstrated mycorrhiza former with Pseudotsuga menziesii (B. Zak, unpublished data) and is closely associated with hemlocks as well as other conifers in the field (Ogawa 1979). We hypothesize that the experimental methods used in pure culture synthesis attempts with these fungi in some way interferred with the mycorrhiza forming process. The physical or chemical characteristics of brown-cubical-rotted wood may be important to the functioning of B. mirabilis and P. atrotomentosus.

The list of probable mycorrhizal associates (Table 7) is not likely representative of the total potential mycorrhizal flora associated with hemlocks. Most of our collections have been opportunistic: Tsuga heterophylla and T. canadensis (L.) Carr are generally mixed with other conifers, so that determining which host is associated with a given sporocarp is often difficult. Tsuga mertensiana forms extensive stands in subalpine zones of the Pacific Northwest, but our

collecting these has focused on hypogeous fungi. Four epigeous genera which appear to be associated with hemlocks in particular abundance are Cortinarius, Inocybe, Russula, and Lactarius. Pacific Northwestern species of the first 3 genera have not been comprehensively monographed, so species identification is difficult at best. The recent monograph of Lactarius in North America by Hesler and Smith (1979) now enables progress in evaluation of mycorrhizal hosts of that genus.

Hypogeous fungi constitute a prominent part of the mycorrhizal fungus flora of hemlocks (Table 7). Elaphomyces granulatus is associated with hemlocks as well as other ectomycorrhizal hosts throughout the temperate and boreal forests of the northern hemisphere. Elaphomyces muricatus is common in Pacific Northwestern hemlock stands. Hysterangius separabile is abundant under western hemlock, as are several species of Martellia and Macowanites, hypogeous relatives of the epigeous genus Russula. Gautieria monticola and several Rhizopogon spp. are widely associated with mountain hemlock.

The fungi reported thus far as mycorrhizal with hemlocks are mostly non-host specific, e.g., Amanita spp. Cenococcum geophilum, Elaphomyces granulatus (Trappe 1962). Only 2 of the 100+ fungi listed in Table 7 appear to fruit only in the presence of hemlocks: Alpova alexsmithii and Boletus mirabilis. Hemlocks for the most part grow in mixed stands, frequently having established as climax species under the canopy of seral overstory species. Host specific mycorrhizal fungi also seem infrequent for other hosts that normally grow in mixed stands, e.g., Arctostaphylos uva-ursi (L.) Spreng. and Arbutus menziesii Pursh. (Molina 1980a). Hosts which characteristically form pure stands,

usually established after fire or other types of deforestation, include Alnus rubra Bong., Pseudotsuga menziesii (Mirb.) Franco, and many of the pines. Host specificity of mycorrhizal fungi seems to be relatively common with these species (Molina 1979, 1980). We hypothesize that host specificity of ectomycorrhizal fungi has evolved primarily with pioneering hosts that tend to grow in pure stands. In contrast, hosts such as hemlocks, regenerating in the understory, must "plug in" to the mycorrhizal system already established with the overstory hosts. A hemlock-specific fungus would have to invade a substrate already occupied by mycorrhizal fungi of the overstory, a difficult prospect. Hence the selection pressure would be against hemlock specificity. Western hemlock typically regenerates in the understory on rotten wood, however, a rather specialized microhabitat in which fungi such as Boletus mirabilis might encounter relatively little competition from previously established mycorrhizal fungi. Rotten wood therefore seems the most likely substrate for evolution of hemlock-specific fungi if they indeed exist.

ACKNOWLEDGEMENTS

These studies were funded in part by the Crown Zellerbach Corporation. We appreciate identification of the Cortinarius spp. by Dr. Alexander H. Smith and Inocybe spp. by Dr. D. E. Stuntz.

LITERATURE CITED

Boullard, B. 1968. Les mycorrhies. Mongr. Bot. Biol. Veg. 2, 135 pp.

Hesler, L. R., and A. H. Smith. 1979. North American species of Lactarius. Univ. Mich. Press, Ann Arbor. 841 pp.

Kinugawa, K., and T. Goto. 1978. Preliminary survey on the "matsutake" (Armillaria ponderosa) of North America. Trans. Mycol. Soc. Japan 19: 91-101.

Kropp, B. R. 1981a. Fungi from decayed wood as mycorrhizal symbionts of western hemlock. Can. J. Forest Res. (in press).

_____. 1981b. Mycorrhiza formation and performance of non-mycorrhizal western hemlock outplanted on rotten wood and mineral soil. Forest Sci. (in press).

_____. 1981c. Rotten wood as mycorrhizal inoculum for containerized western hemlock. Forest Sci. (in press).

Laiho, O. 1970. Paxillus involutus as a mycorrhizal symbiont of forest trees. Acta For. Fenn. 106: 1-72.

Marx, D. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Phytopathol. 59: 153-163.

Molina, R. 1979. Pure culture synthesis and host specificity of red alder mycorrhizae. Can. J. Bot. 57: 1223-1228.

_____. 1980a. Patterns of ectomycorrhizal host-fungus specificity in the Pacific Northwest. Ph.D. Thesis, Oregon State Univ. (in press).

Molina, R. 1980b. Ectomycorrhizal inoculation of containerized western conifer seedlings. U.S.D.A. Forest. Serv. Res. Note PNW-357: 1-10.

Ogawa, M. 1975. Microbial ecology of mycorrhizal fungus Tricholoma matsutake (Ito et Imai) Sing. in pine forest. II. Mycorrhiza formed by Tricholoma matsutake. Bull. Gov. Forest Exp. Sta. Tokyo 278: 21-49.

_____. 1979. Microbial ecology of "shiro" in Tricholoma matsutake and its allied species. IX. Tricholoma ponderosum in Pseudotsuga menziesii-Tsuga heterophylla and Pinus contorta forests. Trans. Mycol. Soc. Japan 20: 370-382.

Pilat, A., and A. Dermek. 1974. Hribovite Hruby. Slovenskej Akad., Bratislava. 207 pp.

Smith, A. H., and H. D. Thiers. 1971. The boletes of Michigan. Univ. Mich. Press, Ann Arbor. 428 pp.

Tanghe, L. J., and D. M. Simons. 1973. Amanita phalloides in the eastern United States. Mycologia 65: 99-108.

Thiers, H. D. 1975a. California mushrooms - a field guide to the boletes. Hafner Press, New York. 261 pp.

_____. 1975b. The status of the genus Suillus in the United States. Nova Hedwigia Beih. 51: 247-278.

Trappe, J. M. 1962. Fungus associates of ectotrophic mycorrhizae. Bot. Rev. 28: 538-606.

_____. 1969. Studies on Cenococcum graniforme. I. An efficient method for isolation from sclerotia. Can. J. Bot. 47: 1389-1390.

Trappe, J. M. 1975. A revision of the genus Alpova with notes on Rhizopogon and the Melanogastraceae. Nova Hedwigia Beih. 51: 279-309.

_____. 1976. Notes on Japanese hypogeous Ascomycetes. Trans. Mycol. Soc. Japan 17: 209-217.

_____. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annu. Rev. Phytopathol. 15: 203-222.

Watling, R. 1980. Mycorrhizal fungi. Bull. Brit. Mycol. Soc. 14: 59-62.

Zak, B. 1969. Characterization and classification of mycorrhizae of Douglas-fir. I. Pseudotsuga menziesii and Poria terrestris (blue- and orange-staining strains. Can. J. Bot. 47: 1833-1840.

_____, and M. J. Larsen. 1978. Characterization and classification of mycorrhizae of Douglas-fir. III. Pseudotsuga menziesii and Byssoporia (Poria) terrestris vars. lilacinorosea, parksii, and sublutea. Can. J. Bot. 56: 1416-1424.

Table 6. Fungi that form ectomycorrhizae with Tsuga heterophylla in pure culture syntheses.

Fungus Species	Culture No. or Literature Citation	Mycorrhiza Abundance[a/]
Amanita aspera (Fr.) Quel.	S-351	?
A. muscaria (L. ex Fr.) Pers. ex Hook.	Molina (1980a)	+++
A. pantherina (DC. ex Fr.) Schum.	S-331	+++
Astraeus pteridis (Shear) Zell.	Molina (1980a)	+++
Boletus edulis Bull. ex Fr.	Molina (1980a)	+
Byssoporia terrestris (DC. ex Fr.) Lars. & Zak	Kropp (1981a)	+++
Cenococcum geophilum Fr.	A-166	+++
	Kropp (1981a)	+++
	Molina (1980a)	+++
Cortinarius delibutus Fr.	S-503	++
C. mutabilis Smith	S-496	+++
Hygrophorus purpurascens (Fr.) Fr.	S-468	+
Hysterangium separabile Zell.	S-388	++
	Molina (1980a)	++

Table 6 continued.

Fungus Species	Culture No. or Literature Citation	Mycorrhiza Abundance[a/]
Laccaria laccata (Scop. ex Fr.) Bk. & Br.	Molina (1980a)	+++
Lactarius deliciosus (Fr.) S. F. Gray	Molina (1980a)	++
L. rufus (Scop. ex Fr.) Fr.	S-334	++
	Kropp (1981a)	++
L. rubrilacteus Hesl. & Smith	S-158	?
Leccinum manzanitae Thiers	Molina (1980a)	++
Melanogaster intermedius (Bk.) Zell. & Dodge	Molina (1980a)	+++
Paxillus involutus (Fr.) Fr.	Molina (1980a)	+++
Piloderma bicolor (Peck) Jul.	Kropp (1981a)	++
Pisolithus tinctorius (Pers.) Cok. & Couch	S-210	+++
	Molina (1980a)	+++
Rhizopogon abletis Smith	Molina (1980a)	++
R. colossus Smith	Molina (1980a)	+
R. cusickensis Smith	Molina (1980a)	++

Table 6 continued.

Fungus Species	Culture No. or Literature Citation	Mycorrhiza Abundance[a]
Rhizopogon ellenae Smith	Molina (1980a)	+++
R. hawkeri Smith	Molina (1980a)	++
R. liui Trappe sp. ined.	Molina (1980a)	++
R. ochraceorubens Smith	Molina (1980a)	+
R. parksii Smith	Molina (1980a)	+
R. rubescens Tul. & Tul.	Molina (1980a)	++
R. semireticulatus Smith	Molina (1980a)	+++
R. subcaerulescens Smith	Molina (1981a)	++
R. subcinnamomeus Smith	S-463	?
	Molina (1980a)	+
R. subclavitisporus Smith	Molina (1980a)	+
R. subgelatinosus Smith	Molina (1980a)	+++
R. villescens Smith	Molina (1980a)	+
R. villosulus Zell.	Molina (1980a)	+
R. vinicolor Smith	Molina (1980a)	+

Table 6 continued.

Fungus Species	Culture No. or Literature Citation	Mycorrhiza Abundance[a/]
Rhizopogon vulgaris (Vitt.) M. Lange	Molina (1980a)	+++
Russula cascadensis Shaff.	S-461	+++
Scleroderma hypogaeum Zell.	Molina (1980a)	+++
Suillus cavipes (Opat.) Smith & Thiers	Molina (1980a)	?
S. granulatus (L. ex Fr.) O. Kuntze	S-300	++
S. lakei (Murr.) Smith & Thiers	Molina (1980a)	+++
S. ponderosus Smith & Thiers	S-242	++
S. tomentosus (Kauffm.) Sing. Snell & Dick	S-313	++
S. umbonatus Dick & Snell	S-299	+++
Thelephora americana Lloyd	S-142	++
Tricholoma flavovilens (Per. ex Fr.) Lund	Molina (1980a)	+++
T. imbricatum (Fr.) Kumm.	S-289	+++
Zelleromyces gilkeyae Sing. & Smith	Molina (1980a)	?

[a/] +++ = 50-100% feeder roots ectomycorrhizal; ++ = 25-50% feeder roots ectomycorrhizal; + = <25 feeder roots ectomycorrhizal; ? = only a few feeder roots colonized, with Hartig nets erratic and shallow and mantles poorly formed or lacking.

Table 7. Fungi associated with *Tsuga* ectomycorrhizae.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Albatrellus flettii (Morse) Pouz.	E	*heterophylla*	288
Alpova alexsmithii Trappe	H	*heterophylla*	4461
		mertensiana	Trappe (1975); 2906, 3749, 3750, 5904
Amanita aspera (Fr.) Quel.	E	*heterophylla*	Trappe (1962); 63 (TENN)
A. fulva (Schaeff. ex) Pers.	E	*heterophylla*	78 (TENN)
A. gemmata (Fr.) Gill.	E	*heterophylla*	Trappe (1962); 62 (TENN)
		mertensiana	163 (TENN), 164 (TENN)
A. muscaria (L. ex Fr.) Pers. ex Hook.	E	*mertensiana*	Trappe (1962)
		mertensiana	167 (TENN), 5102
A. phalloides (Vaill. ex Fr.) Secr.	E	*canadensis*	Tanghe & Simons (1973)
A. vaginata (Bull. ex Fr.) Vitt.	E	*heterophylla*	Trappe (1962); 5338
Barssia oregonensis Gilk.	H	*heterophylla*	5490
Boletus appendiculatus Schaeff. ex Fr.	E	*heterophylla*	32 (SFSU), 52 (SFSU)

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Boletus calopsus var. *frustosus* (Snell & Dick) Mill. & Watl.	E	*mertensiana*	Trappe (1962); 883 (SFSU)
B. edulis Bull. ex Fr.	E	*heterophylla*	Trappe (1962); 5343
		mertensiana	126 (SFSU), 256 (SFSU), 937 (SFSU), 5404
B. fragrans Vitt.	E	*heterophylla*	896 (SFSU)
B. huronensis Smith & Thiers	E	*canadensis*	Smith and Thiers (1971)
B. mirabilis Murr.	E	*canadensis*	Smith and Thiers (1971)
		heterophylla	Thiers 1975a; 235 (SFSU), 248 (SFSU), 249 (SFSU), 250 (SFSU), 251 (SFSU), 4904
		mertensiana	4647, 4649
B. piperatus Fr.	E	*canadensis*	Pilat and Dermek (1974)
		mertensiana	4655, 4720
B. porosporus var. *americana* Smith & Thiers	E	*heterophylla*	893 (SFSU), 935 (SFSU)

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Boletus pulverulentus Opat.	E	heterophylla	Trappe (1962)
		mertensiana	857 (SFSU), 889 (SFSU)
B. rubripes Thiers	E	mertensiana	888 (SFSU)
B. smithii Thiers	E	mertensiana	49 (SFSU), 887 (SFSU)
B. subtomentosus Fr.	E	heterophylla	Trappe (1962); 5322
		mertensiana	4630
B. zelleri Murr.	E	heterophylla	Trappe 1962; 246 (SFSU), 247 (SFSU)
Byssoporia terrestris (D.C. ex Fr.) Lars. & Zak	H	heterophylla	Zak (1969), Zak and Larsen (1978)
Camarophyllus borealis (Peck) Murr.	E	heterophylla	6019
Cantharellus cibarius Fr.	E	heterophylla	Trappe (1962)
Cantharellus tubaeformis Fr.	E	heterophylla	6027
		mertensiana	6034

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Cenococcum geophilum Fr.	H	*canadensis*	Trappe (1962)
		heterophylla	Boullard (1968), Kropp (1981b, 1981c), Molina (1980b), Trappe (1962), 10 (UW), 11 (UW), 18 (UW)
		mertensiana	Trappe (1962)
Chamonixia caespitosa Roll.	H	*heterophylla*	4860
Chroogomphus loculatus Trappe & Mill	E	*mertensiana*	1704
Chroogomphus tomentosus (Murr.) Mill	E	*heterophylla*	Trappe (1962); 6018, 6025
		heterophylla	244 (SFSU), 583 (SFSU)
Cortinarius delibutus Fr.	E	*heterophylla*	5555
C. griseoviolaceus Smith	E	*heterophylla*	5557
C. montanus Kauffm.	E	*mertensiana*	4656
C. mucosus (Fr.) Fr.	E	*heterophylla*	6032
C. mutabilis Smith	E	*mertensiana*	5556
C. phoeniceus var. *occidentalis* Smith	E	*mertensiana*	5336

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Cortinarius vibratilis (Fr.) Fr.	E	*mertensiana*	2295
C. zakii Amm. & Smith	E	*mertensiana*	5320
Dentinum repandum (Fr.) S.F. Gray	E	*mertensiana*	6033
Elaphomyces granulatus Fr.	H	*canadensis*	5677, 5968
		diversifolia	Trappe (1976), 4232, 4358, 4399
		heterophylla	185 (MICH), 1079, 1550, 1875, 1877, 2167, 2168, 2170, 2251, 2321, 2939, 3753, 5039, 5187, 5325, 5678, 5763, 5911, 5912, 6028
		mertensiana	498, 582, 2842, 2939, 4634, 5219, 5473
E. morrettii Vitt.	H	*canadensis*	5972
E. muricatus Fr.	H	*heterophylla*	567, 2008, 5652, 5913
		mertensiana	770, 3502, 5266
Endogone lactiflua Bk. & Br.	H	*mertensiana*	5234

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Gastroboletus imbellus Trappe	E	*mertensiana*	1703
G. subalpinus Trappe & Thiers	H	*heterophylla*	5323
G. turbinatus (Snell) Smith & Sing.	H	*heterophylla*	188 (MICH), 1630
		mertensiana	1940, 2198, 5099
Gautieria monticola (Harkn.) Harkn.	H	*heterophylla*	4473
		mertensiana	4004, 4096, 4662, 5267, 5908, 5938
G. pterosperma Stew. & Trappe sp. ined.	H	*mertensiana*	784
Genea gardneri Gilk.	H	*heterophylla*	1564
G. harknessii Gilk.	H	*heterophylla*	572
Gomphus floccosus (Schw.) Sing.	E	*heterophylla*	Trappe (1962)
Hebeloma crustuliniforme (Bull. ex St. Am.) Quel.	E	*heterophylla*	Trappe (1977)
H. mesophaeum (Fr.) Quel.	E	*heterophylla*	6021

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Hydnotrya cubispora (Bess. & Thomps.) Gilk.	H	*heterophylla*	562, 819, 2350, 2922
H. variiformis Gilk.	H	*heterophylla*	623 (VPI), 2144, 2661, 4544, 5491
		mertensiana	767, 768, 772, 774, 779, 783, 1945, 1962, 1989, 2853, 2854, 4480, 4729, 5262, 5988
Hydnum fuscoindicum Harr.	E	*heterophylla*	5475
Hygrophorus camarophyllus (Fr.) Dum.	E	*heterophylla*	5348
H. goetzii Hesl. & Smith	E	*mertensiana*	584
Hymenogaster parksii Sing. & Smith	H	*heterophylla*	3536
Hysterangium crassum (Tul. & Tul.) Fisch.	H	*heterophylla*	619
H. separabile Zell.	H	*canadensis*	590
		heterophylla	314, 623, 5041
		mertensiana	2855, 5063

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Inocybe calamistrata (Fr.) Gill.	E	*heterophylla*	2274
I. xanthomelas Bours. & Kuhn.	E	*mertensiana*	215 (UW)
Laccaria laccata (Scop. ex Fr.) Bk. & Br.	E	*heterophylla*	Molina (1980b), Trappe (1962, 1977); 5112, 5341
		mertensiana	4648, 4685
Lactarius deliciosus (Fr.) S.F. Gray	E	*heterophylla*	Trappe (1962); 6019
L. fallax var. *concolor* Smith & Hesl.	E	*heterophylla*	243 (SFSU)
L. glutigriseus Wells & Kempt.	E	*heterophylla*	Hesler & Smith (1979)
		mertensiana	Hesler & Smith (1979)
L. kauffmannii Hesl. & Smith	E	*heterophylla*	6017
L. mucidus Burl.	E	*mertensiana*	4651
L. pseudoaffinis Hesl. & Smith	E	*canadensis*	Hesler & Smith (1979)
L. scrobiculatus (Fr.) Fr.	E	*heterophylla*	2374
		mertensiana	4682

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Lactarius subpurpurascens Peck	E	*canadensis*	Helser & Smith (1979)
L. substriatus Smith	E	*heterophylla*	Trappe (1962); 5686, 5687
L. trivialis (Fr.) Fr.	E	*heterophylla*	5649
Leucogaster microsporus Fog. sp. ined.	H	*heterophylla*	2249, 5321
L. rubescens Zell. & Dodge	H	*heterophylla*	95 (MICH)
Leucophleps magnata Harkn.	H	*heterophylla*	831
		mertensiana	1714, 1959
Macowanites chlorinosmus Smith & Trappe	H	*heterophylla*	2349
M. iodiolens Smith & Wells	H	*heterophylla*	568, 2339, 2340, 2348, 2357
Martellia brunnescens Sing. & Smith	H	*mertensiana*	1715, 5054
M. fragrans Smith	H	*mertensiana*	5098
M. idahoensis Sing. & Smith	H	*mertensiana*	1699
M. maculata Sing. & Smith	H	*heterophylla*	4863, 4868, 5651

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Martellia monticola (Harkn.) Sing. & Smith	H	*mertensiana*	2190
M. parksii Sing. & Smith	H	*heterophylla*	1583, 2355, 2544, 3534, 3535, 5654
M. subfulva Sing. & Smith	H	*heterophylla*	1985, 2354
		mertensiana	1993, 5062
M. subochracea Smith	H	*heterophylla*	2250
Melanogaster intermedius (Bk.) Zell. & Dodge	H	*heterophylla*	5923
M. vittadinii Soehn. & Knapp	H	*mertensiana*	5558
Paxillus involutus (Fr.) Fr.	E	*heterophylla*	Laiho (1970), Molina (1980b)
Picoa carthusiana Tul. & Tul.	H	*heterophylla*	5624
Piloderma bicolor (Peck) Jul.	H	*heterophylla*	Kropp (1981c)
Pisolithus tinctorius (Pers.) Cok. & Couch	E	*heterophylla*	Trappe (1977)
Polyozellus multiplex (Underw.) Murr.	E	*heterophylla*	5405

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Rhizopogon abietis Smith	H	*mertensiana*	876, 4654
R. atrogleba Zell.	H	*mertensiana*	1645
R. atroviolaceus Smith	H	*mertensiana*	5269
R. cokeri Smith	H	*mertensiana*	350, 4652, 5264
R. colossus Smith	H	*heterophylla*	5650
R. evadens var. *subalpinus* Smith	H	*mertensiana*	351, 1663, 2273, 5937
R. hawkeri Smith	H	*heterophylla*	2346
R. milleri Smith	H	*mertensiana*	347
R. obscurus Smith	H	*mertensiana*	301
R. parksii Smith	H	*heterophylla*	2305, 2347, 5324, 5653
R. pseudovillosulus Smith	H	*heterophylla*	5980
R. rubescens Tul. & Tul.	H	*heterophylla*	Trappe (1962)
R. smithii Hosf.	H	*mertensiana*	879, 4481, 4489, 4723, 5057, 5936

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Rhizopogon subcaerulescens Smith	H	*mertensiana*	2182, 2195, 4006, 4437, 4440, 5053, 5559
R. subgelatinosus Smith	H	*heterophylla*	4468
R. subpurpurascens Smith	H	*mertensiana*	322, 4440, 4662
R. subsalmoneus Smith	H	*mertensiana*	782, 1652, 2841, 2905, 2932, 4439, 5214, 5268, 5901
R. villosulus Smith	H	*heterophylla*	5655
R. vinicolor Smith	H	*heterophylla*	4905, 5040, 5894
R. vulgaris (Vitt.) M. Lange	H	*mertensiana*	4653, 4661, 4699, 4724
Rozites caperata (Pers. ex Fr.) Karst.	E	*mertensiana*	4680
Russula atrata Shaff.	E	*heterophylla*	1058
R. brevipes Peck	E	*heterophylla*	155 (UW), 204 (UW), 338 (MICH)
R. cascadensis Shaff.	E	*heterophylla*	154 (UW), 6023
R. decolorans Fr.	E	*heterophylla*	43 (MICH), 152 (MICH), 6014
R. dissimulans Shaff.	E	*heterophylla*	203 (UW)

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Russula emetica (Schaeff. ex Fr.) Pers. ex Fr.	E	*heterophylla*	Trappe (1962)
R. laurocerasi Melz.	E	*heterophylla*	Trappe (1962, as *R. foetens*)
R. nigricans (Bull. ex. Fr.) Fr.	E	*heterophylla*	6013, 6024
Suillus flavoluteus (Snell) Snell & Dick	E	*canadensis*	Thiers (1975b)
S. imitatus Smith & Thiers	E	*heterophylla*	Thiers (1975b)
S. punctatipes (Smell & Dick) Smith & Thiers	E	*mertensiana*	50 (SFSU), 4650, 4655
Thelephora americana Lloyd	E	*heterophylla*	Kropp (1981c); 4657, 4659
T. terrestris Ehrh. ex Fr.	E	*chinensis*	Watling (1980)
		heterophylla	Trappe (1977)
Tricholoma matsutake (Ito & Imai) Sing.	E	*diversifolia*	Ogawa (1975)
		sieboldii	Ogawa (1975)
T. ponderosum (Peck) Sing.	E	*canadensis*	Kinugawa and Goto (1978), Ogawa (1979)

Table 7 continued.

Fungus Species	Epigeous or Hypogeous	Host *Tsuga* sp.	Literature Reports and Fungus Collection Numbers
Tricholoma ponderosum (Peck) Sing.		*heterophylla*	Kinugawa and Goto (1978), Ogawa (1979)
T. robustum (Alb. & Schw. ex Fr.) Rick.	E	*sieboldii*	Trappe (1962)
Tricholoma vaccinum (Fr.) Kumm.	E	*heterophylla*	6026
T. virgatum (Fr.) Kumm.	E	*mertensiana*	478
Truncocolumella rubra Zell.	H	*mertensiana*	674, 5056, 5900, 5902
Tylopilus pseudoscaber (Secr.) Smith & Thiers	E	*heterophylla*	33 (SFSU), 54 (SFSU), 56 (SFSU)
Zelleromyces gilkeyae Sing. & Smith	H	*heterophylla*	2370

B. Fungi from decayed wood as ectomycorrhizal symbionts of western hemlock.

ABSTRACT

Five of 18 wood inhabiting fungi tested formed mycorrhizae with western hemlock in pure culture synthesis. Field observations indicate that four more are mycorrhizal with hemlock growing in rotten wood.

INTRODUCTION

Decayed wood is an important substrate for ectomycorrhizal activity. Harvey et al. (1976) have shown that rotten wood in Douglas-fir/larch stands in Montana supports a substantial portion of the total number of ectomycorrhizae in the forest floor. They also found that rotten wood supports more active mycorrhizae during the drier period of the year than soil humus (1978). Western hemlock (Tsuga heterophylla (Raf.) Sarg.) commonly regenerates on decaying logs and stumps in old growth forests in the Pacific Northwest (Minore 1972). Natural hemlock seedlings on decayed wood are mycorrhizal. Nonmycorrhizal seedlings outplanted in rotten wood in clearcuts become mycorrhizal during the first 2-3 months after planting (Kropp 1981). Rotten wood from a clearcut and from a forest has also been used successfully to inoculate containerized hemlock seedlings with mycorrhizal fungi (Kropp 1980).

Although many fungi have been identified as being mycorrhizal with hemlock (Trappe 1962, Kropp and Trappe unpublished data, Zak 1969,

Molina unpublished data) almost no attempt has been made to identify rotten wood inhabiting fungi that form hemlock mycorrhizae. Trappe (1965) reported that tuberculate mycorrhizae occuring on Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) are found most abundantly in well rotted stumps and logs. The fungus forming this mycorrhiza was later identified as Rhizopogon vinicolor A. H. Smith (Zak 1971). Zak (1976) has found the wood inhabiting fungus, Piloderma (Corticium) bicolor to be mycorrhizal with Douglas-fir and Pacific madrone (Arbutus menziesii Pursh). Another wood inhabiting fungus, Piloderma byssinum (Karst.) Julich, is mycorrhizal on Douglas-fir (Froidevaux 1975). Byssoporia terrestris has been shown to be mycorrhizal in rotten wood with both Douglas-fir and western hemlock (Zak 1969, Larsen and Zak 1978). This is the only report of an identified wood inhabiting fungus mycorrhizal with western hemlock.

The objective of this research was to identify additional wood inhabiting fungi which form mycorrhizae with western hemlock.

MATERIALS AND METHODS

Field observations and pure culture syntheses were used to determine whether fungi were mycorrhizal with western hemlock. Sporocarps occurring on rotten wood near hemlock seedlings were collected and identified. If the sporocarps occurred within about 50 cm of a hemlock seedling on rotten wood, the fungi were considered as possible symbionts with the seedlings (Fig. 6). Although no definite rhizomorph or mycelial connections between the sporocarps and seedlings were found, some of the seedlings had mycorrhizae that were covered with mycelium

similar to that at the base of the sporocarps. If mycorrhizae from the seedlings were found at the base of the sporocarps or if the mycelia at the base of the sporocarp and on the mycorrhizae were the same, the fungus was considered to be mycorrhizal with the seedlings. This type of observation is somewhat subjective and mycorrhizal association was not considered conclusive unless mycorrhizae could be obtained with the fungus in pure culture synthesis. Most of the pure cultures of the fungi were obtained by tissue explant from the sporocarps. Cenococcum geophilum was obtained from surface sterilized sclerotia (Trappe 1969). Isolate B and Piloderma bicolor were obtained from hemlock mycorrhizae in rotten wood (Zak 1964). Isolate C, and Byssoporia terrestris were cultured from surface sterilized rhizomorphs. Cultures of seven additional fungi, commonly found in decaying logs, were obtained from the Forest Products Laboratory at Madison, Wisconsin.

Each fungus obtained in culture was tested in pure culture synthesis tubes containing a peat-vermiculite mix and a nutrient solution following as closely as possible the methods used by Molina (1979). Hemlock seeds were rinsed in running tap water for 1 hour and shaken in Tween 20 for 30 minutes. They were again rinsed in running water for an hour before being surface sterilized by shaking in 30% H_2O_2 for 30 minutes. The seeds were then rinsed aseptically with 2 liters of sterile distilled H_2O and placed on agar in vials. After one week incubation at room temperature and disposal of contaminated vials, sterile seeds were stratified in the vials for 3-4 weeks at 3-4°C. They were then germinated under fluorescent-incandescent light at room temperature and planted aseptically into pure culture

synthesis tubes and inoculated with the desired fungus. Seedlings were grown in the tubes, which were half submerged in a 16°C waterbath under fluorescent-incandescent lighting with a 15-h photoperiod. After 4-5 months, they were examined for mycorrhiza formation. The presence of a Hartig net on roots squash mounted in 5% KOH was taken as evidence of mycorrhiza formation.

RESULTS

Fourteen fungi (Table 8) were observed on rotten wood with western hemlock seedlings. Nine of these were tested with hemlock in pure culture synthesis; the remaining five were not obtained in culture. Cultures of ten additional wood inhabiting fungi were also tested in pure culture synthesis with western hemlock. Pure culture synthesis showed that five of the fungi form mycorrhizae with hemlock. Field observations strongly indicated that four additional fungi are mycorrhizal on hemlock growing in wood.

Cenococcum geophilum, a common forest soil fungus also found in decayed wood, formed mycorrhizae with hemlock in pure culture. It was also observed on natural hemlock seedlings in wood. Cenococcum was the predominant fungus forming mycorrhizae on nonmycorrhizal seedlings planted into rotten wood in clearcuts and on containerized seedlings inoculated with rotten wood (Kropp 1980, 1981). Cenococcum was also reported to be mycorrhizal with western hemlock in the field by Trappe (1962).

Piloderma bicolor is mycorrhizal with Douglas-fir both in nature and pure culture (Zak 1976, 1971a). These results show that it also

forms mycorrhizae with hemlock in pure culture synthesis and in nature. In addition, inoculations of containerized western hemlock with rotten wood containing Piloderma bicolor resulted in Piloderma mycorrhizae being formed (Kropp 1980). Zak (1969) and Larsen and Zak (1978) reported field observations of Byssoporia terrestris being mycorrhizal with western hemlock and Douglas-fir. Zak (1969) reported difficulty in obtaining mycorrhizae on Douglas-fir with Byssoporia in pure culture synthesis, although one attempt was successful. He made no attempt to test Byssoporia with hemlock in pure culture. These results support his field observations of an association between hemlock and Bryssoporia by demonstrating mycorrhiza formation in pure culture synthesis.

Isolate B was cultured from the mycorrhizae of a hemlock nursery seedling planted in rotten wood on a clearcut. The seedling was heavily colonized in the field by this distinctive fungus, which formed mycorrhizae in pure culture synthesis. However, formation was poor, with only one mycorrhiza developing on one of the seedlings tested. No sporocarps were available for identification of this fungus. The artificial conditions of pure culture synthesis possibly prohibited the thorough colonization of the seedlings that was noted in the field.

Lactarius rufus was observed on rotten wood with hemlock seedlings and was tested in pure culture with western hemlock. The isolate of L. rufus from wood failed to form mycorrhizae in pure culture synthesis. However, a successful synthesis was made with another culture of L. rufus obtained from R. Molina. Attempts were made to synthesize mycorrhizae in pure culture with both Boletus mirabilis and Paxillus

atrotomentosus. Both fungi gave negative results. Hemlock mycorrhizae found with the collection of P. atrotomentosus appeared to be formed with Paxillus mycelium. Field indications are strong enough to consider Paxillus to be mycorrhizal with western hemlock. Boletus mirabilis, on the other hand, is less likely to be mycorrhizal with hemlock. Examination of hemlock mycorrhizae at the base of the sporocarp showed no mycorrhizae with mycelium resembling that of the sporocarp. Although, interestingly enough, some of the mycorrhizae found appressed to the sporocarp base were formed by Cenococcum.

Cantharellus infundibuliformis, Russula sp., and Galerina sp. were not obtained in culture but were closely associated with hemlock on rotten wood. Mycorrhizae on hemlock seedlings close to these sporocarps were apparently formed with the same mycelium found at the base of the sporocarps. Except for Galerina these fungi belong to mycorrhizal genera and should probably be considered mycorrhizal with western hemlock. Chroogomphus tomentosus was observed with hemlock on wood along with mycorrhizae formed with mycelium from the sporocarp. However, these mycorrhizae could not be positively identified as belonging to western hemlock. Two fungi, Xeromphalina brunneola and Pleurotus porrigens, both considered saprophytic, were found with hemlock seedlings on rotten logs. Mycorrhizae were found within the tissue of the Pleurotus sporocarp. Since no culture grew from tissue explants from this fungus, it could not be tested in pure culture synthesis. Since Pleurotus is a saprophytic genus, it is unlikely to be mycorrhizal with hemlock. However, there are instances of fungi from saprobic genera which have been shown to be mycorrhizal with

orchids (Furman and Trappe 1971). Xeromphalina brunneola was also found with hemlock along with mycorrhizae having similar mycelium. This fungus grew in culture but did not form mycorrhizae in pure culture synthesis. Tricholomopsis rutilans from wood was cultured and tested with hemlock in pure culture with negative results. It was not observed with seedlings in the field and should be considered saprophytic. Fomes annosus was tested in pure culture synthesis with hemlock because it had been reported to form an unusual type of mycorrhiza in culture with pine seedlings by Orlos and Dominik (1960). Hemlock seedlings were killed by this fungus.

Cultures of seven other wood inhabiting fungi obtained from the Forest Products Laboratory in Madison, Wisconsin were tested with hemlock in pure culture synthesis. These fungi were selected because they are commonly isolated from decaying logs. It was thought that these fungi might be able to persist in downed logs and eventually become mycorrhizal with seedlings becoming established on the logs. All seven of these fungi failed to form mycorrhizae in pure culture.

DISCUSSION

Some fungi forming mycorrhizae on hemlock seedlings in decayed wood on clearcuts are the same as those forming mycorrhizae with hemlock in the soil (Kropp 1981). It is likely that as logs and stumps decay and become incorporated into the soil, soil inhabiting mycorrhizal fungi are able to colonize the rotten wood. There they become mycorrhizal with hosts growing on the wood. Cenococcum

geophilum is an example of a mycorrhizal fungus occurring in both environments. Other fungi are restricted to rotten wood (Kropp 1981). However, even some of these fungi are able to colonize soil as reported for Byssoporia terrestris by Zak (1969). The author has observed mycelia of Piloderma bicolor growing in soil near rotten wood.

Numerous decaying logs in old growth forests in the Pacific Northwest are suspended above contact with the soil. These logs are often covered with mycorrhizal western hemlock seedlings. The fungi forming these mycorrhizae may have been in the log before it fell or may have colonized it afterwards. In this research, seven fungi commonly isolated from standing or recently felled trees did not form mycorrhizae, suggesting that fungi necessary to form mycorrhizae colonize the wood after the log has fallen. Nine fungi examined in this research can be considered mycorrhizal with western hemlock on rotten wood. Five of these have been conclusively shown to be mycorrhizal by pure culture synthesis. Field observations of mycorrhizae on western hemlock seedlings indicate that there are additional mycorrhizal fungi that inhabit rotten wood. Further collections and pure culture syntheses are needed to identify additional wood inhabiting fungi mycorrhizal with hemlock.

LITERATURE CITED

Furman, Thomas E., and J. M. Trappe. 1971. Phylogeny and ecology of mycotrophic achlorophyllous angiosperms. Quarterly Rev. Biol. 46:219-225.

Froidevaux, L. 1975. Identification of some Douglas-fir mycorrhizae. Eur. J. For. Pathol. 5:212-216.

Harvey, A. E., M. J. Larsen, and M. F. Jurgensen. 1976. Distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. For. Science 22:393-398.

Harvey, A. E., M. F. Jurgensen, and M. J. Larsen. 1978. Seasonal distribution of ectomycorrhizae in a mature Douglas-fir/larch forest in western Montana. For. Science 24:203-208.

Kropp, B. R. 1980. Rotten wood as mycorrhizal inoculum for containerized western hemlock. In press.

Kropp, B. R. 1981. Mycorrhiza formation and performance of non-mycorrhizal western hemlock outplanted on rotten wood and mineral soil. In press.

Larsen, M. J., and B. Zak. 1978. Byssoporia gen. nov.: taxonomy of the mycorrhizal fungus Poria terrestris. Can. J. Bot. 56:1122-1129.

Minore, D. 1972. Germination and early growth of coastal tree species on organic seed beds. USDA Forest Service Res. Pap. PNW-135. Pacific Northwest Forest and Range Exp. Sta., Portland, Oregon.

Molina, R. 1979. Pure culture synthesis and host specificity of red alder mycorrhizae. Can. J. Bot. 57:1223-1228.

Orlos, H., and T. Dominik. 1960. Some Notes on Biology of the Root Fomes (Fomes annosus (Fr.) Cooke). Sylwan 104:1-13.

Trappe, J. M. 1962. Fungus associates of ectotrophic mycorrhizae. Bot. Rev. 28:538-605.

_____. 1965. Tuberculate mycorrhizae of Douglas-fir. For. Sci. 11:27-32.

_____. 1979. Studies on Cenococcum graniforme. I. An efficient method for isolation from sclerotia. Can. J. Bot. 47:1389-1390.

Zak, B., and D. H. Marx. 1964. Isolation of mycorrhizal fungi from roots of individual slash pines. For. Sci. 10:214-222.

Zak, B. 1969. Characterization and classification of mycorrhizae of Douglas-fir. I. Pseudotsuga menziesii + Poria terrestris (blue- and orange-staining strains). Can. J. Bot. 47:1833-1840.

_____. 1971a. Detoxification of autoclaved soil by a mycorrhizal fungus. USDA Forest Service Res. Note PNW-159. Pacific Northwest Forest and Range Exp. Sta., Portland, Oregon.

_____. 1971b. Characterization and classification of mycorrhizae of Douglas-fir. II. Pseudotsuga menziesii + Rhizopogon vinicolor. Can. J. Bot. 49:1079-1084.

_____. 1976. Pure culture synthesis of Pacific madrone ectendomycorrhizae. Mycologia 68:362-369.

Table 8. Fungi occurring in rotten wood and attempted pure culture syntheses of mycorrhizae with Tsuga heterophylla.

Species	Field Observations[a/]	Pure Culture Synthesis[b/]
Boletus mirabilis Murrill	a	-,-
Byssoporia terrestris (D.C. per Fries) Larsen et Zak	b	+
Cantharellus infundibuliformis Fr.	b	no culture
Cenococcum geophilum Fr.	b	+
Chroogomphus tomentosus (Murr.) O.K. Miller	a	no culture
Coniophora puteana (Schum. ex. Fr.) Karst.	c	-
Fomes annosus (Fr.) Cooke	c	-
Fomitopsis officinalis (Vill. ex Fr.) Bond et Sing.	c	-
Fomitopsis pinicola (Swartz ex Fr.) Karst.	c	-
Galerina sp.	b	no culture
Ganoderma applanatum (Pers. ex Wallr.) Pat.	c	-
Ganoderma tsugae Murr.	c	-
Isolate B	b	+
Isolate C	a	-
Lactarius rufus (Scop. ex Fr.) Fries	a	+,-
Paxillus atrotomentosus (Batsch ex Fr.) Fries	b	-,-
Perrenniporia subacida (Peck) Donk	c	-
Phellinus pini (Thore ex Fr.) Pilat	c	-

Table 8 continued.

Species	Field Observations[a/]	Pure Culture Synthesis[b/]
Piloderma bicolora (Peck) Julich	b	+
Pleurotus porrigens (Fr.) Gillet	a	no culture
Russula sp.	b	no culture
Tricholomopsis rutilans (Fr.) Sing.	c	-
Xeromphalina brunneola O. K. Miller	b	-

[a/] a = observed with hemlock, b = observed with hemlock as an apparent mycorrhizal associate, c = inhabits wood, not observed with hemlock.

[b/] Synthesis attempted twice with certain fungi; + = ectomycorrhizae formed; - = no ectomycorrhizae formed.

LITERATURE CITED

Arnott, J. T. 1975. Field performance of container-grown and bareroot trees in coastal British Columbia. Can. J. For. Res. 5:186-194.

Arnott, J. T. 1976. Survival and growth of western hemlock in British Columbia. IN Proceedings: Western hemlock management conference, p. 196-200. William A. Atkinson and Robert J. Zasoski, eds. Univ. Wash., Seattle.

Berntsen, C. M. 1955. Seedling distribution on a spruce-hemlock clearcut. USDA For. Serv. Pac. Northwest For. and Range Exp. Stn. Res. Note 119. 7 p.

Berntsen, C. M. 1960. Planting Sitka spruce and Douglas fir on decayed wood in coastal Oregon. USDA For. Serv. Pac. Northwest For. and Range Exp. Stn. Res. Note 197. 5 p.

Boullard, B. 1968. Les mycorrhizes. Mongr. Bot. Biol. Veg. 2. 135 p.

Briscoe, C. B. 1959. Early results of mycorrhizal inoculation of pine in Puerto Rico. Caribbean Forester 20:73-77.

Clements, J. B. 1938. Trials in Nyasaland of introduced pines. Austral. Forestry 1:21-33.

Farr, W. A. and A. S. Harris. 1971. Partial cutting of western hemlock and Stika spruce in southeast Alaska. USDA For. Serv. Pac. Northwest For. and Range Exp. Stn. Res. paper PNW-124.

Fowells, H. A. 1965. Silvics of forest trees of the Unites States. USDA For. Serv. Div. Timber Management Research. Agr. Handbook 271.

Frank, A. B. 1885. Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Ber. Dtsch. bot. Ges. 3, 27-33.

Froidevaux, L. 1975. Identification of some Douglas-fir mycorrhizae. Eur. J. For. Pathol. 5:212-216.

Furman, T. E. and J. M. Trappe. 1971. Phylogeny and ecology of mycotrophic achlorophyllous angiosperms. The Quarterly Rev. of Biol. 46:219-225.

Harlow, W. M. and E. S. Harrar. 1969. Textbook of dendrology. McGraw-Hill Book Company, New York. 512 p.

Harris, A. S. 1967. Natural reforestation on a mile-square clearcut in southeast Alaska. USDA For. Serv. Pac. Northwest For. and Range Exp. Stn. Res. paper PNW-52.

Harvey, A. E., M. J. Larsen, and M. F. Jurgensen. 1976. Distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. For. Sci. 22:393-398.

Harvey, A. E., M. F. Jurgensen, and M. J. Larsen. 1978. Seasonal distribution of ectomycorrhizae in a mature Douglas-fir/larch soil in western Mon-ana. For. Sci. 24:203-208.

Harvey, A. E., M. J. Larsen, and M. F. Jurgensen. 1979. Comparative distribution of ectomycorrhizae in soils of three western Montana forest habitat types. For. Sci. 24:350-358.

Heilman, P. E. and G. Ekuan. 1980a. Effects of phosphorus on growth and mycorrhizal development of Douglas-fir in greenhouse pots. Soil Sci. Soc. Am. J. 44:115-119.

Heilman, P. E. and G. Ekuan. 1980b. Phosphorus response of western hemlock seedlings on Pacific coastal soils from Washington. Soil Sci. Soc. Am. J. 44:392-395.

Kinugawa, K. and T. Goto. 1978. Preliminary survey on the "matsutake" (Armillaria ponderosa) of North America. Trans. Mycol. Soc. Japan 19:91-101.

Kropp, B. R. 1981a. Fungi from decayed wood as mycorrhizal symbionts of western hemlock. Can. J. Forest Res. (in press)

Kropp, B. R. 1981b. Mycorrhiza formation and performance of non-mycorrhizal western hemlock outplanted on rotten wood and mineral soil. Forest Sci. (in press)

Kropp, B. R. 1981c. Rotten wood as mycorrhizal inoculum for containerized western hemlock. Forest Sci. (in press)

Laiho, O. 1967. Field experiments with ectendotrophic Scotch pine seedlings. Pap., IUFRO, 14th Congress, Munich, Vol. 5, pp. 149-157.

Laiho, O. 1970. Paxillus involutus as a mycorrhizal symbiont of forest trees. Acta For. Fenn. 106:1-72.

Larsen, M. J. and B. Zak. 1978. Byssoporia gen. nov.: taxonomy of the mycorrhizal fungus Poria terrestris. Can. J. Bot. 56:1122-1129.

Levisohn, I. 1957. Researches on mycorrhiza. Gt. Brit. Forestry Commission Report on Forest Research for the year ended March 1957. London.

McBean, A. P. 1941. A study of the factors affecting the reproduction of western hemlock and its associates in the Quatsino region, Vancouver Island. Brit. Columbia Forest Serv. Tech. Publ. 25. 38 p.

Marx, D. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Phytopathol. 59:153-163.

Marx, D. H. and J. P. Barnett. 1974. Mycorrhizae and containerized forest tree seedlings. In Proc. North Amer. Containerized Forest Tree Seedling Symp. (R. W. Tinus, W. I. Stein, and W. E. Balmer, eds.) p. 85-92. Great Plains Agr. Counc. Publ. 68, 458 p.

Marx, D. H. 1975. Mycorrhizae and establishment of trees on strip-mined land. The Ohio J. of Sci. 75:288-297.

Marx, D. H., W. C. Bryan, and C. E. Cordell. 1977. Survival and growth of pine seedlings with Pisolithus ectomycorrhizae after two years on reforestation sites in North Carolina and Florida. For. Sci. 23:363-373.

Marx, D. H. 1977. The role of mycorrhizae in forest production. TAPPI Conference papers, Annual meeting, Feb. 14-16, Atlanta, GA. p. 151-161.

Marx, D. H., J. G. Mexal, and W. G. Morris. 1979. Inoculation of nursery seedbeds with Pisolithus tinctorius spores mixed with hydromulch increases ectomycorrhizae and growth of loblolly pines. Southern J. of Applied Forestry 3:175-178.

McComb, A. L. 1938. The relation between mycorrhizae and the development and nutrient absorption of pine seedlings in a prairie nursery. J. Forestry 36:1148-1154.

Meyer, F. H. 1973. Distribution of ectomycorrhizae in native and man-made forests. IN Ectomycorrhizae, their ecology and physiology. p. 79-105. Marks, G. C. and T. T. Kozlowski, eds. Academic Press, New York and London.

Mikola, P. 1970. Mycorrhizal inoculation in afforestation. Int. Rev. of For. Res. Vol. 3. Romberger, J. A. and P. Mikola, eds. Academic Press, New York and London.

Mikola, P. 1973. Application of mycorrhizal symbiosis in forestry practice. IN Ectomycorrhizae, their ecology and physiology. p. 383-411. Marks, G. C. and T. T. Kozlowski, eds. Academic Press, Yew York and London.

Minore, D. 1972. Germination and early growth of coastal tree species on organic seed beds. USDA Forest Service, USDA Res. Pap. PNW-135, 18 p. Pac. Northwest For. and Range Exp. Stn., Portland, Oregon.

Molina, R. 1979a. Ectomycorrhizal inoculation of containerized Douglas-fir and lodgepole pine seedlings with six isolates of Pisolithus tinctorius. For. Sci. 25:585-590.

Molina, R. 1979b. Pure culture synthesis and host specificity of red alder mycorrhizae. Can. J. Bot. 57:1223-1228.

Molina, R. 1980a. Patterns of ectomycorrhizal host-fungus specificity in the Pacific Northwest. Ph.D. thesis, Oregon State University. (in press)

Molina, R. 1980b. Ectomycorrhizal inoculation of containerized western conifer seedlings. USDA Forest Serv. Res. Note PNW-357. p. 1-10.

Newton, M. 1976. Site preparation requirements for western hemlock. IN Proceedings: Western hemlock management conference, p. 191-195. William A. Atkinson and Robert J. Zasoski, eds. Univ. Wash., Seattle.

Newton, M. 1978. Test of western hemlock wildlings in brushfield reclamation. Res. Pap. 39, 22 p. Ore. State Univ. For. Res. Lab., Corvallis.

Ogawa, M. 1975. Microbial ecology of mycorrhizal fungus Tricholoma matsutake (Ito et Imai) Sing. in pine forest. II. Mycorrhiza formed by Tricholoma matsutake. Bull. Gov. Forest Exp. Sta. Tokyo 278:21-49.

Ogawa, M. 1979. Microbial ecology of "shiro" in Tricholoma matsutake and its allied species. IX. Tricholoma ponderosa in Pseudotsuga menziesii-Tsuga heterophylla and Pinus contorta forests. Trans. Mycol. Soc. Japan 20:370-382.

Orlos, H. and T. Dominik. 1960. Some notes on biology of the root Fomes (Fomes annosus (Fr.) Cooke). Sylwan 104:1-13.

Pilat, A. and A. Dermek. 1974. Hribovité Hruby. Slovenskey Akad., Bratislava. 207 p.

Reid, C. P. P. and F. W. Woods. 1969. Translocation of C^{14} labeled compounds in mycorrhizae and its implications in interplant nutrient cycling. Ecology 50:179-187.

Richen, C. W. 1976. Attitudes of management toward western hemlock. IN Proceedings: Western hemlock management conference, p. 7-9. William A. Atkinson and Robert J. Zasoski, eds. Univ. Wash., Seattle.

Roeloffs, J. W. 1930. Over Kunstmatige Verjonging van Pinus merkusii Jungh. et de Vr. en Pinus khasya Royle. Tectona 23:874-907.

Ruth, R. H. 1964. A problem analysis and program for silvicultural research in the Sitka spruce-western hemlock type of the Pacific Northwest. USDA For. Serv. Pac. Northwest For. and Range Exp. Stn.

Ruth, R. H. and A. S. Harris. 1973. Western hemlock-Sitka spruce. IN Silvicultural systems for the major forest types of the United States. pp. 5-7. USDA For. Serv. Div. Timber Management Research. Agr. Handbook 445.

Ruth, R. H. and A. S. Harris. 1979. Management of western hemlock-Sitka spruce forests for timber production. USDA For. Serv. Pac. Northwest For. and Range Exp. Stn. Gen. Tech. Report PNW-88. 197 p.

Schramm, J. R. 1966. Plant colonization studies on black wastes from anthracite mining in Pennsylvania. Trans. Amer. Phil. Soc. 56(1): 194.

Shemakhanova, N. M. 1962. Mycotrophy of wood plants. USDA and NSF, Washington, D.C. Transl. by the Israel Program for Scientific Translations. 329 p.

Skinner, M. F. and G. D. Bowen. 1974. The uptake and translocation of phosphate by mycelial strands of pine mycorrhizas. Soil Biol. and Biochem. 6:53-56.

Slankis, V. 1973. Hormonal relationships in mycorrhizal development. IN Ectomycorrhizae their ecology and physiology. p. 231-298. Marks, G. C. and T. T. Kozlowski, eds. Academic Press, New York and London.

Smith, A. H. and H. D. Thiers. 1971. The boletes of Michigan. Univ. Mich. Press, Ann Arbor. 428 p.

Steinbrenner, E. C. 1976. Soil-site relationships and comparative yields of western hemlock and Douglas-fir. IN Proceedings: Western hemlock management conference, p. 236238. William A. Atkinson and Robert J. Zasoski, eds. Univ. Wash., Seattle.

Tanghe, L. J. and D. M. Simons. 1973. Amanita phalloides in the eastern United States. Mycologia 65:99-108.

Thiers, H. D. 1975a. California mushrooms - a field guide to the boletes. Hafner Press, New York. 261 p.

Thiers, H. D. 1975b. The status of the genus Suillus in the United States. Nova Hedwigia Beih. 51:247-278.

Trappe, J. M. 1962. Fungus associates of ectotrophic mycorrhizae. Bot. Rev. 28:538-606.

Trappe, J. M. 1965. Tuberculate mycorrhizae of Douglas-fir. For. Science 11:27-32.

Trappe, J. M. 1969. Studies on Cenococcum graniforme I. An efficient method for isolation from sclerotia. Can. J. Bot. 47:1389-1390.

Trappe, J. M. and R. F. Strand. 1969. Mycorrhizal deficiency in a Douglas-fir region nursery. Forest Sci. 15:381-389.

Trappe, J. M. 1975. A revision of the genus Alpova with notes on Rhizopogon and the Melanogastraceae. Nova Hedwigia Beih. 51: 279-309.

Trappe, J. M. 1976. Notes on Japanese hypogeous ascomycetes. Trans. Mycol. Soc. Japan 17:209-217.

Trappe, J. M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annu. Rev. Phytopathol. 15:203-222.

Viereck, L. A. and E. L. Little. 1975. Atlas of United States trees. Vol. 2. Alaska trees and common shrubs. USDA For. Serv., Washington, D.C. Misc. Public. 1293.

Vozzo, J. A. and E. Hacskaylo. 1971. Inoculation of Pinus caribaea with ectomycorrhizal fungi in Puerto Rico. Forest Sci. 17:239-245.

Watling, R. 1980. Mycorrhizal fungi. Bull. Brit. Mycol. Soc. 14: 59-62.

White, D. P. 1941. Prairie soil as a medium for tree growth. Ecol. 22:398-407.

Wiley, K. N. 1976. Site index and yield of western hemlock. IN Proceedings: Western hemlock management conference. p. 228-235. William A. Atkinson and Robert J. Zasoski, eds. Univ. Wash., Seattle.

Williamson, R. L. 1976. Natural regeneration of western hemlock. IN Proceedings: Western hemlock management conference. p. 166-169. William A. Atkinson and Robert J. Zasoski, eds. Univ. Wash., Seattle.

Wright, E. 1957. Importance of mycorrhizae to ponderosa pine seedlings. For. Sci. 3:275-280.

Zak, B. 1964. Role of mycorrhizae in root disease. Ann. Rev. Phytopath. 2:377-392.

Zak, B. 1969. Characterization and classification of mycorrhizae of Douglas-fir. I. Pseudotsuga menziesii + Poria terrestris (blue- and orange-staining strains). Can. J. Bot. 47:1833-1840.

Zak, B. 1971a. Detoxification of autoclaved soil by a mycorrhizal fungus. USDA Forest Service Res. Note PNW-159. Pacific Northwest Forest and Range Exp. Sta., Portland, Oregon.

Zak, B. 1971b. Characterization and classification of mycorrhizae of Douglas-fir. II. Pseudotsuga menziesii + Rhizopogon vinicolor. Can. J. Bot. 49:1079-1084.

Zak, B. 1976. Pure culture synthesis of Pacific madrone ectendomycorrhizae. Mycologia 68:262-269.

Zak, B. and D. H. Marx. 1964. Isolation of mycorrhizal fungi from roots of individual slash pines. For. Sci. 10:214-222.

Zak, B. and M. J. Larsen. 1978. Characterization and classification of mycorrhizae of Douglas-fir. III. Pseudotsuga menziesii + Byssoporia (Poria) terrestris vars. lilacinorosea, parksii and sublutea. Can. J. Bot. 56:1416-1424.