EFFECTS OF *LACCARIA PROXIMA* AND FIBROUS PULP WASTES ON THE GROWTH OF CONTAINER-GROWN CONIFERS

by

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ABSTRACT

Li, D.W. 1989. Effects of *Laccaria proxima* and fibrous pulp wastes on the growth of container-grown conifers. 117 pp. Major advisor: Dr. E.C. Setliff.

Key Words: conifers, containerized seedlings, ectomycorrhiza, fibrous pulp waste, *Laccaria proxima*, tree nurseries.

Nine conifer species, Japanese larch (*Larix kaempferi*), white spruce (*Picea glauca*), black spruce (*P. mariana*), red spruce (*P. rubens*), jack pine (*Pinus banksiana*), mugo pine (*P. mugo*), red pine (*P. resinosa*),Japanese black pine (*P. thunbergii*) and Douglas-fir (*Pseudotsuga menziesii* var *menziesii*), were tested to determine the effective host range of the ectomycorrhizal fungus *Laccaria proxima* and the possibility of utilizing pulp waste as potting medium for containerized seedling production. *L. proxima* significantly improved the growth of container grown jack pine, mugo pine, black spruce, red spruce and Douglas-fir, improved the growth of Japanese black pine and white spruce and slightly improved the growth of red pine and Japanese larch. Rye grain spawn inoculum was used for mycorrhizal establishment on jack pine seedlings. Pulp waste (33% by volume) usually had negative effects on tree seedlings except for Japanese black pine (positive) and Douglas-fir (no significant effect). The interactions of *L. proxima* and pulp waste varied with host tree species, but were significantly positive ($P < 0.01$) for jack pine and black spruce, and nearly significant for red spruce ($P < 0.08$). Negative effects were found with Japanese black pine.

Up to 8% pulp waste by volume without mycorrhiza improved the growth of jack pine seedlings over the controls, but over 8% pulp waste hindered jack pine seedling growth. Ten to 30% of pulp waste was effectively utilized in jack pine seedling production in association with *L. proxima*. Pulp waste with malt extract amendment was used as a spawn medium. Pulp waste in seedling production of black spruce, mugo pine, red spruce and Douglas-fir in association with *L. proxima* and of Japanese black pine without is feasible, but further research is needed to find out the optimal percentage of pulp waste which can be utilized in seedling production for most tree species.

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CHAPTER 1. INTRODUCTION

Laccaria proxima (Boud.) Pat.*sensu* Boud. is a native ectomycorrhizal fungus that is widely distributed in North America from Canada to Mexico (Singer, 1977; Aquirre-Acosta and Perez-Silva, 1978; Danielson *et al.*, 1984). Unlike some ectomycorrhizal species, such as *Pisolithus tinctorius*, *Paxillus involutus*, *Hebeloma crustuliniforme*, and *Thelephora terrestris,* which all have been researched intensively, *Laccaria proxima* has been studied very little.

Considering its extensive geographical distribution, wide host range, and trait as a pioneer ectomycorrhizal fungus of many coniferous species (Mason *et al.*, 1987), *Laccaria proxima* is a potentially useful ectomycorrhizal fungus in forestry. The full potential value of this ectomycorrhizal fungus remains to be found and explored. One use might be in developing low cost mycorrhizal spawn substrates or mycorrhizal wood waste composts from materials such as fibrous pulp waste, bark and sawdust.

Fibrous pulp waste is a problem for the pulp and paper industry and in the environment, and although some methods of waste utilization have been developed, economical and technical problems still restrain them from being implemented. Even landfill as a conventional disposal method is still far from a good method due to its expense, limited disposal sites and negative impact on the environment (Dolar *et al.*, 1972, Sawhney and Kozloski, 1984; D'Arcy, 1988). New approaches are needed to solve the pulp waste problem more effectively.

One approach to the problem is to utilize fibrous pulp waste as a potting medium component in the production of containerized tree seedlings. This waste compost material would supply a cheap growing medium to growers of seedlings and would serve as a partial substitute for the more expensive peat moss and vermiculite now being used. Furthermore, the resulting pulp mulch from adequate composting with a mycorrhizal fungus such as *L. proxima*, could be transformed into a suitable potting additive that could result in higher quality, stress tolerant seedlings.

In recent years, the production of containerized seedlings has increased dramatically. In 1982, 31 million containerized seedlings were produced in Ontario, and in 1983, the production rose to 54.7 million, an increase of 78% (Scarratt, 1985a). In 1984, the production of container stock, at 81.5 million total seedlings was almost 50% greater than in 1983 (Scarratt, 1985b). The production of containerized jack pine (*Pinus banksiana* Lamb.) seedlings was 52.5 million in 1984 (Scarratt, 1985b). Based upon the 1984 level of jack pine seedling production, if 20% by volume of pulp waste is added to the medium for seedling production, 315 tonnes of pulp waste could be utilized in Ontario yearly.

Preliminary tests indicated that when only pulp waste, peat moss and vermiculite were used as the potting medium, there was a negative effect on jack pine seedling growth (D'Arcy, 1988). However, composting the pulp waste fibers with ectomycorrhizal fungi, prior to using it as a component of a potting mix might theoretically reduce the toxic effects of the raw pulp. Because of the mycorrhizal component, the result may be healthier trees that would be able to withstand environmental stresses better after outplanting. On adverse sites, outplanted pine seedlings with *Pisolithus tinctorius* ectomycorrhiza grew 180-480% better than seedlings with naturally formed mycorrhia (Marx *et al.*, 1988). They also found that the survival and growth were positively related to the initial amount of *P. tinctorius* ectomycorrhiza on seedling roots at time of planting.

The primary objectives of this research project were as follows:

1) to determine the effective host range of *L. proxima* [including nine conifer species: white spruce (*Picea glauca* (Moench) Voss), black spruce (*P. mariana* (Mill.) B.S.P.), red spruce (*P. rubens* Sarg.), jack pine, mugo pine (*Pinus mugo* Turra), red pine (*P. resinosa* Ait.), Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirbel) Fr.), Japanese larch (*Larix kaempferi* (Lamb.) Carr.) and Japanese black pine (*Pinus thunbergii* Parl.)],

2) to determine if fibrous pulp waste can be upgraded with *L. proxima* and be used as a potting medium for producing containerized conifer seedlings in the greenhouse,

3) to test the proportions of pulp waste that might be used with peat and vermiculite as a suitable medium for containerized jack pine seedling production, and

4) to test rye grain and pulp waste as a spawn medium.

Secondary experimentation included: (1) the influence of rye grain in potting medium on jack pine seedling growth (Appendix I), (2) a potting mixture experiment-extreme vertice design effects of various mixtures of *L. proxima*, pulp waste, peat moss and vermiculite on jack pine seedling growth (Appendix II), (3) the effects of drying pulp waste on effectiveness of inoculum (Appendix III), (4) forest soil as a natural mycorrizal inoculum (Appendix IV), (5) the growth of *L. proxima* in response to temperature (Appendix V), and (6) the effects of pulp waste supernatant and nutrients on *L. proxima* growth (Appendix VI).

CHAPTER 2. LITERATURE REVIEW

ECTOMYCORRHIZA

Hosts and Mycorrhizal Fungi

Approximately 95% of the world's vascular plant species belong to families that are characteristically mycorrhizal (Trappe, 1977). The hosts for ectomycorrhizae include both gymnosperms and angiosperms. Although only about 3% of the phanerogams form ectomycorrhizae, these fungi are the most important types in forestry (Meyer, 1973) and occur naturally on many important forest tree species around the world (Marx, 1977). Ectomycorrhizal plants are mainly woody perennials and trees found in 43 families (Meyer, 1973; Harley and Smith, 1983). Ectomycorrhizae are most common in the Betulaceae, Ericaceae, Fagaceae, Juglandaceae, Myrtaceae, Pinaceae, and Salicaceae (Harley, 1969; Meyer, 1973 and Marx and Schenck, 1983). All members of the gymnosperm family Pinaceae are ectomycorrhizal; i.e., *Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, and *Tsuga*. Some important angiosperm genera with ectomycorrhizae are *Betula*, *Carya*, *Fagus*, *Populus*, *Quercus*, and *Salix* (Schultz, Isebrands and Kormanik, 1983).

Ectomycorrhizal fungi include members of many families of Basidiomycetes, and only a few Ascomycetes, sterile

Fungi Imperfecti, and Phycomycetes (Trappe, 1971; Harley and Smith, 1983).

It is estimated that more than 2,100 species of ectomycorrhizal fungi exist in North America and over 5,000 in the world. Most ectomycorhizal fungi of forest trees are Basidiomycetes that produce mushrooms or puffballs as reproductive structures (Marx, 1980a; Schultz, Isebrands and Kormanik, 1983).

Geographical Distribution

Ectomycorrhizae exist around the world, but most of them are found mainly in north and south temperate zones and in sub-arctic regions (Harley, 1969 and Harley and Smith 1983). In the subalpine timberline of temperate regions, especially in the colder boreal forests, ectomycorrhizal trees dominate. A climax forest with predominantly ectomycorrhizal trees is distributed mainly in areas with a large amplitude in the annual periodicity of temperature. In the temperate zones, the timberline is formed almost entirely by ectomycorrhizal trees (Singer and Morello, 1960; Moser, 1967; Meyer, 1973; and Harley and Smith, 1983). In boreal forests, members of the Pinaceae are abundant. Broad-leaved trees of this zone, such as *Betula*, and *Populus,* are also ectomycorrhizal. The ectomycorrhizal trees are often pioneers on wastelands. Ectomycorrhizal trees occur in lowland tropical forests only sporadically.

Benefits to the Hosts

As early as 1894, experiments showed that ectomycorrhizal plants of pine grew faster than non-mycorrhizal plants (Franks, 1894 cited by Harley and Smith, 1983). During nearly one century, it was fully demonstrated that ectomycorrhizae are essential in the growth of forest trees. The effects of ectomycorrhizae on seedlings are much more obvious. In the absence of ectomycorrhiza, some golden larch (*Pseudolarix* spp.) and some pines (*Pinus* spp.) were unable to survive (Wang *et al.*, 1985).

Under natural conditions, trees of the pioneer group are incapable of developing without ectomycorrhiza (Mikola, 1973). In many parts of the world, ectomycorrhizal trees and their symbiotic fungi fail to occur naturally. Introduction of exotic pines to many countries and afforestation in higher altitudes, in prairies and steppes, drained bogs, calluna heaths, on coal spoils, strip mined coal wastes, and other similar adverse conditions failed until ectomycorrhizal fungi were introduced (Harley, 1969; Marx and Schenck, 1983; Gulden and Hølland, 1985). Moser (1967) estimated that the world's forested areas would have been reduced by at least 20 to 30% if ectomycorrhizal fungi did not exist, and that the tree's altitudinal limit would be several hundred metres lower (Gulden and Hølland, 1985).

It is quite obvious that ectomycorrhizae are vital to forest tree growth. Ectomycorrhizae affect host trees positively in several ways.

1) Enhancing the ability of hosts to absorb mineral nutrients and water

Mycorrhizae increase mineral and water availability as a result of nutrient exchange with the fungus and increased root surface. Plants with abundant ectomycorrhizae have a much larger root surface area for nutrient and water absorption than plants with few or no ectomycorrhiza. Not only does the multi-branching habit increase the root surface area, but hyphae of the symbiotic fungi grow out of the ectomycorrhiza into adjacent soil and function as additional absorbing organs. Ectomycorrhizae are able to absorb and accumulate in the fungus mantle various essential elements such as nitrogen, phosphorus, potassium, calcium and magnesium, as well as trace elements such as, manganese, boron, and molybdenum more rapidly and efficiently and for longer periods of time than nonmycorrhizal feeder roots. They then translocate these elements to host root tissues. At the same time, ectomycorrhizae are able to break down certain complex minerals and organic substances in the soil and make essential nutrients from these materials available to their host (Marx, 1972, 1977; Bowen, 1973; Theron, 1982; Marx and Schenck, 1983; Gulden and Hølland, 1985). A more important point is that many ectomycorrhizal fungi have the ability to extract nutrients at lower soil availabilities than those available to their plant hosts (Hewitt and Smith, 1975; Bannister, 1976).

2) Protecting hosts against fungal pathogens

Mycorrhizae establish resistance to certain feeder root pathogens (Marx, 1973; Theron, 1982; Marx and Schenck, 1983). The production of bacteriostatic and fungistatic substances by mycorrhizae has been known for a long time (Harley and Smith, 1983). Numerous publications have shown that mycorrhizae can protect plants successfully against fungal pathogens (Perrin, 1983). These pathogens include *Cylindrocarpon destructans, Cylindrocladium scoparium, Fusarium oxysporum, F. solani, Mycelium radicis altovirens, Phytophthora* spp., *P. cinnamomi, P. infestans, Inonotus tomentosus* var. *circinatus, Phellinus weirii; Pythium* spp. such as *P. aphanidermatum, P. debaryanum, P. irregulare, P. spinnosum,* and *P. ultimum; Rhizoctonia* spp. such as, *R. praticola, R. solani; Sclerotium bataticola, S. rolfsii,* and *Thanatephorus* spp.(Marx, 1969a, 1969b; Marx *et al.*, 1970; Richard *et al.*, 1971; Park, 1980; Perrin, 1983; Sylvia, 1983 and Chakravarty and Unestam, 1987).

Duchesne *et al.* (1988) stated that pine root exudate stimulates the synthesis of antifungal compounds by some ectomycorrhizal fungi. Chakravarty and Unestam (1987) found that protection by mycorrhizal fungi took place even before visible mycorrhizae were found on the roots and may not only be effective inside the root, but probably also extend out into the rhizoplane or rhizosphere.

The resistance mechanisms of mycorrhizae to pathogenic infections are evident in several ways *viz.*, (a) utilizing surplus carbohydrates in the root and thereby reducing the amount of chemicals stimulatory to pathogens, (b) providing a physical barrier (the fungal mantle) to penetration by the pathogen, (c) secreting antibiotics inhibitory to pathogens, (d) supporting, along with the root, a protective microbial rhizosphere population, and (e) producing chemical inhibitors by the host cortex cell in response to symbiotic infection that may function as inhibitors to infection and spread of pathogens in mycorrhizal roots (Zak, 1964; Marx, 1969a).

3) Improving rhizosphere conditions

Mycorrhizal activities increase populations of nonpathogenic microorganisms in the rhizosphere of the plant (Manion, 1981). These nonpathogenic microorganisms in turn may improve rhizosphere conditions. Microorganisms associated with the mycorrhizae have an important task in maintaining symbiosis and survival of the fungus (Rambelli, 1973).

4) Producing plant growth hormones and regulators

Mycorrhizae produce auxin, cytokinins, gibberellins and other plant growth hormones and growth regulators (Slankis, 1973). Most of the known ectomycorrhizal fungi can produce auxin (Harley and Smith, 1983). In pine mycorrhizae, abundant growth hormones are present in the fungal hyphae, particularly in the fungal mantle. Various auxins are localized in the vacuoles of hyphae that are intermeshed with cortical cells and in the endodermis and pericycle of the roots. Dichotomous branching is induced in pines (Slankis, 1973; Hanley and Greene, 1987). Some ectomycorrhizal fungi, such as *Rhizopogon reseolus*, *Suillus* (*Boletus*) *cothurnatus*, *S.* (*Boletus.*) *punctipes*, and *Amanita rubescens* are known to produce cytokinins, although little work has been done on the exudation of gibberellins (GA) by ectomycorrhizal fungi. It was proved earlier that *Boletus edulis* var. *pinicolus* produced GA in the fungal mycelium and fruiting body (Gogala, 1971). Hanley and Greene (1987) likewise found that *Pisolithus tinctorius* and *Thelephora terrestris* produced GA that increased shoot growth of Scotch pine over time.

Auxin and cytokinins were detected as products of ectomycorrhizal fungi many years ago. Auxin and cytokinins are produced universally, at least in the ectomycorrhizal fungus-root complex. They probably are produced in small quantities as secondary metabolites, that are synthesized from root-originated substrates and are present at fairly constant, low concentrations (Hacskaylo, 1983).

5) Increasing the tolerance of hosts to adverse conditions

Mycorrhizae increase the tolerance of trees to low and high temperatures, soil toxins, extremes of soil pH, and resistance to drought (Theron, 1982; Marx and Schenck, 1983; Gulden and Hølland, 1985; Crum, 1988). The presence of mycorrhizae may be an important factor in lessening the plant's susceptibility to pollution (Kowalski, 1987). Kowalski's research showed that mycorrhizae with a relatively thick fungal mantle are more resistant to pollution of industrial emissions than mycorrhizae devoid of the mantle. There are some indication that mycorrhizal fungi can protect the roots against toxic effects of O_3 and SO_2 (Garrett *et al.*, 1982), against heavy metals from pollutant emissions (McCreight and Schroeder, 1982), and against mobilized Al from acidified soil (perhaps by formation of metal-organic complexes) (Meyer, 1984; Stroo and Alexander, 1985). This is the reason why some ectomycorrhizal fungi improved outplanting performances of seedling by increasing survival and early rapid growth after planting (Gagnon *et al.*, 1987). Survival and initial growth of pines up to an age of at least four years can be significantly improved on a wide range of sites if the root systems of transplants are adequately infected by selected superior strains of mycorrhizae.

This is why Marx *et al.* (1982) indicated that future production of containerized seedlings for reforestation should stress not only production of favorable shoot/root ratios, but also well developed mycorrhizae.

LACCARIA PROXIMA (BOUD.) PAT. *SENSU* BOUD.

The genus *Laccaria* Berk. & Br. includes 26 fungal species (Høiland, 1976; Singer, 1977; Kuhner, 1976; Last *et al.*, 1984; Ballero, 1987; Gagnon, 1987; Lee and Yang, 1987; Mueller, 1987; Mueller, 1989). *Laccaria* is classified in the subclass Basidiomycetes, order Agaricales and family Tricholomataceae (Singer, 1977).

Within this genus, most research has focused on *L. laccata*. Only few studies have been made on *L. proxima*. But *L. proxima*, like *L. laccata*, is a species native to North America with an extensive geographical distribution. In North America, it is found in Canada, the United States and Mexico (Aquirre-Acosta and Perez-Silva 1978, Danielson *et al.*, 1984). Worldwide, the fungus is reported to occur in Australia (May *et al.*, 1987), New Zealand (McNabb, 1972), Korea (Lee and Yang, 1987), Japan (Sagara, 1981), Britain (Last *et al.*, 1984), Romania (Salageanu and Stefureac, 1972), Italy (Giovanni, 1980; Ballero and Contu, 1987), and Norway (Høiland, 1976). Its host range is still very unclear, but scientists have found *L. proxima* on Fraser fir

(*Abies fraseri*) (Kenerley *et al.*, 1984), paper birch (*Betula papyrifera*) (Jones and Hutchinson, 1986), European white birch (*B. pendula, B. pubescens*) (Last *et al.*, 1984; Pelham *et al.*, 1988), Japanese chestnut (*Castanea crenata*) (Lee and Yang, 1987), tamarack (*Larix laricina*) (Zhu, 1985), white spruce, black spruce, Sitka spruce (*P. sitchensis*), jack pine, lodgepole pine (*P. contorta*), and Scotch pine (*P. sylvestris*) (Kampert and Strzelczyk, 1978; Danielson, 1984; Mason *et al.*, 1987, Cote and Thiboult, 1988). Thus, the host range of *L. proxima* is very broad and therefore of potential economical significance to tree seedling production.

Laccaria proxima isolated from mycorrhiza of Scotch pine produced cytokinins (Kampert and Strzelczyk, 1978). Danielson (1984) found that *L. proxima* improved the growth of jack pine. Danielson *et al.* (1984) showed that the infection of containerized jack pine by *L. proxima* was reduced when fertilized with 60 mg N/L (also expressed as 400 mg/L of 15-15-18) twice weekly. This suggests that high N concentrations may restrict the formation of the *L. proxima* and jack pine symbion. Jones *et al.* (1986) studied the influence of mycorrhizal associations between *L. proxima* and paper birch and jack pine seedlings which were exposed to elevated copper, nickel or aluminum, but the results were uncertain. He considered that mycorrhizal and non-mycorrhizal seedlings responded differently to Al, Ca, Cu, and Ni, but in general, the ectomycorrhizal fungi were least tolerant to Cu in liquid culture. *Laccaria proxima* and *Lactarius rufus* were proved most detrimental to birch grown in the presence of Cu (Jones *et al.*, 1988).

Being a pioneer fungus of a broad host range, *L. proxima* colonised new trees that developed in open prairies. This explains why *L. proxima* established mycorrhiza from spores in soil from treeless sites. Fruiting bodies of *L. proxima* took two years to appear after a stand of birches were planted at Bush estate near Edinburgh (Mason *et al.*, 1987).

FIBROUS PULP WASTE

A Problem

In most wood pulping processes, only about one half of the original wood is converted to usable products (Dolar *et al.*, 1972). There are 143 pulp and paper mills in Canada, of which 35 exist in Ontario (Canadian Pulp and Paper Association, 1989). In 1987, 23 million tonnes of wood pulp were produced and 102 million m^3 of wood were consumed by pulp and paper mills. Producing one tonne of wood pulp consumes 4.37 m^3 of wood (Canadian Pulp and Paper Association, 1989). The leftover fiber and by-products of the pulping process are largely disposed of as waste (Dolar *et al.*, 1972). In northwestern Ontario alone, thousands of tonnes of pulp and fibrous pulp waste are produced each day (D'Arcy, 1988). The liquid and solid wastes pose a serious problem to a society that is becoming increasingly aware of the detrimental influences of pollution on the environment and the need for recycling and conservation.

There are other aspects that relate to the wood waste problem. One aspect is that the wood pulp waste is contaminated with chemicals used in the pulping process *viz.* kaolinite, sulphite liquor and certain excess elements such as Na^+ which are known to reach toxic levels to oak plants (Dolar *et al.*, 1972). Chlorinated compounds and many other chemicals might also be of a toxic nature to the environment. If fibrous pulp waste is disposed of as landfill directly into the environment, it could become a serious source of pollution (Sawhney and Kozloski, 1984).

Disposal Methods

Pulp waste constitutes a disposal problem and a heavy economical burden for pulp companies. In 1987, the Canadian Pacific Forest Products Ltd. mill of Thunder Bay was paying approximately $ 13 per tonne for disposal of pulp waste as landfill (D'Arcy, 1988), the simplest disposal method.

There are five ways by which pulp waste may be utilized or disposed. These are as follows:

a) as landfill,

b) as organic fertilizer when mixed with high nutrient municipal wastes (D'Arcy, 1988),

c) as a mulch over the land (Dolar *et al.*, 1972),

d) conversion to liquid oil, and

e) gasification for burning and energy production (Maloney, 1978).

Disposing of pulp waste in a landfill is attractive in that it has historically been cost effective and minimized the impact on the mill's production processes. Paper mills dispose of approximately three quarters of their pulp waste sludge in landfills (Miner and Marshall, 1976) while the other quarter is combusted in a power boiler or incinerator (Edde, 1984). With limited loading, the waste can be successfully incorporated into the soil as a result of the high organic matter content (Gehm, 1973). However, increasing concern over potential leachates from landfills, limited landfill capacity, and increasing transportation costs have increased interest in other disposal methods (Dolar *et al.*, 1972; Edde, 1984).

A reasonable method to utilize pulp waste would be in conjunction with high-nutrient municipal waste, but little research has been conducted up to this time. When municipal waste alone or municipal waste associated with ectomycorrhizal fungus was used as a soil amendment, seedling growth and revegetation in adverse soil conditions were promoted significantly (Berry and Marx, 1976). A number of investigations have been carried out in recent years to evaluate the feasibility of municipal sludge disposal by using hydromulching and inorganic soil amendments for crops and for pulpwood, with generally encouraging results. Recent studies also have been developed relative to land reclamation and long term consolidation properties of landfilled sludges (Edde, 1984).

The conversion of pulp waste into liquid oil or combustible gases is another good concept, but a large capital investment is needed to implement this method.

CHAPTER 3. THE EFFECTS OF *L. PROXIMA* AND PULP WASTE ON TREE HOSTS

INTRODUCTION

As a native ectomycorrhizal fungus to North America (Singer, 1977; Aquirre-Acosta and Perez-Silva, 1978; Danielson *et al.*, 1984) and a pioneer ectomycorrhizal fungus of many coniferous species (Mason *et al.*, 1987), *Laccaria proxima* has a broad host range. The fungus is found on gymnosperms and angiosperms (Kenerley *et al.;* 1984, Last *et al.*, 1984; Jones and Hutchinson, 1986; Lee and Yang, 1987; Cote and Thiboult, 1988), but information about its specific host, especially important tree species, is limited. However, detailed host ranges of mycorrhiza must be established (Navratil *et al.*, 1981). It is necessary to explore the effective host range of *L. proxima* so as to provide better understanding of its potential availability and economical significance to forestry.

Disposal methods of fibrous pulp waste, as a fast-growing problem due to its expense, limited disposal sites and negative impact on the environment (Dolar *et al.*, 1972, Sawhney and Kozloski, 1984; D'Arcy, 1988), need to be solved based on environmental concerns. A feasible approach to the problem is to utilize fibrous pulp waste as a potting medium component in the production of containerized tree seedlings. In order to determine the possibility of utilizing pulp waste as a potting medium component, it is important to understand the effect of pulp waste and the interactive effects of pulp waste in association with *L. proxima* on certain main conifers.

The following experiments were designed to test the influence of the mycorrhizal fungus *L. proxima* and pulp waste as a potting medium at 33% v/v on the growth of nine conifer species.

MATERIALS AND METHODS

Laccaria proxima was obtained (original source unknown) from the pathology laboratory, Lakehead University. It was grown with seven tree species established from the following Lakehead University seed lots:

white spruce (*Picea glauca* (Moench) Voss), seed lot 48.400 S81086;
black spruce (*P. mariana* (Mill.) B.S.P.), seed lot 48.600 S83032;
red spruce (*P. rubens* Sarg.), seed lot 48.700 S86031;
jack pine (*Pinus banksiana* Lamb.), seed lot 50.200 S83034;
mugo pine (*P. mugo* Turra), seed lot 50.600 S80270;
red pine (*P. resinosa* Ait.), seed lot 50.700 S83053;
and Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirbel) Fr.), seed lot 55.150 S82007.

Japanese larch [*Larix kaempferi* (Lamb.) Carr.] and Japanese black pine [*Pinus thunbergii* Parl.] seed were obtained from the Qingdao Forestry Bureau, Shandong, the People's Republic of China.

The seeds were surface sterilized with 1% sodium hypochlorite for 20 minutes, and rinsed in water.

Fibrous pulp waste was obtained from the Canadian Pacific Forest Products Ltd. mill of Thunder Bay. The pulp waste contains most macro- and micro nutrients as well as heavy metals and the particle size of the pulp waste mainly distributes from 2 mm to 12 mm (Appendix VII).

Sphagnum peat moss (Sunshine brand) and vermiculite (Terra-Lite brand) were obtained from the greenhouse of Lakehead University.

The potting medium: peat moss, vermiculite and pulp waste were autoclaved at 121°C for 20 minutes before use.

The seedlings were grown in Can-Am multipots (M-2). The multipot size is 22 x 35 cm and has 67 cavities. The individual cavity size is 3.3 x 12.0 cm and 67 cm^3. The cavity diameter: height ratio is 1:3.6. Spacing between cavities is 3.5 cm.

The inoculum was *L. proxima* permeated rye grain. Rye grain (180 g & 250 cm^3) with 100 mL water was added to 500 mL fruit jars (total 10) and autoclaved at 121 °C for 20 minutes. Every jar was inoculated with about 20 cm^3 prepared rye grain inoculum. After inoculation, all jars were stirred to assure mixing of rye grain with *L. proxima*.

Before being used, the jars were incubated at 25°C for *ca.*15 days for mycelial establishment.

A 2^2 factorial experiment design was used in the experiment (Table 1). The two factors were *L. proxima* and pulp waste. The two levels were absence (0) and presence (1) of the two factors (Tables 1 and 2).

Table 1. A 2^2 factorial experiment design pertaining to the effects of *L. proxima*/pulp waste medium on nine conifers.

Pulp Waste	*L. proxima*	
	Without	With
Without	00	01
With	10	11

Table 2. Treatment combinations of the experiment of the effects of *L. proxima* and pulp waste on hosts.

Treatment	*L.proxima*	Pulp Waste
Control	0	0
Pulp Waste(Pw)	0	1
L. proxima(Lp)	1	0
Lp with Pw	1	1

For the control, the potting medium composition was, by volume, equal parts peat moss and vermiculite (Riffle and Tinus, 1982; Hung and Molina, 1986a). For the pulp waste treatment, the medium composition was pulp waste, peat moss and vermiculite in equal amounts by volume. In the *L. proxima* treatment, a potting medium composed of peat moss and vermiculite in equal amounts by volume (1500 cm^3 in total) was inoculated with 20 cm^3 of *L. proxima* rye seed inoculum. In the *L. proxima* plus pulp waste treatment, a medium composed of pulp waste, peat moss and vermiculite in proportions of 1:1:1 by volume (1500 cm^3 in total) was inoculated with 20 cm^3 of *L. proxima* rye seed inoculum. Replications ranged from six to 19 for the nine tree species. Tree species and treatments were randomly assigned to multipots. Jack pine was grown from March 6 to May 2, 1988, and the Japanese black pine and larch were grown from July 10 to Dec. 16, 1988. The other species were grown from May 20 to Dec. 21, 1988.

The experiment (including eight containerized seedling experiments) was conducted in the Lakehead University greenhouse and followed the same procedure as described above. After complete mixing and wetting with tap water, media prepared according to each experiment, were carefully put into multipots. Two to three seeds were sown into each multipot cavity. Seeds were covered with vermiculite to a depth of 5 mm. After watering to saturation, the multipots were covered with black plastic polyethylene for 3 days to maintain moisture and to induce seed germination. When germination was complete and seed coats shed, seedlings were thinned to one seedling per cavity. The seedlings were watered for 10 seconds every 16 min by an automatic sprinkling system. Since August 1988 the seedlings were watered twice each day with an automatic overhead irrigation system. The seedlings were fertilized once a week by hand with a TERR-LIFE Sphon Mixer at 300 ppm of 20-20-20 from a stock solution of 7500 ppm. The fertilizer solution was allowed to drip freely through the drain holes of multipots. The fertilizer was applied once weekly. Temperature ranged from about 17 to 32°C in winter and 19 to 38°C in summer. Relative humidity varied from about 80 to 90%. The seedlings were exposed to natural sunlight in the greenhouse (No. 1). The day length in Thunder Bay varies from 8 hr and 20 min to 16 hr and 19 min each year. (Jack pine seedlings were grown in a different greenhouse (No. 2) under daylight and 16 hr High Pressure Sodium Lamp (400W) and watered for 10 sec every 16 min by an automatic sprinkling system).

To eliminate position effects on the benches, 18 multipots on the southeast bench in the greenhouse were systematically rotated in position weekly throughout the experiments. After the seedlings were harvested, length and dry weights of shoot, roots and total (shoot+root) were determined. Dry weights were obtained by oven-drying seedlings at 102°C±2°C for 24 hr.

SPSS-X on the Lakehead University microvax computer was used for the statistical analyses.

Statistical comparison were made between (1) mycorrizal and no-mycorrhizal plants (the effects of *L. proxima*), (2) seedling grown in media with pulp waste or without pulp waste (the effects of pulp waste) and (3) seedling grown in media with mycorrhiza and pulp waste or without mycorrhiza and pulp waste (the interaction of *L. proxima* and pulp waste).

RESULTS

Microscopic examination of the feeder roots of jack pine confirmed the ectomycorrhizal nature of *L. proxima* (Appendix VIII). The root systems of other species were examined macroscopically for mycorrhiza.

White Spruce

Seedling Length

Laccaria. proxima had no significant effects on shoot, root and total length of white spruce. The addition of pulp waste to the peat/vermiculite had significantly negative effects on shoot and total lengths but not root length (Table 3). *L. proxima* and pulp waste had no significant interaction on shoot and total length of white spruce, but had a significantly negative interaction on root length. Among all four treatments, there were no significant differences in root length (Table 3 and Fig. 1).

Table 3. Average length (mm) of white spruce seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 221 days.

Growth Parameter	Pw (%)	*Laccaria proxima* (SE)		*Pr* > F		
		Absent	Present	Lp*	Pw	Lp-Pw
Shoot Length (mm)	0	81±3	79±4	0.771	**0.003**	0.954
	33	68±4	67±5			
Root Length (mm)	0	123±2	128±3	0.687	0.615	**0.032**
	33	127±2	121±2			
Total Length (mm)	0	204±4	207±5	0.664	**0.007**	0.327
	33	194±6	188±5			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 45 degrees of freedom.

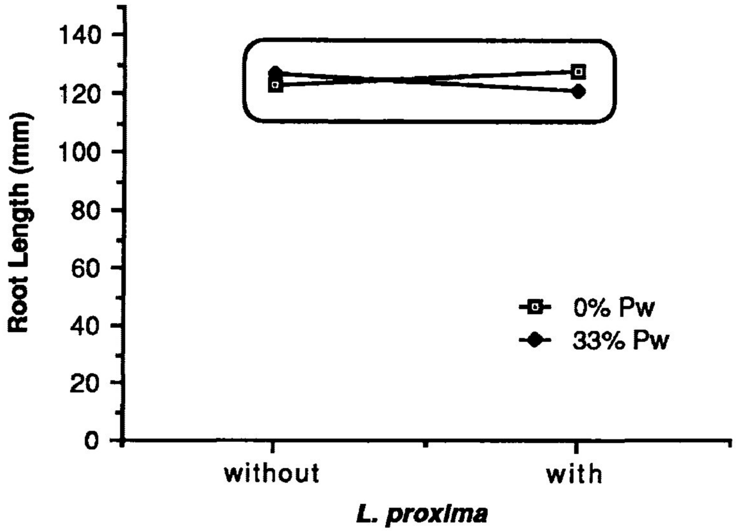

Fig. 1. Interaction of *L. proxima* with pulp waste on root length of white spruce and multicomparison among the treatments.

Seedling Dry Weight

Laccaria proxima had a significantly positive effect on root dry weight, but not on shoot and total dry weights. *Laccaria proxima* did stimulate the seedling growth in total dry weight evidently. Pulp waste without *L. proxima* had significantly negative effects on shoot, root, and total dry weights of the white spruce. *L. proxima* with pulp waste had no interactions on dry weights of white spruce (Table 4).

Table 4. Average dry weight (mg) of white spruce seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 221 days.

Growth	Pw	*Laccaria proxima* (SE)		*Pr* > F		
Parameter	(%)	Absent	Present	Lp*	Pw	Lp-Pw
Shoot Dry Wt. (mg)	0	259±27	262±27	0.195	**0.000**	0.307
	33	114±15	165±24			
Root Dry Wt. (mg)	0	174±22	196±21	**0.036**	**0.001**	0.362
	33	84±18	144±19			
Total Dry Wt. (mg)	0	433±46	458±44	0.075	**0.000**	0.301
	33	198±31	308±41			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 45 degrees of freedom.

Black Spruce

Seedling Length

L. proxima significantly improved the shoot and root length of black spruce, but had no significant effect on total length (Table 5). Pulp waste had significantly negative effects on shoot and total length but not root length. Although *L. proxima* and pulp waste had no interactive effects on shoot and total lengths of black spruce, there was a negative interaction on root length (Table 5 and Fig. 2). Black spruce seedlings grown without *L. proxima* and pulp waste was better than the treatment with *L. proxima* and pulp waste in root length. Longer root systems were found with seedlings grown with either *L. proxima* or pulp waste than with both *L. proxima* and pulp waste (Fig. 2). There were no significant differences among *L. proxima*, pulp waste and the control treatments in regard to the root systems.

Seedling Dry Weight

While *L. proxima* significantly increased shoot, root and total dry weights of black spruce in media without pulp waste and with pulp waste (Table 6), the overall effects of the pulp waste were negative. Interactions of *L. proxima* and pulp waste significantly improved shoot, root and total dry weights of black spruce (Table 6 and Figs. 3, 4, and 5).

Table 5. Average length (mm) of black spruce seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 221 days.

Growth Parameter	Pw (%)	*Laccaria proxima* (SE) Absent	Present	*Pr* > F Lp*	Pw	Lp-Pw
Shoot Length (mm)	0	134±3	143±5	**0.019**	**0.005**	0.323
	33	109±7	131±8			
Root Length (mm)	0	126±2	127±2	**0.009**	0.287	**0.001**
	33	131±2	119±6			
Total Length (mm)	0	260±4	270±5	0.105	**0.001**	0.957
	33	240±7	250±7			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 55 degrees of freedom.

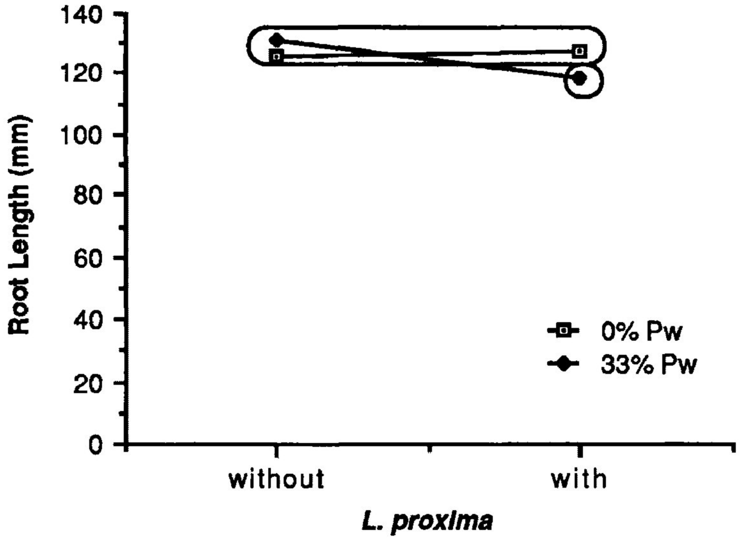

Fig. 2. Interaction of *L. proxima* with pulp waste on root length of black spruce and multicomparison among the treatments.

Table 6. Average dry weight (mg) of black spruce seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 221 days.

Growth	Pw	*Laccaria proxima* (SE)		*Pr* > F		
Parameter	(%)	Absent	Present	Lp*	Pw	Lp-Pw
Shoot Dry Wt. (mg)	0	439±21	471±18	**0.000**	**0.000**	**0.012**
	33	163±18	318±33			
Root Dry Wt. (mg)	0	266±21	290±18	**0.001**	**0.000**	**0.031**
	33	84±18	144±19			
Total Dry Wt. (mg)	0	706±38	761±27	**0.000**	**0.000**	**0.007**
	33	256±35	517±45			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 55 degrees of freedom.

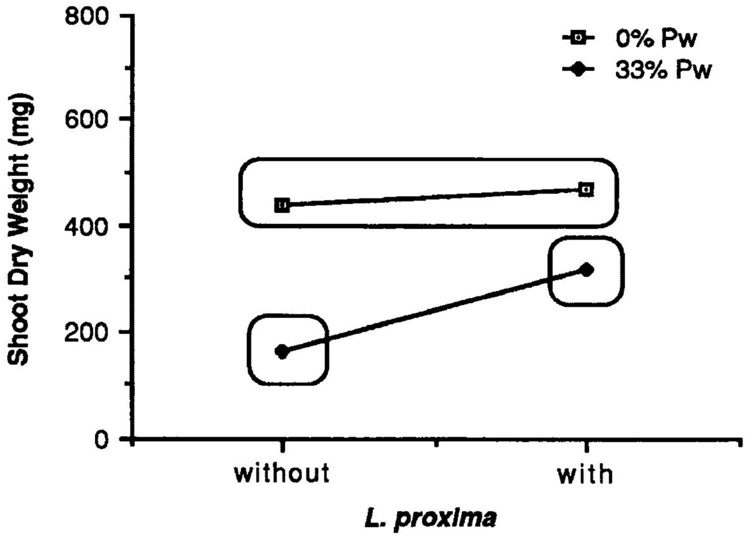

Fig. 3. Interaction of *L. proxima* with pulp waste on shoot dry weight of black spruce and multicomparison among the treatments.

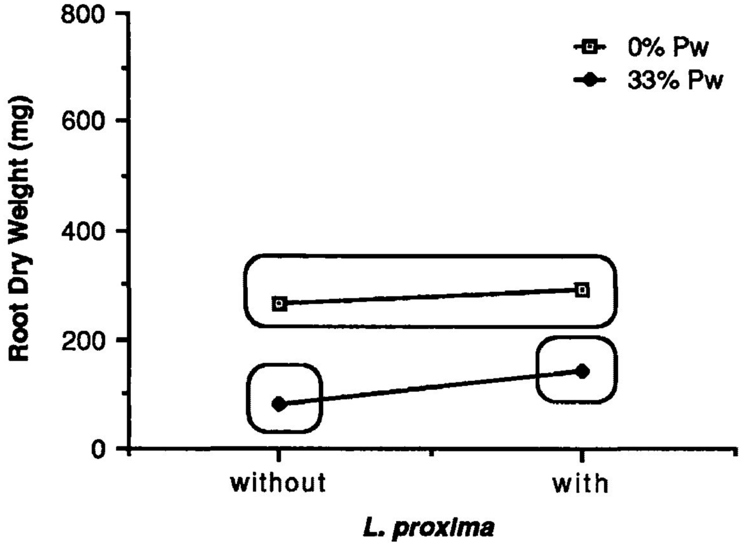

Fig. 4. Interaction of *L. proxima* with pulp waste on root dry weight of black spruce and multicomparison among the treatments.

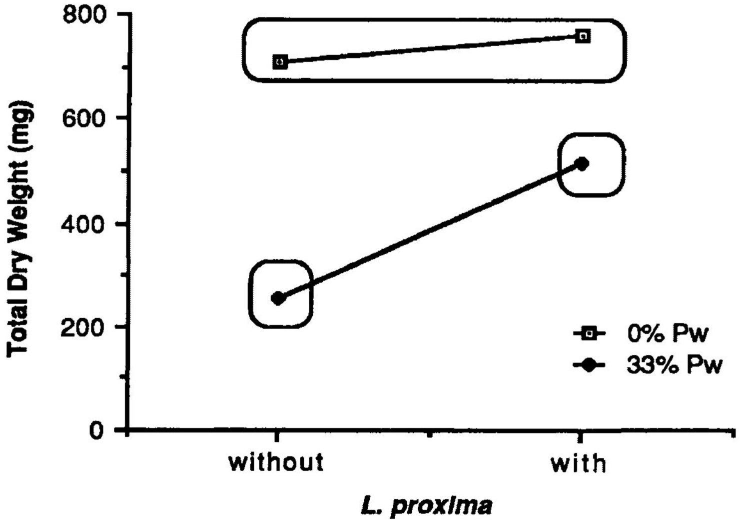

Fig. 5. Interaction of *L. proxima* with pulp waste on total dry weight of black spruce and multicomparison among the treatments.

The effects of *L. proxima* and pulp waste as well as the interaction of *L. proxima* with pulp waste showed a similar trend in that shoot, root and total dry weights of black spruce grown in peat moss and vermiculite with or without

L. proxima treatment were significantly heavier than those with the pulp waste amendment. In the *L. proxima* plus pulp waste treatment, dry weights of black spruce were significantly greater than in pulp waste without *L. proxima* treatment (Figs. 3, 4, and 5).

Red Spruce

Seedling Length

L. proxima significantly increased the root length of red spruce, but not the shoot and total lengths (Table 7). Pulp waste had significantly negative effects on shoot and total lengths of red spruce but not on root length. *L. proxima* and pulp waste had no interactive effects on seedling lengths of red spruce (Table 7).

Table 7. Average length (mm) of red spruce seedlings grown with and without *L. proxima* and pulp waste and *Pr > F* values after 221 days.

Growth Parameter	Pw (%)	*Laccaria proxima* (SE) Absent	Present	*P > F* Lp*	Pw	Lp-Pw
Shoot length (mm)	0	174±6	165±6	0.729	**0.000**	0.057
	33	130±6	144±6			
Root Length (mm)	0	119±2	128±3	**0.012**	0.750	0.363
	33	121±2	125±2			
Total Length (mm)	0	293±6	293±6	0.183	**0.000**	0.132
	33	251±6	269±7			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 68 degrees of freedom.

Seedling Dry Weight

L. proxima significantly increased root and total dry weights of red spruce seedlings, but not shoot dry weight. Pulp waste had significantly negative effects on dry weights of red spruce. *L. proxima* and pulp waste had no interactive effects on red spruce seedling dry weights (Table 8).

Table 8. Average dry weight (mg) of red spruce seedlings grown with and without *L. proxima* and pulp waste and *Pr > F* values after 221 days.

Growth	Pw	*Laccaria proxima* (SE)		*P* > F		
Parameter	(%)	Absent	Present	Lp*	Pw	Lp-Pw
Shoot Dry Wt. (mg)	0	604±23	588±31	0.216	**0.000**	0.056
	33	284±34	383±32			
Root Dry Wt. (mg)	0	226±17	269±22	**0.002**	**0.000**	0.335
	33	117±18	198±20			
Total Dry Wt. (mg)	0	830±31	857±44	**0.027**	**0.000**	0.081
	33	401±50	581±48			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 68 degrees of freedom.

Jack Pine

Seedling length

L. proxima significantly improved shoot and total lengths of jack pine seedlings, but not the root length (Table 9). Pulp waste without mycorrhiza was markedly detrimental to both root and shoot lengths; however there were significantly positive interactive effects of *L. proxima* and pulp waste on shoot and total lengths of jack pine seedlings, but not on root length (Table 9 and Figs. 6 and 7). Shoot lengths of jack pine seedlings grown with or without *L. proxima* and *L. proxima* with pulp waste were significantly greater than those in pulp waste without *L. proxima* (Fig. 6). Total length of jack pine seedlings grown in the control and *L. proxima* treatment were significantly greater than in pulp waste and *L. proxima* with pulp waste treatments. Total lengths of seedlings grown in media with *L. proxima* with pulp waste were significantly greater than those grown with pulp waste in the media and without *L. proxima* (Table 9, Fig. 7).

Table 9. Average length (mm) of jack pine seedlings grown with and without *L. proxima* and pulp waste and *Pr* > *F* values after 58 days.

Growth Parameter	Pw (%)	*Laccaria proxima* (SE)		*Pr* > F		
		Absent	Present	Lp*	Pw	Lp-Pw
Shoot Length (mm)	0	79±2	78±3	**0.000**	**0.000**	**0.000**
	33	52±2	76±3			
Root Length (mm)	0	112±3	115±3	0.500	**0.005**	0.683
	33	106±2	107±2			
Total Length (mm)	0	191±3	193±3	**0.000**	**0.000**	**0.000**
	33	158±3	183±3			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 85 degrees of freedom.

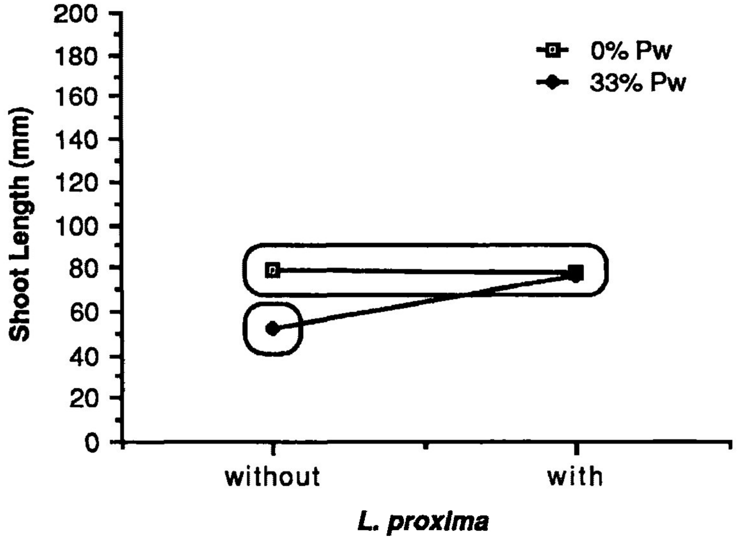

Fig. 6. Interaction of *L. proxima* with pulp waste on shoot length of jack pine and multicomparison among the treatments.

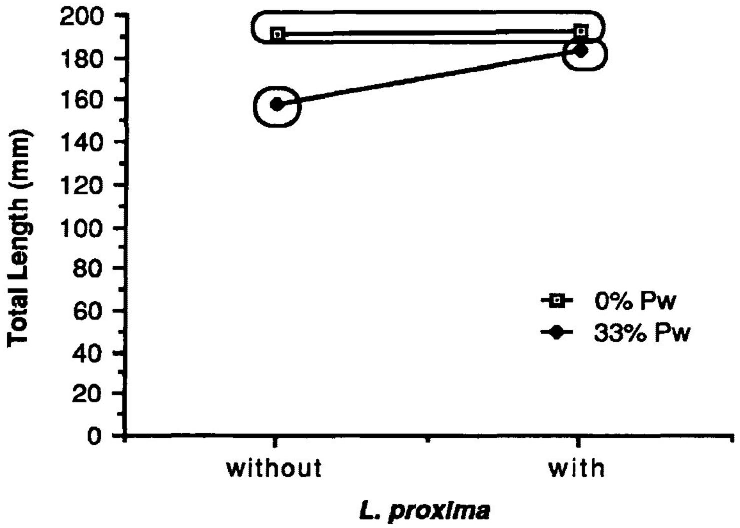

Fig. 7. Interaction of *L. proxima* with pulp waste on total length of jack pine and multicomparison among the treatments.

Seedling Dry Weight

Both *L. proxima* and pulp waste had significant effects on jack pine seedling dry weight, but the effects of pulp waste were negative (Table 10). *L. proxima* and pulp waste had significant interactive effects on dry weights of jack pine seedlings (Table 10 and Figs. 8, 9 and 10). Dry weights (including shoot, root and total) of jack pine grown in *L. proxima* with or without pulp waste were significantly heavier than in nonmycorrhizal control and pulp waste treatments. Among control and pulp waste treatments, dry weights in the controls were significantly heavier than in the pulp waste treatment (Figs. 8, 9, and 10). The jack pine seedlings were smaller than the other species tested because they were grown for a 3 months to 5 months shorter period of time.

Table 10. Average dry weight (mg) of jack pine seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 58 days.

Growth Parameter	Pw (%)	*Laccaria proxima* (SE) Absent	Present	*Pr > F* Lp*	Pw	Lp-Pw
Shoot Dry Wt. (mg)	0	53±2	82±8	**0.000**	**0.000**	**0.008**
	33	14±1	77±10			
Root Dry Wt. (mg)	0	26±1	36±4	**0.000**	**0.007**	**0.002**
	33	11±1	38±4			
Total Dry Wt. (mg)	0	79±3	117±11	**0.000**	**0.000**	**0.007**
	33	25±2	114±13			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 85 degrees of freedom.

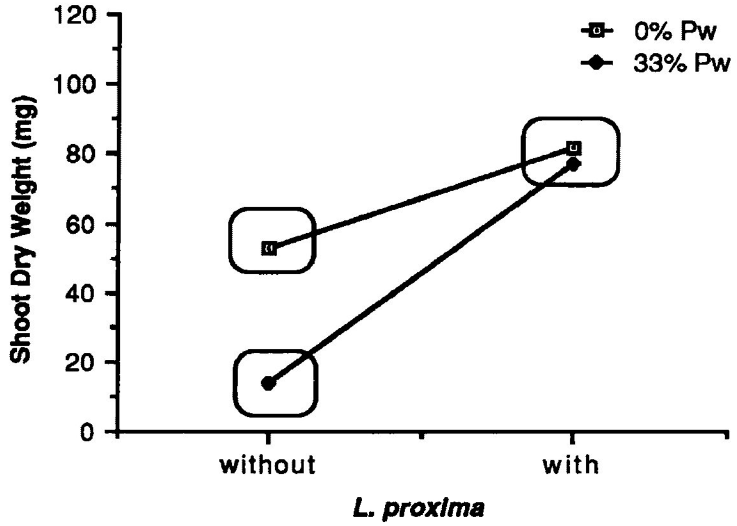

Fig. 8. Interaction of *L. proxima* with pulp waste on shoot dry weight of jack pine and multicomparison among the treatments.

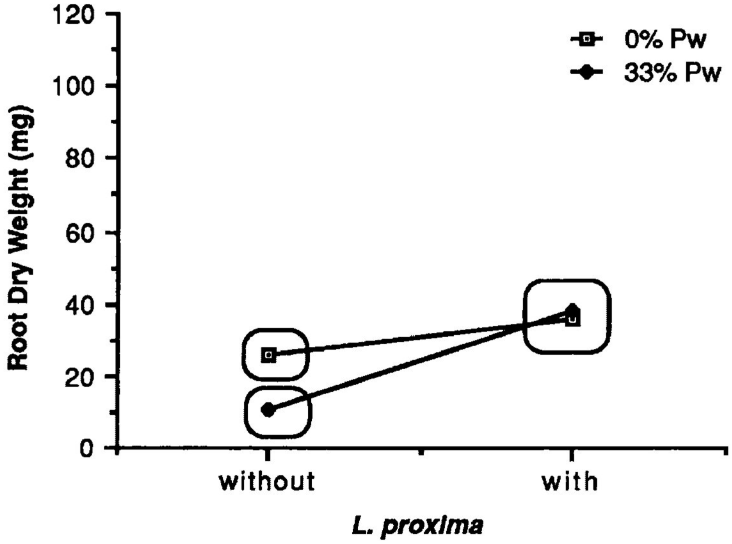

Fig. 9. Interaction of *L. proxima* with pulp waste on root dry weight of jack pine and multicomparison among the treatments.

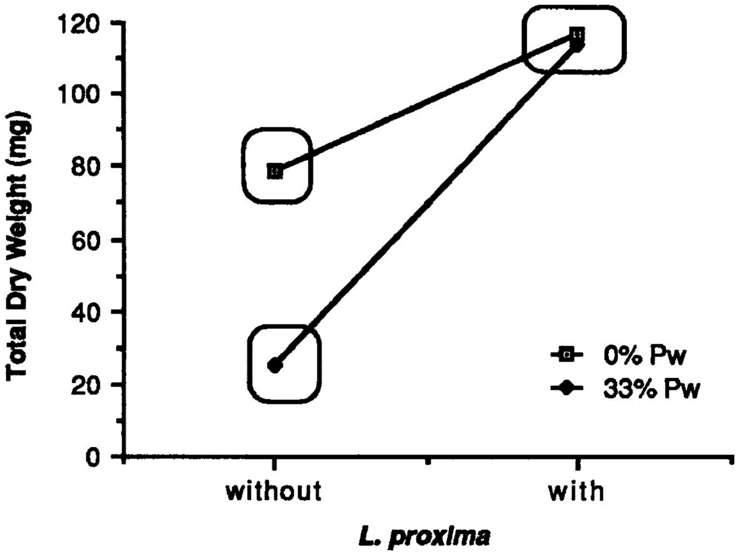

Fig. 10. Interaction of *L. proxima* with pulp waste on total dry weight of jack pine and multicomparison among the treatments.

Mugo Pine

Seedling Length

L. proxima and pulp waste had no individual or interactive effects on seedling shoot, root and total lengths of mugo pine (Table 11).

Table 11. Average length (mm) of mugo pine seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 221 days.

Growth Parameter	Pw %	*Laccaria proxima* (SE) Absent	Present	*Pr* > F Lp*	Pw	Lp-Pw
Shoot Length (mm)	0	130±5	132±6	0.709	0.501	0.922
	33	127±6	128±3			
Root Length (mm)	0	120±2	123±2	0.059	0.155	0.596
	33	122±3	128±6			
Total Length (mm)	0	249±5	255±6	0.124	0.648	0.509
	33	248±6	256±9			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 65 degrees of freedom.

Seedling Dry Weight

Both *L. proxima* and pulp waste had significant effects on shoot, root and total dry weights of mugo pine seedlings. The difference was that the effect of pulp waste was negative and there were no interactive effects (Table 12).

Table 12. Average dry weight (mg) of mugo pine seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 221 days.

Growth Parameter	Pw %	*Laccaria proxima* (SE) Absent	Present	*Pr* > F Lp*	Pw	Lp-Pw
Shoot Dry Wt. (mg)	0	488±44	640±47	**0.000**	**0.000**	0.239
	33	202±25	446±31			
Root Dry Wt. (mg)	0	223±27	342±25	**0.000**	**0.000**	0.939
	33	80±7	201±20			
Total Dry Wt. (mg)	0	711±67	982±67	**0.000**	**0.000**	0.400
	33	281±31	647±49			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 65 degrees of freedom.

Red Pine

Seedling Length

Both *L. proxima* and pulp waste had no significant effects on shoot, root and total length of red pine seedlings. *L. proxima* with pulp waste had a significantly negative interaction on root length, but not on shoot and total lengths (Table 13 and Fig. 11). Root lengths of red pine grown with pulp waste were significantly greater than those in the peat moss and vermiculite control with or without *L. proxima* and *L. proxima* with pulp waste. Among these three treatments, there were no significant differences (Fig. 11).

Table 13. Average length (mm) of red pine seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 221 days.

Growth Parameter	Pw %	*Laccaria proxima* (SE) Absent	Present	*Pr* > F Lp*	Pw	Lp-Pw
Shoot Length (mm)	0	154±6	165±6	0.416	0.426	0.389
	33	155±8	152±9			
Root Length (mm)	0	118±1	119±2	0.227	0.127	**0.039**
	33	127±4	118±3			
Total Length (mm)	0	273±6	284±6	0.636	0.736	0.099
	33	282±7	270±7			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 65 degrees of freedom.

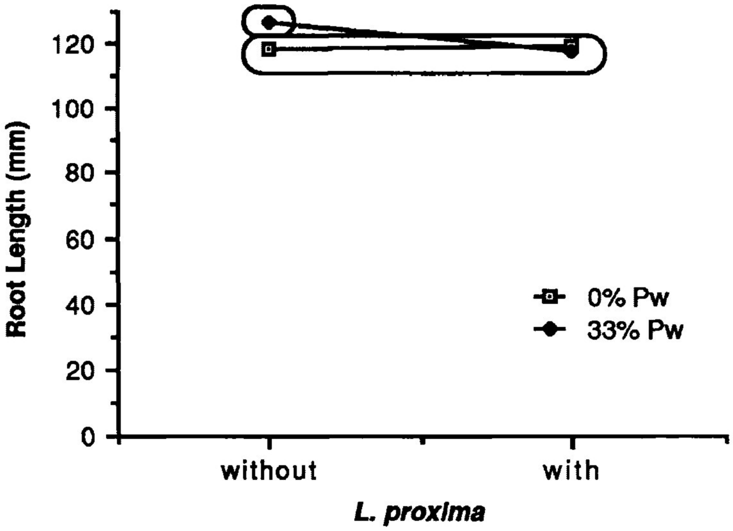

Fig. 11. Interaction of *L. proxima* with pulp waste on root length of red pine and multicomparison among the treatments.

<u>Seedling Dry Weight</u>

L. proxima had no significant effects on shoot, root and total dry weights of red pine. But pulp waste still had significantly negative effects on dry weights of red pine. There was no statistically significant interaction of *L. proxima* and pulp waste on dry weights of red pine (Table 14).

Table 14. Average dry weight (mg) of red pine seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 221 days.

Growth Parameter	Pw %	*Laccaria proxima* (SE)		*Pr* > F		
		Absent	Present	Lp*	Pw	Lp-Pw
Shoot Dry Wt. (mg)	0	510±52	583±53	0.225	**0.005**	0.997
	33	323±57	397±80			
Root Dry Wt. (mg)	0	335±21	332±37	0.404	**0.002**	0.227
	33	179±31	262±46			
Total Dry Wt. (mg)	0	845±66	916±82	0.247	**0.002**	0.635
	33	502±86	657±125			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 38 degrees of freedom.

Douglas-fir

Seedling Length

Laccaria proxima had significant effects on the shoot lengths of Douglas-fir but not on root and total lengths. However, *L. proxima* obviously improved the seedling growth in total length. Pulp waste had significantly negative effects on the shoot and total lengths but not on root length. *L. proxima* and pulp waste had no statistically significant interactive effects on Douglas-fir seedling length (Table 15).

Table 15. Average length (mm) of Douglas-fir seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 221 days.

Growth Parameter	Pw %	*Laccaria proxima* (SE) Absent	Present	*Pr* > F Lp*	Pw	Lp-Pw
Shoot Length (mm)	0	155±7	189±13	**0.028**	**0.007**	0.232
	33	137±7	149±9			
Root Length (mm)	0	109±1	109±1	0.974	0.788	0.799
	33	110±7	109±4			
Total Length (mm)	0	264±8	298±14	0.088	**0.033**	0.323
	33	247±11	258±10			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 24 degrees of freedom.

Seedling Dry Weight

L. proxima significantly increased the shoot, root and total dry weights of Douglas-fir seedlings. Pulp waste decreased the dry weight of Douglas-fir seedlings but insignificantly. There was no interaction of *L. proxima* with pulp waste on dry weights (Table 16).

Table 16. Average dry weight (mg) of Douglas-fir seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 221 days.

Growth	Pw	*Laccaria proxima* (SE)		*Pr* > F		
Parameter	%	Absent	Present	Lp*	Pw	Lp-Pw
Shoot Dry Wt. (mg)	0	364±32	506±55	**0.010**	0.108	0.799
	33	297±47	416±42			
Root Dry Wt. (mg)	0	277±36	384±41	**0.009**	0.589	0.924
	33	260±30	361+32			
Total Dry Wt. (mg)	0	640±53	890±96	**0.007**	0.227	0.846
	33	558±75	777±62			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 24 degrees of freedom.

Japanese Black Pine

Seedling Length

L. proxima had a significant effect on root length of Japanese black pine seedlings but not on shoot and total length. Pulp waste and *L. proxima* with pulp waste had significantly negative individual or interactive effects on shoot and total length respectively, but there was no difference with root length (Table 17). Shoot length of Japanese black pine seedlings grown with *L. proxima* was significantly greater than in the other three treatments. Among the three treatments, shoot length in the control and pulp waste treatments were significantly greater than in *L.*

proxima with pulp waste treatment (Fig. 12). Total length of Japanese black pine grown in *L. proxima* treatment was significantly greater than in the other three treatments. There were no significant differences among the three treatments in total seedling length (Fig. 13).

Table 17. Average length (mm) of Japanese black pine seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 160 days.

Growth Parameter	Pw %	*Laccaria proxima* (SE) Absent	Present	*Pr* > F Lp*	Pw	Lp-Pw
Shoot Length (mm)	0	160±3	170±3	0.721	**0.002**	**0.001**
	33	159±3	147±4			
Root Length (mm)	0	120±2	126±3	**0.003**	0.731	0.704
	33	118±2	126±2			
Total Length (mm)	0	280±4	296±4	0.109	**0.005**	**0.009**
	33	278±3	273±4			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 64 degrees of freedom.

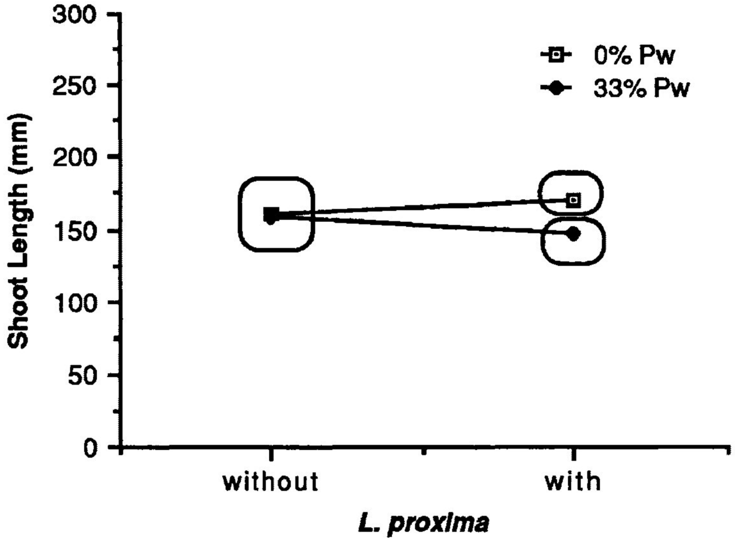

Fig. 12. Interaction of *L. proxima* with pulp waste on shoot length of Japanese black pine and multicomparison among the treatments.

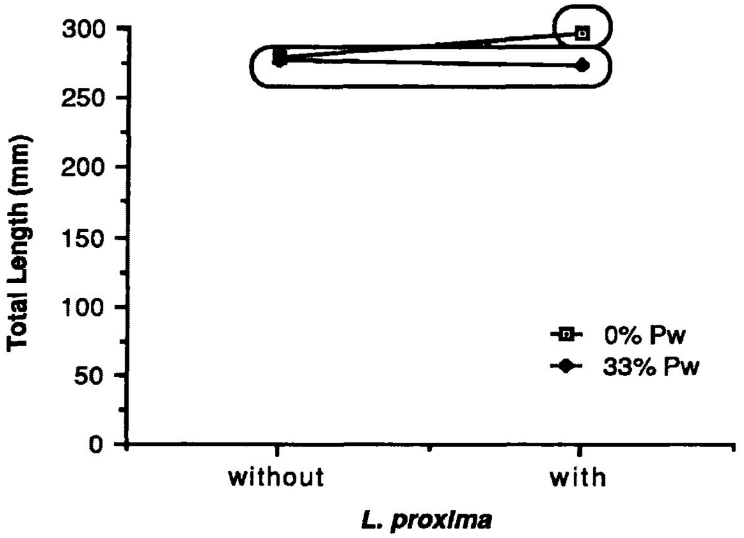

Fig. 13. Interaction of *L. proxima* with pulp waste on total length of Japanese black pine and multicomparison among the treatments.

Seedling Dry Weight

L. proxima significantly improved the root dry weight of Japanese black pine, but not the shoot and total dry weights. Pulp waste had significantly negative effects on shoot and total dry weights except root dry weight and *L. proxima* with pulp waste had significantly negative interactions on shoot, root and total dry weights (Table 18 and Figs. 14, 15 and 16). Shoot and total dry weights of Japanese black pine grown in *L. proxima* treatment were significantly heavier than in the other three treatments. Total dry weight of seedlings grown with pulp waste showed a slight increase compared with those grown without pulp waste and mycorrhiza, but there were no significant differences among the three treatments (Figs. 14 and 16). Root dry weight in *L. proxima* treatment without pulp waste was significantly heavier than those in the control and pulp waste treatments and heavier than *L. proxima* with pulp waste but insignificantly. In the *L. proxima* with pulp waste treatment, root dry weight was significantly heavier than in the pulp waste treatment without *L. proxima,* but not in the control and *L. proxima* treatment (Fig. 15).

Table 18. Average dry weight (mg) of Japanese black pine seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 160 days.

Growth Parameter	Pw %	*Laccaria proxima* (SE)		*Pr* > F		
		Absent	Present	Lp*	Pw	Lp-Pw
Shoot Dry Wt. (mg)	0	518±25	617±38	0.451	**0.007**	**0.005**
	33	512±16	453±28			
Root Dry Wt. (mg)	0	92±9	147±14	**0.000**	0.767	**0.034**
	33	112±7	127±10			
Total Dry Wt. (mg)	0	610±31	763±47	0.106	**0.035**	**0.005**
	33	624±21	580±37			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 64 degrees of freedom.

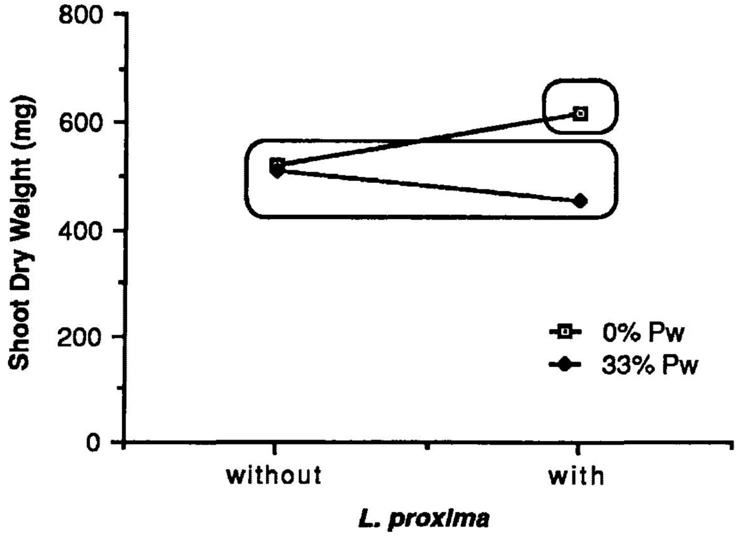

Fig. 14. Interaction of *L. proxima* with pulp waste on shoot dry weight of Japanese black pine and multicomparison among the treatments.

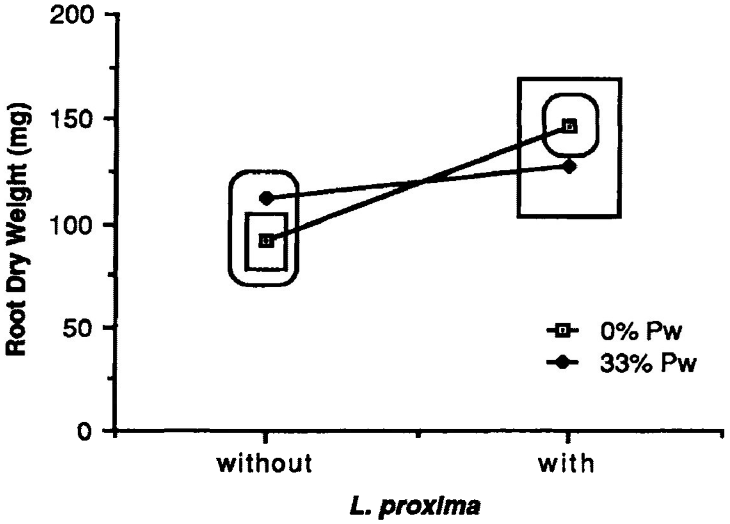

Fig. 15. Interaction of *L. proxima* with pulp waste on root dry weight of Japanese black pine and multicomparison among the treatments.

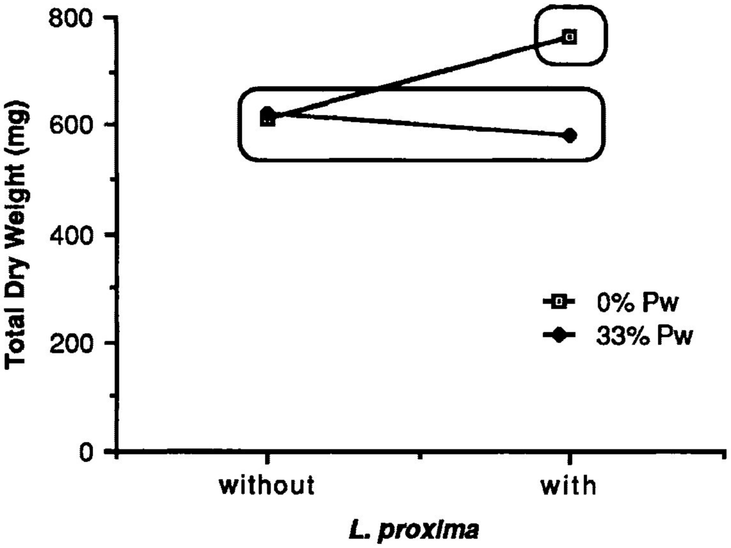

Fig. 16. Interaction of *L. proxima* with pulp waste on total dry weight of Japanese black pine and multicomparison among the treatments.

Japanese Larch

Seedling Length

L. proxima had no significant effects on seedling lengths of Japanese larch. Pulp waste had significantly negative effects on seedling lengths, and the presence of *L. proxima* with pulp waste failed to improve the seedlings. There were no interactions of *L. proxima* and pulp waste on seedling lengths (Table 19).

Table 19. Average length (mm) of Japanese larch seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 160 days.

Growth Parameter	Pw %	*Laccaria proxima* (SE) Absent	Present	*Pr* > F Lp*	Pw	Lp-Pw
Shoot Length (mm)	0	175±5	173±5	0.743	**0.009**	0.960
	33	156±9	153±6			
Root Length (mm)	0	124±3	123±3	0.599	**0.010**	0.825
	33	114±6	111±1			
Total Length (mm)	0	290±3	296±7	0.555	**0.002**	0.824
	33	270±12	265±6			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 47 degrees of freedom.

Seedling Dry Weight

L. proxima had no significant effects on seedling dry weights of Japanese larch. Pulp waste had a significantly negative effect on shoot dry weight, but not on root and total dry weights. There were no significant interactions of *L. proxima* with pulp waste on seedling dry weights (Table 20).

Table 20. Average dry weight (mg) of Japanese larch seedlings grown with and without *L. proxima* and pulp waste and *Pr* > F values after 160 days.

Growth	Pw	*Laccaria proxima* (SE)		*Pr* > F		
Parameter	%	Absent	Present	Lp*	Pw	Lp-Pw
Shoot Dry Wt. (mg)	0	383±31	406±25	0.512	**0.025**	0.950
	33	289±39	318±55			
Root Dry Wt. (mg)	0	69±7	80±7	0.266	0.736	0.943
	33	112±7	127±10			
Total Dry Wt. (mg)	0	452±35	486±31	0.441	0.058	0.948
	33	354±49	395±70			

*Lp = *L. proxima*, Pw = pulp waste, Lp-Pw = *L. proxima* by pulp waste. Experimental error was determined with 47 degrees of freedom.

Summary of the Results

L. proxima significantly increased the seedling weight and length of most of the tree species. Although *L. proxima* had no significant effects on Japanese larch and red pine growth, it did slightly improve the growth of these two species (Table 21).

Pulp waste usually significantly decreased the seedling growth of tree species except for Douglas-fir. Even with Douglas-fir, the effect of pulp waste was slightly negative (Table 21).

The interaction of *L. proxima* with pulp waste on tree species was quite different. There were highly positive significant interactions with black spruce and jack pine, less positive significant interactions with red spruce, no significant interactions with white spruce, mugo pine, red pine, Douglas-fir and Japanese larch, and highly negative interactions with Japanese black pine (Table 21). In other words, the apparent detrimental effects of the pulp waste is at least partially negated by the mycorrhizal fungus.

Table 21. The effects and interactions of *L. proxima* and pulp waste on the growth biomass of nine conifer species.

Tree Species	df	*L. proxima*	Pulp Waste	Lp by Pw
Larix kaempferi	47	n.d.[1]	(-)[6] +	n.d.
Picea glauca	45	(+) +	(-) +++	n.d.
Picea mariana	55	(+) +++[2]	(-) +++	(+) +++
Picea rubens	68	(+) ++[3]	(-) +++	(+) +
Pinus banksiana	85	(+) +++	(-) +++	(+) +++
Pinus mugo	65	(+) +++	(-) +++	n.d.
Pinus resinosa	38	n.d.	(-) +++	n.d.
Pinus thunbergii	64	(+) +[4]	(-) ++	(-) +++
Pseudotsuga menziesii	24	(+)[5] +++	n.d.	n.d.

[1]n.d.indicates no difference from controls, [2]+++, highly sig.at $P \le 0.01$, [3]++, sig. at $p \le 0.05$, [4]+, nearly sig. ($p \le 0.11$), [5](+) means positive effect, [6](-) means negative effect.

DISCUSSION

Seedling dry weight biomass is considered a more important value than seedling length for evaluating the experimental results because it more accurately reflects the photosynthetic activities of the seedlings. In this experiment, the evaluations were mainly based on this point.

The experiments show that mycorrhiza can increase the size of various conifer species in the greenhouses. Because of the negative effect of pulp waste in terms of the environment and economic liability, it is extremely important to explore new avenues to transform pulp waste into more useful products such as a potting medium ingredient for containerized seedling culture. In so doing, there would be a cost benefit to the forest industry, a savings on natural resources and the production of higher quality tree seedlings. This is the reason why *L. proxima* mycorrhiza and pulp waste were introduced into the experiments of containerized seedling production at the same time.

The successful utilization of pulp waste as a potting medium component for seedling production in association with *L. proxima* depends on three aspects. Firstly, *L. proxima* improved seedling growth; secondly, the interaction of *L. proxima* with pulp waste was significantly positive; and thirdly, the harmful effects of pulp waste on seedling growth was partially negated.

Any one of these three aspects can make the utilization of pulp waste possible. A good performance of *L. proxima* can off-set the negative effect of pulp waste. If the effect of *L. proxima* is mediocre, but there is a significant interaction of *L. proxima* with pulp waste, the interaction can partially make up for the negative effect of pulp waste and make the utilization of pulp waste feasible for use as a component of the seedling growth medium. If pulp waste had less harmful effects on seedling growth, the utilization of pulp waste would be highly desirable.

As for the utilization of pulp waste, its use at 33% v/v is feasible in the seedling production of jack pine, black spruce, mugo pine, Douglas-fir and red pine among the nine conifers tested as demonstrated by the growth results of these species. However, the crucial aspect is to find out the optimal proportion of pulp waste that can be used in the potting medium to *L. proxima* that must be used to minimize the negative effects of the pulp waste on the seedling growth. Among the nine tree species tested at the 33% pulp waste level, utilization of pulp waste for jack pine and black spruce seedling production was most promising. To jack pine, the result was confirmed by a potting mixture experiment (Appendix II).

In the conventional procedure of ectomycorrhizal fungi evaluation, only optimal conditions were adopted (Valdés, 1986 and Gagnon *et al.*, 1987). This procedure did not permit the evaluation of how the mycorrhiza would perform under adverse environmental conditions. This was why some mycorrhizae had improved seedling growth in the greenhouse, but after seedlings were outplanted, mycorrhizae failed to improve growth performance. The reason was that when a seedling is transplanted from the nursery into the field its environmental conditions are changed dramatically, and adaptation to the new environment may be difficult. To reduce possible shock, the change should be as small as possible and the seedling should be resistant to adverse conditions of the new habitat (Mikola, 1973). In order to get a better understanding of the ectomycorrhizal fungi evaluation, it is important to introduce both positive and negative factors into the evaluation procedure. This is one of the reasons why pulp waste, the negative factor in general, was introduced into the experiments. Based on the results about black spruce, if pulp waste were not introduced into the experiment, *L. proxima* as a promising ectomycorrhizal fungus could have been ignored due to no significant different between the *L. proxima* treatment and the control. Actually, with pulp waste introduced, *L. proxima* exhibited unusual advantage in improving black spruce seedling growth. Ectomycorrhizal fungi vary in their resistance to adverse conditions such as drought and waterlogged conditions (Trappe, 1977) and certain species of fungal symbionts are more beneficial than others to pines on certain sites, especially on adverse sites (Marx, 1977). For example, loblolly pine seedlings with *Pisolithus tinctorius* survived and grew as well at 40°C as they did at 24°C. Seedlings with *Thelephora terrestris* or without ectomycorrhiza survived poorly and did not grow at 40°C (Marx and Bryan, 1971). Although in nurseries the performance of pine with both *P. tinctorius* and *T. terrestris* were very good, on adverse sites, such as coal spoils, severely eroded sites, borrow pits, prairie soil and clay spoils, the performance of pine seedling with *P. tinctorius* was much better than with *T. terrestris* or other naturally occurring ectomycorrhizae (Marx, 1977; Marx *et al.*, 1984). Nevertheless, soil and site characteristics markedly influence the types of mycorrhizal fungi that develop on tree roots (Mason *et al.*, 1987). Soil factors exert selective pressures on symbiotic fungi. Fungi that can tolerate these factors and ecologically adapt to these sites should be used to tailor seedlings prior to planting (Marx, 1977).

Therefore, a promising ectomycorrhizal fungus should be one that is adapted to the specific conditions, especially adverse conditions. Therefore when we test and screen mycorrhizal fungi for seedling production and reforestation, if certain adverse factors are introduced under natural conditions into the experiment, one can obtain more meaningful results and have greater confidence for outplanting success. The most important aspect is that this method could avoid missing promising mycorrhizal fungi, especially those that could improve the tolerance of trees to specific adverse conditions.

CHAPTER 4. THE INFLUENCE OF *L. PROXIMA* AND VARIOUS AMOUNTS OF PULP WASTE ON JACK PINE SEEDLING GROWTH

INTRODUCTION

According to the results in Chapter 3, it is feasible to utilize pulp waste as a potting medium component in containerized jack pine seedling production. At the same time, *L. proxima* could upgrade pulp waste by minimizing the negative effect of pulp waste. Based on environmental concerns, in order to make the utilization of pulp waste practical, it is crucial to investigate how much pulp waste upgraded with *L. proxima* can be used in jack pine seedling production in comparison with the conventional seedling production. For this reason, this experiment was established to determine the suitable quantity range of pulp waste that might be used as a component of the potting medium in association with *L. proxima* for jack pine seedling growth.

MATERIALS AND METHODS

Jack pine seedlings were grown in multipots filled with different ratios of a mixture containing fibrous pulp waste, peat moss, and vermiculite with and without spawn of *L. proxima*. Fibrous pulp waste representing 0%, 10%, 20%, 30%, 40%, and 50% of the final volume were added to a 1:1

peat/vermiculite mixture. All treatments were inoculated with *L. proxima* rye seed spawn except for the control (treatment 1) which was 1:1 peat moss/vermiculite (Table 22). There were 19 single seedling replications in each treatment. Seedlings grew in the greenhouse from May 19 to Aug. 18, 1988.

The seeds, peat moss, vermiculite, pulp waste, multipots and rye grain inoculum preparation as well as seedling growing conditions and data analysis maintained the same procedure as was followed in the experiment of effects of *L. proxima* and pulp waste on tree hosts.

Table 22. Treatment combinations and medium composition of the experiment of pulp waste with *L. proxima* as a component of jack pine seedling potting medium.

Treatment	Rep.	*L. proxima* (cm^3)	Pulp Waste (%)	Peat Moss (%)	Vermiculite (%)
1	19	0	0	50	50
2	19	20	0	50	50
3	19	20	10	45	45
4	19	20	20	40	40
5	19	20	30	35	35
6	19	20	40	30	30
7	19	20	50	25	25

RESULTS

Seedling Length

Shoot length and total seedling length at treatment level of 0%, 10% and 20% pulp waste with *L. proxima* were significantly greater than the non-mycorrhizal control seedling growing in a 1:1 peat moss-vermiculite (Table 23). The 30% and 40% pulp waste treatments were not significantly different from the control and the addition of 50% pulp waste was significantly worse than the control treatment. For root length, none of the treatments were significantly different from the control (Table 23 and Figure 17).

Table 23. Length of jack pine seedlings grown with *L. proxima* and pulp waste after 91 days.

Pw[1] %	*Lp*	n	Shoot Length (mm)	Root Length (mm)	Total Length (mm)
0		19	109±3 d[2]	125±2 ab	234±3 c
0		19	146±3 b	125±1 ab	271±4 b
10		19	157±3 a	131±2 a	288±3 a
20		19	134±4 c	130±4 a	264±6 b
30		19	109±4 d	124±3 ab	233±6 c
40		19	103±3 d	130±3 a	233±4 c
50	+	19	67±4 e	121±2 b	188±3 d

[1] Pw = pulp waste, *Lp* = *L. proxima*. [2] All data presented as mean±standard error. Means in the same column with different letters are significantly different (*P* = 0.05) by one-way ANOVA.

Figure 17. The growth of jack pine seedlings on 0% to 50% pulp waste by volume after 91 days.

<u>Seedling Dry Weight</u>

Seedling shoot and total dry weights at pulp waste ratios of 0% and 10% with *L. proxima* were significantly heavier than the non-mycorrhizal control. There was no significant difference between 20% pulp waste with *L. proxima* treatment and the non-mycorrhizal control treatment, but 30%, 40% and 50% pulp waste with *L. proxima* were significantly worse than the non-mycorrhizal control. Seedling root dry weight at the 0% and 10% pulp waste with *L. proxima* treatment were not significantly different from the non-mycorrhizal control treatment. All higher proportions of pulp waste were significantly worse than the control (Table 24).

Table 24. Dry weight of jack pine seedlings grown with *L. proxima* and pulp waste after 91 days.

Pw[1] (%)	*Lp*	n	Shoot Dry Weight (mg)	Root Dry Weight (mg)	Total Dry Weight (mg)
0		19	280±18 b[2]	178±14 a	458±31 b
0	+	19	350±13 a	175±11 a	525±22 a
10	+	19	397±12 a	168±6 a	545±17 a
20	+	19	283±16 b	139±9 b	422±25 b
30	+	19	145±12 c	80±7 c	225±18 c
40	+	19	105±10 d	55±5 cd	160±15 d
50	+	19	43±10 e	30±5 d	73±14 e

[1] Pw = pulp waste, *Lp* = *L. proxima*. [2] All data presented as mean±standard error. Mean in the same column with different letters are significantly different ($P = 0.05$) by one-way ANOVA.

DISCUSSION

The addition of *L. proxima* greatly improved the growth of jack pine seedling. Even adding 40% pulp waste in association with *L. proxima* in a culture medium still provided results equal to the control of 1:1 peat moss-vermiculite in seedling length. According to the biomass of jack pine seedling, if 10% pulp waste were utilized in jack pine production, much better quality of seedlings which photosynthesize more biomass could be produced. But 20% pulp waste could be utilized too, if the main purpose were to utilize pulp waste as an economic incentive and for environmental concerns. In this experiment, the effect of

L. proxima was more remarkable on the seedling length growth than on the dry weight. The reason may be that, although pulp waste at 30% dramatically decreased the biomass of jack pine seedling, *L. proxima* greatly improved efficiency of the root system so that even fewer roots, in terms of mass, allowed for increased seedling height growth. Also there is the possibility of auxin production as a factor in improving tree growth.

However, in the host range test, the results were a little different on jack pine. Here, the effects of *L. proxima* were more striking on the dry weight than on the growth length. Based on seedling dry weight, even more than 33% pulp waste (by volume) in association with *L. proxima* could be utilized in jack pine production. The performance of *L. proxima* was not so obvious in seedling growth length as in seedling dry weight growth. The reason may lie in variations of pulp waste quality. Lack of consistent inoculum quality was identified as a problem (Marx *et al.*, 1982). Considering the results of these experiments, there are many reasons for variations among experiments. There may be differences in duration of inoculum storage (quality). Rooting media, seed treatments, fertility regimes, temperature, CO_2 concentration, and light conditions could lead to differences in seedling growth (Marx *et al.*, 1982). Also, because these two experiments were performed at different times, Mar. 16 to May 2, 1988 for the host test experiment and May 19 to Aug. 18, 1988 for the pulp waste experiment, different environmental conditions were present in the greenhouse, most notably, temperature and sunlight duration. The temperature in the earlier experiment varied from 17°C to 32°C daily, whereas the temperature in the latter experiment ranged from 19°C to 38°C. The earlier experiment received much less sunlight than the latter experiment.

Another difference was the age of the inoculum used. In the host test experiment, the inoculum was just produced and fresh, whereas for the 2nd experiment this same inoculum was stored in an incubator at 25°C for two months. The two-month storage of the *L. proxima* inoculum might have decreased the viability of the inoculum. Temperature and time in storage are known to influence the efficacy of ectomycorrhizal inoculum greatly (Hung and Molina, 1986b). Storage of laboratory-produced *Pisolithus tinctorius* mycelial inoculum at 3°C for 6 days drastically reduced its ability to form mycorrhiza with seedlings of European spruce (*Picea abies*), Canadian hemlock (*Tsuga canadensis*), and black pine (*Pinus nigra*) (Maronek and Hendrix, 1980) and after storage at 5°C for 4 wks, the effectiveness of some batches of *P. tinctorius* inoculum decreased significantly (Marx *et al.*, 1984). Hung and Molina (1986b) found that, in general, fresh inocula of *L. laccata* and *H. crustuliniforme* were the most effective and retained their effectiveness for a month of storage, which then declined rapidly in a short time. Storage at 2°C prolonged inoculum viability for at least 2 months over that held at 21°C storage.

Therefore, it was probably these differences in temperature, sunlight and duration of *L. proxima* inoculum storage that were responsible for the differences in the effects of *L. proxima* in the two experiments of *L. proxima* as they related to jack pine seedlings. According to the results a better approach which combines the advantages of these two experiments might be found in order to produce better quality of jack pine seedlings.

CHAPTER 5. THE INFLUENCE OF LOW LEVELS OF PULP WASTE WITHOUT *L. PROXIMA* ON JACK PINE SEEDLING GROWTH

INTRODUCTION

The negative effects of pulp waste at 33% by volume level may be due to complex factors such as excess micronutrients, toxins, heavy metals, decomposition products, and salt toxicity, as well as problems related to structure, porosity and aeration (Dolar *et al.*, 1972). However, the main factor influencing yield was probably N immobilization by microbes in the high C/N pulp waste (Dolar *et al.*, 1972). This does not mean that the pulp waste at any level will produce the same negative effects on seedling growth as at the 33% by volume level. King (1979) found that waste wood fibre as a soil amendment at low level (below 6% by oven dry weight) increases yields of fescue grass (*Festuca arundinacea* Schreb.) for 8 to 10 months thereafter. This fact raised the possibility that the effects of these detrimental elements might vary with the change of their concentrations because both pulp waste and waste wood fibre are similar in the main composition--wood fibre. The following experiment was carried out to see if pulp waste without mycorrhiza at low level (below 10%) might at some point stimulate seedling growth and be used as a partial potting medium substrate.

MATERIALS AND METHODS

Pulp waste in the amounts of 0%, 2%, 4%, 6%, 8% and 10% by volume was added to the 1:1 peat moss/vermiculite potting medium. There were 19 replications (seedlings) in each treatment (Table 25). Seedlings without *L. proxima* grew from July 10 to Dec. 12, 1988.

The seeds, potting medium, pulp waste, multipots, experiment preparation, seedling growing conditions and data analysis used were the same as described in Chapter 3.

Table 25. Treatment combinations and medium composition of an experiment that used low levels of pulp waste as a component of a jack pine seedling potting medium.

Treatment	Rep.	Pulp Waste (%)	Peat Moss (%)	Vermiculite (%)
1	19	0	50	50
2	19	2	49	49
3	19	4	48	48
4	19	6	47	47
5	19	8	46	46
6	19	10	45	45

RESULTS

Seedling Length

Seedlings grown on 4% pulp waste had significantly greater shoot lengths than the control seedlings without pulp waste. The remainder of the treatments were not significantly different from the control. In terms of root length, 2%, 4% and 6% pulp waste treatments were not significantly different from the control, and at 8% and 10% pulp waste, the seedling root lengths were significantly less than control seedlings. As for total length, none of the treatments were significantly different from the control (Table 26).

Table 26. Length of jack pine seedlings grown with various levels of pulp waste after 155 days.

Pw[1] (%)	n	Shoot Length (mm)	Root Length (mm)	Total Length (mm)
0	19	136±4 a[2]	133±4 c	269±6 ab
2	19	148±4 a	125±2 abc	273±4 ab
4	19	151±5 b	127±2 bc	278±5 b
6	19	138±4 a	127±3 bc	265±5 ab
8	19	139±5 a	121±2 ab	260±6 a
10	19	140±3 a	118±2 a	258±4 a

[1] Pw = pulp waste. [2] All data presented as mean±standard error. Means in the same column with different letters are significantly different (*P* = 0.05) by one-way ANOVA.

Seedling Dry Weight

In terms of shoot dry weight, 4% pulp waste was significantly better than the control. The remainder of the treatments were insignificantly different. For root dry weight, 4% pulp waste treatment was significantly better than the control. The treatments of 2%,6% and 8% pulp waste were insignificantly different from the control. The treatment of 10% pulp waste was significantly worse than the control (Table 27). From the standpoint of total dry weight, the treatments of 2% and 4% pulp waste were significantly better than the control. The treatments of 6% and 8% pulp waste were insignificantly different from the control. The treatment of 10% pulp waste was significantly worse than the control.

Table 27. Dry weight of jack pine seedlings grown with various levels of pulp waste after 155 days.

Pw[1] (%)	n	Shoot Dry Weight (mg)	Root Dry Weight (mg)	Total Dry Weight (mg)
0	19	346±18 abc[2]	123±10 bc	469±26 cd
2	19	411±12 cd	136±5 cd	547±15 ab
4	19	431±18 d	149±7 d	580±21 a
6	19	377±11 bc	133±6 cd	510±16 bc
8	19	321±20 abc	110±6 ab	431±24 de
10	19	299±15 abc	94±6 a	393±20 e

[1] Pw = pulp waste. [2] All data presented as mean±standard error. Means in the same column with different letters are significantly different (*P* = 0.05) by one-way ANOVA.

DISCUSSION

Based on both length and dry weight of jack pine seedlings, small portions of pulp waste up to as much as 4% seemed to stimulate the biomass of jack pine seedlings and levels as high as 8% were insignificantly different from the controls. This means that, although in general, pulp waste had significantly negative effects on tree seedling growth at the higher levels shown in the previous experiment, it may be possible to use pulp waste without mycorrhiza at levels of 4 to 8%.

The reason for improved tree growth is unknown but may be due to a reduction of toxic elements in pulp waste to a non-detrimental level; whereas the microbial nutrients and inorganic compounds contributed to the improved growth. Further research is required to characterize the chemical and microbial components in pulp waste from the standpoint of both tree and human health.

CHAPTER 6. THE EFFECTS OF PULP-WASTE-BASED INOCULUM AND RYE-GRAIN-BASED INOCULUM ON JACK PINE SEEDLING GROWTH

INTRODUCTION

Whether the utilization of pulp waste in association with *L. proxima* becomes successful in containerized seedling production depends on whether a cheap and commercialized inoculum can be produced with a simple production procedure. A reliable and effective commercial source of fungal inoculum must be available before large scale ectomycorrhizal inoculum of nursery seedlings is feasible (Hung and Molina, 1986b). Thus, it is desirable to explore a method to produce *L. proxima* commercially. Inocula made from peat moss, vermiculite, cereal grain, peat-vermiculite, perlite-peat moss, perlite-corn flour and peat moss-cotton seed skin-perlite-wheat bran successfully formed ectomycorriza on pine seedlings (Marx *et al.*, 1982; Wang *et al.*, 1985 and Valdés, 1986). Pulp waste as a cheap inoculum substrate source might be a good candidate for inoculum production.

The objectives of the following experiment were (1) to determine whether pulp waste or rye seed represented the best *L. proxima* inoculum source and (2) to determine whether pulp waste can substitute for rye seed as a *L. proxima* inoculum substrate, thereby providing a method for further utilizing pulp waste.

MATERIALS AND METHODS

One of two treatments (vermiculite/peat moss, 750:750 cm^3) were inoculated with either 20 cm^3 of rye-grain inoculum or pulp-waste inoculum, respectively. There were 19 seedlings used as replications in each treatment. Jack pine seedlings grew from June 4 to Aug. 24. 1988.

The rye-grain inoculum was made from *L. proxima* permeated rye grain. The pulp-waste inoculum was made from pulp waste inoculated with *L. proxima* rye grain (180 g-250 cm^3) with water 100 mL, or pulp waste (40 g with 100 mL 1% malt extract solution) was added to each 500 mL fruit jar and autoclaved 121°C for 20 minutes. Every jar was inoculated with 20 cm^3 rye grain inoculum prepared before, no matter which inoculum was made. After inoculation, all jars were stirred to assure mixing of rye grain or pulp waste with *L. proxima*. Before use, the jars were incubated at 25 °C for ca. 15 days for mycelial establishment.

The materials not described in detail here and the process of the experimental performance were the same as those described in the experiment of effects of *L. proxima* and pulp waste on tree hosts (page 16-19).

RESULTS

Shoot and total length of jack pine seedlings grown with rye-grain inoculum were significantly greater than seedlings grown on pulp-waste inoculum. There was no significant difference in root length between rye and pulp waste inocula (Table 30).

As for seedling dry weight, rye-grain inoculum was significantly better than the pulp-waste inoculum in shoot dry weight. There were no significant differences between rye-grain and pulp waste inocula in root and total dry weights (Table 31).

Table 28. Length of jack pine seedlings grown for 81 days with peat moss and vermiculite (1:1 v/v) medium inoculated with rye grain and pulp waste inocula.

Inocula	n	Shoot Length (mm)	Root Length (mm)	Total Length (mm)
Pw[1]	19	125±3 b[2]	133±2 a	258±4 b
Rye	19	150±4 a	132±3 a	282±5 a

[1] Pw = pulp waste. [2] All data presented as mean±standard error. Means in the same column with different letters are significantly different ($P = 0.05$) by one-way ANOVA.

Table 29. Dry weight of jack pine seedlings grown for 81 days with peat moss and vermiculite (1:1 v/v) medium inoculated with rye grain and pulp waste inocula.

Inocula	n	Shoot Dry Weight (mg)	Root Dry Weight (mg)	Total Dry Weight (mg)
Pw[1]	19	253±11 b[2]	123±7 a	376±16 a
Rye	19	328±22 a	116±8 a	444±29 a

[1] Pw = pulp waste. [2] All data presented as mean±standard error. Means in the same column with different letters are significantly different ($P = 0.05$) by one-way ANOVA.

DISCUSSION

Although pulp-waste inoculum was slightly worse than rye-grain inoculum in improving the growth of jack pine seedlings, there was no significant difference in seedling dry weight. The reason was, as the pulp waste leaching study indicated, that the pulp waste may contain toxic agents that may be harmful to tree growth. The detrimental effect of these chemicals may be, in part, mitigated by the fungus. Another reason was that pulp-waste inoculum was not leached with water before use in the experiments. Marx *et al* (1982) found that the inoculum was most effective after leaching with water to remove nutrients. In any case, it appears to be feasible to utilize pulp waste as a *L. proxima* inoculum. It will be an economical way for pulp waste utilization. However, rye-grain inoculum is a better method for jack pine seedling production in that it was easier to establish and handle.

CHAPTER 7. SUMMARY

L. proxima significantly improved the growth of jack pine, mugo pine, black spruce, red spruce and Douglas-fir and possibly improved the growth of Japanese black pine and white spruce. However, for these latter two species, more research is needed to determine whether the effects of *L. proxima* might induce significant improvements. Clearly, however, *L. proxima* is an excellent candidate ectomycorrhizal fungus of importance to forestry.

Pulp waste (33% by volume) usually had significantly negative effects on the growth of seedlings except Japanese black pine (positive) and Douglas-fir (no significant effect).

Interactions of *L. proxima* pulp waste varied with host tree species. Highly significant growth improvements were observed with jack pine and black spruce. Positive results with red spruce were not significant. Detrimental effects were observed with Japanese black pine; whereas white spruce, mugo pine, red pine, Douglas-fir and Japanese larch were unaffected.

Pulp waste was found to hinder jack pine seedling growth except at levels < 8%, but below 8% pulp waste stimulated the growth of the seedlings. When *L. proxima* was added to the pulp waste, 10 to 33% of pulp waste could be utilized as a potting medium component for jack pine seedling production.

Pulp waste utilization is feasible in the seedling production of black spruce, mugo pine, red spruce and Douglas-fir seedlings in association with *L. proxima* and Japanese black pine without *L. proxima*, However, it is important to find out the optimal percentages of pulp waste that can be utilized in the production of these tree seedlings. Pulp waste amended with malt extract solution could be used as a *L. proxima* spawn medium. No doubt, this is one possible approach for pulp waste upgrading and utilization.

Rye seed spawn seems to be a better inoculum than pulp waste, but pulp waste as an inoculum substrate is feasible

The results, with regard to the growth of containerized seedlings, especially of the important species of jack pine and black spruce, need to be verified by pilot scale testing in commercial greenhouses.

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APPENDIX I

THE INFLUENCE OF RYE GRAIN IN POTTING MEDIUM ON JACK PINE SEEDLING GROWTH

INTRODUCTION

In most of the experiments, the inoculum used was made from rye grain. Should we add rye grain in peat/vermiculite medium as a control in all our experiments? Or simplify the experimental procedure by setting the control without adding rye grain into the potting medium? If the same results can be obtained with the second method in comparison with the first one, it will be more convenient to conduct the experiments. The following experiment was designed to answer this question.

MATERIALS AND METHODS

The effects of autoclaved rye grain (without *L. proxima*) on jack pine growth were studied in comparison to peat moss and vermiculite 1:1 by volume. Twenty cm^3 of rye grain autoclaved for 20 minutes was added to 1500 cm^3 of peat and vermiculite (1:1 by volume) medium. There were 19 replications in each treatment. Treatments were randomly assigned to the multipot. Containerized jack pine seedlings grew from June 3 to Aug. 23, 1988.

Materials used in the experiment, experiment preparation, seedling growing conditions and data analysis, are the same as those used to study the effects of *L. proxima* and pulp waste on tree hosts.

RESULTS

Rye grain had no significant influence on seedling shoot, root and total lengths and dry weights (Tables 28 and 29).

Table 30. Length of jack pine seedlings grown for 81 days in peat moss and vermiculite (1:1 v/v) medium with and without rye grain.

Rye Grain	n	Shoot Length (mm)	Root Length (mm)	Total Length (mm)
	19	143±3 a[1]	128±2 a	271±4 a
+	19	144±4 a	130±2 a	274±4 a

[1] All data presented as mean±standard error. Means in the same column with different letters are significantly different ($P = 0.05$) by one-way ANOVA.

Table 31. Dry weight of jack pine seedlings grown for 81 days in peat moss and vermiculite (1:1 v/v) medium with and without rye grain.

Rye Grain	n	Shoot Dry Weight (mg)	Root Dry Weight (mg)	Total Dry Weight (mg)
–	19	335±11 a[1]	146±6 a	481±16 a
+	19	320±31 a	103±12 a	423±42 a

[1] All data presented as mean±standard error. Means in the same column with different letters are significantly different ($P = 0.05$) by one-way ANOVA.

DISCUSSION

In most of the experiments rye grain by itself was not used as control, because this simplified the experimental procedures. Also the rye seed might have complicated the analysis by becoming a food base for detrimental microbes. This test showed no significant influence of the rye seed on the growth of seedlings. Therefore, rye seed can be used as an inoculum source without any apparent detrimental effects.

APPENDIX II

POTTING MIXTURE EXPERIMENT-EXTREME VERTICE DESIGN EFFECTS OF VARIOUS MIXTURES OF *L. PROXIMA*, PULP WASTE, PEAT MOSS AND VERMICULITE ON JACK PINE SEEDLING GROWTH

INTRODUCTION

The extreme vertice design is useful for establishing the best mixture combinations (Anderson and McLean, 1974). The following experiment utilizes this mathematical approach in determining the best pulp waste: peat moss: vermiculite mixture.

MATERIALS AND METHODS

The range of rye grain inoculum tested was from 0% to 10%, pulp waste from 0% to 40%, peat moss from 0% to 100%, vermiculite from 0% to 80% by volume. The 17 mixture combinations are shown in Table 32. Each treatments had three replications and these were randomized into three multipots so that one replication of all treatments was randomly assigned to one multipot. Jack pine seedlings were grown from Feb. 19 to June 21, 1989.

Experimental procedures are those described in Chapter 3 except that the seedlings were grown in the greenhouse

(No. 2) under daylight and 16 hr High Pressure Sodium Lamp (400W). The seedlings were watered for 10 sec every 16 min by an automatic sprinkling system.

Table 32. Treatment combinations of the effects of *L. proxima*, pulp waste, peat moss and vermiculite on jack pine growth.

Vertices	Rep	Peat Moss %	Vermiculite %	Pulp Waste %	Inoculum %
1	3	100.0	0.0	0.0	0.0
2	3	0.0	80.0	20.0	0.0
3	3	0.0	80.0	10.0	10.0
4	3	0.0	60.0	40.0	0.0
5	3	0.0	50.0	40.0	10.0
6	3	60.0	0.0	40.0	0.0
7	3	50.0	0.0	40.0	10.0
8	3	20.0	80.0	0.0	0.0
9	3	10.0	80.0	0.0	10.0
10	3	0.0	67.5	27.5	5.0
11	3	70.0	0.0	26.7	3.3
12	3	7.5	80.0	7.5	5.0
13	3	43.3	53.4	0.0	3.3
14	3	27.5	27.5	40.0	5.0
15	3	36.0	44.0	20.0	0.0
16	3	15.0	52.5	22.5	10.0
17	3	26.7	47.8	21.1	4.4

RESULTS

The model of the experiment was obtained by multiple regression:

$$Y = \underset{(0.00058)}{0.00307X_1} + \underset{(0.0008)}{0.00143X_2} + \underset{(0.0055)}{0.0142X_3} + \underset{(0.126)}{0.13X_4}$$

$$+ \underset{(0.0000268)}{0.000042X_1{*}X_2} - \underset{(0.0000907)}{0.000318X_1{*}X_3} - \underset{(0.001379)}{0.00064X_1{*}X_4}$$

$$- 0.000242X_2{*}X_3 - 0.00124X_2{*}X_4 - 0.002X_3{*}X_4$$
$$\quad (0.0000886) \quad (0.001395) \quad (0.001432)$$

Y = biomass (g),

X_1 = peat moss (%),

X_2 = vermiculite (%),

X_3 = pulp waste (%),

X_4 = *L. proxima* rye grain inoculum (%),

$X_1{*}X_2$ = the interaction of peat moss and vermiculite,

$X_1{*}X_3$ = the interaction of peat moss and pulp waste,

$X_1{*}X_4$ = the interaction of peat moss and *L. proxima*,

$X_2{*}X_3$ = the interaction of vermiculite and pulp waste,

$X_2{*}X_4$ = the interaction of vermiculite and *L. proxima*,

$X_3{*}X_4$ = the interaction of pulp and *L. proxima*.

R square was 96.6%.

In the light of the model, when the normal medium, 1:1 peat moss-vermiculite by volume was applied, the biomass of jack pine seedling would be 0.33 g. Based on this biomass and the pulp waste utilization concerns, the medium composition of peat moss 58%, pulp waste 32% and *L. proxima* inoculum 10% can reach this yield level. This meant that 32% pulp waste upgraded by *L. proxima* could be utilized in jack pine seedling production according to the environmental concerns. The optimal composition of the potting medium was peat moss 90% and *L. proxima* 10%. With this composition, the yield of seedlings was predicted to be able to reach 1 g.

Original data of the experiment (replication mean) are shown in Table 33).

Table 33. Original data of potting mixture experiment-extreme vertice design effects of various mixtures of *L. proxima*, pulp waste, peat moss and vermiculite on jack pine seedling growth.

Treat-ment	Biomass (g)	Peat Moss	Vermiculite	Pulp Waste	*L. proxima*
		% by Volume			
1	0.31667	100.0	0.0	0.0	0.0
2	0.03867	0.0	80.0	20.0	0.0
3	0.09867	0.0	80.0	10.0	10.0
4	0.03967	0.0	60.0	40.0	0.0
5	0.04233	0.0	50.0	40.0	10.0
6	0.03500	60.0	0.0	40.0	0.0
7	0.28633	50.0	0.0	40.0	10.0
8	0.21167	20.0	80.0	0.0	0.0
9	0.46367	10.0	80.0	0.0	10.0
10	0.04867	0.0	67.5	27.5	5.0
11	0.03967	70.0	0.0	26.7	3.3
12	0.16733	7.5	80.0	7.5	5.0
13	0.43300	43.3	53.4	0.0	3.3
14	0.04200	27.5	27.5	40.0	5.0
15	0.06767	36.0	44.0	20.0	0.0
16	0.18567	15.0	52.5	22.5	10.0
17	0.17633	26.7	47.8	21.1	4.4

DISCUSSION

According to the regression coefficients of the model, it is quite obvious that *L. proxima* had the strongest effect on the biomass of jack pine seedlings. The second was pulp waste followed by peat moss and vermiculite.

The effects of *L. proxima* and peat moss were positive. Based on the results of previous experiment, the effect of pulp waste was negative in general (over 10% without *L. proxima*) and the interaction of pulp waste and *L. proxima* was positive, but the regression coefficients of pulp waste and pulp waste in association with *L. proxima* was not consistent with the actual effects of them. It was due to the correlations of pulp waste and pulp waste-*L. proxima* with other factors.

The results of the experiment exhibited that there was a great potential in improving the growth of jack pine seedlings and utilizing more pulp waste by adjusting the composition of the medium. At the same time, this experiment verified the results of previous experiments that up to 30% pulp waste in association with *L. proxima* could be utilized in jack pine seedling production. Further research is needed to find out the proper medium compositions for certain purposes and to verify the results of this experiment.

APPENDIX III

THE DRYING OF MYCORRHIZAL PULP WASTE ON INOCULUM EFFECTIVENESS

INTRODUCTION

The practical use of a mycorrhizal mulch made from pulp waste will require a material that is dried to the point of reduced biological activity and threats from contamination during shipping and storage. Marx *et al.* (1982) found that a substrate permeated by *P. tinctorius* and dried for 60-90 hours at 25°C to 30°C to a moisture content of 12 to 20% based on ovendry weight was still viable, after being stored in small quantities for 5 weeks at room temperature and up to 9 weeks at 5°C.

The objectives of the following experiment were to try to find a way to determine the effect of drying on *L. proxima* inoculum quality and at the same time, to find the best approach of inoculating multipots with *L. proxima*.

MATERIALS AND METHODS

Pulp-waste inoculum was dried for 15 days until it was air-dry and contained 13.5% moisture based on oven-dry weight. Twenty percent (v/v) dried pulp-waste inoculum was added to 1:1 vermiculite/peat moss in one of two ways. Technique I (20% pulp waste I) involved mixing the pulp-waste inoculum with the medium completely and evenly. In Technique II (20% pulp waste II), the pulp waste was added in the peripheral part of medium next to the wall of the multipot cavities. The control treatment was comprised of 1:1 vermiculite/peat moss without inoculum. Each treatment had 19 replications. Jack pine seedlings grew from Oct. 11, 1988 to July 3, 1989.

The growing conditions for the experiment and the data analysis described in the experiment of effects of *L. proxima* and pulp waste on tree hosts (page 18-22).

RESULTS

Seedling Length

In terms of shoot length, both 20% pulp waste I and 20% pulp waste II treatments were significantly worse than the control without pulp waste and *L. proxima*. There was no significant difference between the 20% pulp waste I and 20% pulp waste II treatments. Root length of jack pine seedling grown in 20% pulp waste I treatments was not significantly different from the control. Root length in 20% pulp waste II was significantly worse than in the control and 20% pulp waste I treatment. In terms of total length, both 20% pulp waste I and 20% pulp waste II treatments were significantly worse than the control. Between 20% pulp waste I and 20%

pulp waste II treatments, 20% pulp waste I was significantly better (Table 34).

Table 34. Shoot, root and total length of jack pine seedlings grown for 296 days with 20% dried pulp waste.

Pw[1] (%)	*Lp*	n	Shoot Length (mm)	Root Length (mm)	Total Length (mm)
0		20	95±2 a[2]	134±2 a	229±3 a
20I		20	67±2 b	140±3 a	207±3 b
20II	+	20	65±2 b	127±2 b	192±2 c

[1] Pw = pulp waste, 20I means that pulp waste was mixed with medium completely and evenly, 20II means that pulp waste was used in the peripheral area of the pot. [2] All data presented as mean±standard error. Means in the same column with different letters are significantly different ($P = 0.05$) by one-way ANOVA.

Seedling Dry Weight

Based on seedling shoot, root and total dry weights, both 20% pulp waste I and 20% pulp waste II treatments were significantly worse than the control. Between 20% pulp waste I and 20% pulp waste II treatments, there were no significant differences (Table 35).

Table 35. Shoot, root and total dry weight of jack pine seedlings grown for 296 days with 20% dried pulp waste.

Pw[1] (%)	*Lp*	n	Shoot Dry Weight (mg)	Root Dry Weight (mg)	Total Dry Weight (mg)
0		20	280±16 a[2]	229±13 a	509±27 a
20I		20	110±11 b	100±10 b	210±20 b
20II	+	20	103±8 b	115±7 b	218±14 b

[1] Pw = pulp waste, 20I means that pulp waste was mixed with medium completely and evenly, 20II means that pulp waste was used in the peripheral area of the pot. [2] All data presented as mean±standard error. Means in the same column with different letters are significantly different (*P* = 0.05) by one-way ANOVA.

DISCUSSION

Based upon the result that the quality of seedlings grown with 20% dried pulp waste medium was much worse than in the control, it is obvious that the viability of the dried *L. proxima* inoculum decreased dramatically, or even lost viability completely. There might be two reasons responsible for the result. One is that the duration of the air-drying was too long for the inoculum to maintain viability. Another is that *L. proxima* is not tolerant to the low moisture content levels. No doubt, the moisture level in *L. proxima* inoculum is an important factor in maintaining the viability of *L. proxima* inoculum during storage. The key problem will be to determine how tolerant *L. proxima* is to drying and how long it will remain viable and under what temperature conditions. More research is needed in order to determine the optimal conditions for *L. proxima* packaging, storage, transportation and keeping effectiveness.

APPENDIX IV

FOREST SOIL AS A NATURAL MYCORRHIZAL INOCULUM

INTRODUCTION

The use of soil inoculum to form ectomycorrhizae on seedlings for establishing plantations of pine, eucalyptus, and oak is currently the main example of the practical use of ectomycorrhizae in the world today (Marx and Schenck, 1983). Furthermore, soil inoculum would be easy to handle in seedling production. As a mycorrhizal inoculum, it might have potential economic significance to the utilization of pulp waste by reducing the cost of seedling production. This experiment was conducted to test the effectiveness of forest soil in forming mycorrhiza on jack pine seedling roots and to improve the growth of jack pine seedlings.

MATERIALS AND METHODS

Forest soil was collected at Lakehead University Jack Haggerty Forest, June 1988, in an area containing black spruce, balsam fir, jack pine and aspen. Peat moss (130 g and 1000 cm^3) and vermiculite (130 g and 1000 cm^3) were used as the jack pine seedling culture medium for each treatment. There were two treatments in this experiment. For treatment one the above medium was inoculated with 166 g forest soil as a natural mycorrhizal inoculum. The second treatment was a control wherein 166 g of forest soil was sterilized (autoclaved at 121°C for 20 minutes). Each treatment had 20 replications. Jack pine seedlings grew from Sept. 13, 1988 to May 2, 1989.

Other materials and experiment preparation were described in detail on page 18-22.

RESULTS

In this experiment, forest soil as a mycorrhizal additive was compared with the standard peat moss and vermiculite mixture with sterilized soil. Jack pine seedlings grown with unsterilized forest soil were significantly better than those grown with sterilized forest soil in terms of seedling lengths and dry weights (Tables 36 and 37).

Table 36. Shoot, root and total length of jack pine seedlings grown for 263 days with forest soil.

Soil	n	Shoot Length (mm)	Root Length (mm)	Total Length (mm)
Sterilized	20	106±2 a[1]	129±0 a	235±3 a
Unsterilized	20	111±2 b	132±3 b	243±3 b

[1] All data presented as mean±standard error. Means in the same column with different letters are significantly different (*P* = 0.05) by one-way ANOVA.

Table 37. Shoot, root and total dry weight of jack pine seedlings grown for 263 days with forest soil.

Soil	n	Shoot Dry Weight (mg)	Root Dry Weight (mg)	Total Dry Weight (mg)
Sterilized	20	207±10 a[1]	178±7 a	385±14 a
Unsterilized	20	248±9 b	213±9 b	461±13 b

[1] All data presented as mean±standard error. Mean in the same column with different letters are significantly different (*P* = 0.05) by one-way ANOVA.

DISCUSSION

The forest soil contained some kind of mycorrhizal fungi which improved the growth of jack pine seedling. Even after partial drying and storage for 3 months, the efficacy was maintained. It would be an efficient and economical method to use forest soil as a natural inoculum to produce seedlings with mycorrhiza in old forest areas such as in the boreal forests. However, caution should be exercised because of the possibility of introducing soil pests into the greenhouse environment (Riffle and Tinus, 1982), although there was no evidence of the occurrence for this kind of problem.

Soil inoculum has been used in many other areas of the world to improve survival and growth of pines (Mikola, 1970, 1973). Forest soil inoculum is often applied to correct ectomycorrhizal fungus deficiencies on young nursery seedlings before outplanting (Mikola, 1973; Marx, 1980b). A sufficient amount is 10% of the volume (soil inoculum) (Mikola, 1973).

In many instances, soil inoculum for the establishment of exotic pine plantations has been initially introduced in small quantities from another country. Such inoculum may initially contain several species of ectomycorrhizal fungi from the native country but often only one or two will persist in the exotic locations (Marx *et al.*, 1985).

Soil inoculum from plantations and natural stands contains a mixture of fungi. One likely reason for success with soil inoculum is the possible introduction of more than one ectomycorrhizal fungus and this increases the chances of having at least one effective symbiont. Soil inoculum provided seedling production with a simple and effective mycorrhizal establishment method.

Except for the disadvantage of introducing soil borne plant pests, another major disadvantage in using forest soil inoculum is that technology does not exist for selection and management of superior species of fungi from the usually mixed populations present in the soil inoculum. The dominant and persistent fungi in the soil inoculum may function poorly in outplantings (Marx *et al.*, 1985). For these reasons, caution should be exercised in the use of soil inoculum. In order to use pulp waste for environmental concerns, forest soil might provide much cheaper mycorrhizal inoculum to pulp waste utilization in seedling production. It is worth conducting more research on this aspect to overcome these two potential disadvantages.

APPENDIX V

GROWTH OF *L. PROXIMA* IN RESPONSE TO TEMPERATURE

INTRODUCTION

The determination of the optimum temperature for growth is very important for *L. proxima* inoculum production, storage, shipping and application. As mentioned earlier, temperature in storage are known to influence the efficacy of ectomycorrhial inoculum greatly. This experiment was designed to investigate the range of temperatures for *L. proxima* growth and survival.

MATERIALS AND METHODS

Temperatures were set at 20, 25, 30, 35, and 40°C in each of five incubators, respectively. Plastic petri dishes containing 25 mL of 1.25% malt extract plus 2% agar medium were individually inoculated with one grain of rye-grain inoculum. There were 7 replications in each treatment. All petri dishes were placed in incubators without light for 12 days from Aug. 31 to Sept. 12, 1988. The diameter of the colonies were measured from two directions at right angles.

To eliminate rye grain size error, rye grain diameters were subtracted from the fungal colony diameters.

RESULTS

Laccaria proxima grew best at 30°C. The second was at 25°C followed by 20°C and 35°C. At 40°C *L. proxima* failed to grow (Figure 18).

After data collection for analysis, petri dishes of 40°C treatment were put in the incubator for another 18 days (a total of one month at 40°C), then returned to the 30°C incubator. Only one petri dish contained a viable colony of *L. proxima*.

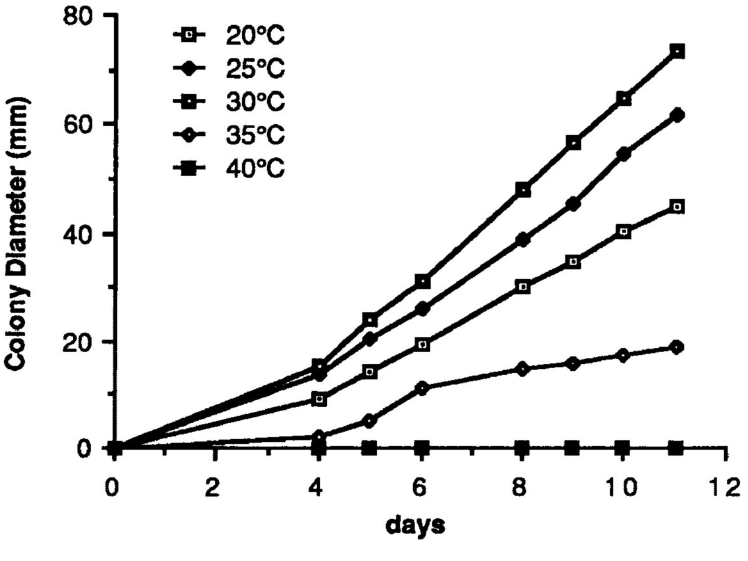

Fig. 18. The growth of *L. proxima* on malt extract agar medium at five temperatures.

DISCUSSION

The optimum temperature for most ectomycorrhizal species lies between 18 and 30°C. The majority of ectomycorrhizal species grow best at pH ranging from 4 to 6 (Harley, 1969).

The optimum temperature for *L. proxima* growth was 30°C and even at 35°C the growth was relatively rapid. This means that the effective growth/temperature regime is broad enough to accommodate the temperature variations one might find during the winter and summer months in the greenhouse.

APPENDIX VI

EFFECTS OF PULP WASTE SUPERNATANT AND NUTRIENTS ON *L. PROXIMA* GROWTH

INTRODUCTION

The negative effects of pulp waste were perhaps due to various soluble and detrimental toxins it contained which directly acted on seedling growth or on *L. proxima,* and thus indirectly influenced the growth of the seedlings. The information about the mechanism of the action of the detrimental elements on seedling are limited. The following experiment was designed to test the effects of unknown soluble pulp waste substances on *L. proxima* growth and to find an approach to minimize the negative effects of pulp waste. Another objective of the experiment was to find carbohydrate/nutrient sources suitable for *L. proxima* growth and inoculum production.

MATERIALS AND METHODS

A 3x4 factorial experimental design was used to determine the harmful or beneficial effects of aqueous pulp waste supernatant on the growth of *L. proxima.* Three fungal nutrients and four concentration levels of supernatant were tested. The three kinds of nutrients were malt extract, glucose and sucrose. Four concentrations of pulp waste supernatant were 0% (deionized water), 1%, 10% and 100%. The supernatant was prepared by soaking 100 g pulp waste in 2 L deonized water for 24 hours at room temperature. The pulp waste fibers were squeezed and filtered out to obtain the supernatant. The supernatant was further diluted to 10% and 1%. Culture media and treatment combinations were prepared as shown in Table 38. All media were autoclaved for 20 minutes. Each treatment had eight replications. Paper discs containing mycelial fragments were used in the experiment. After autocalving for 20 minutes, filter paper discs (6 mm in diameter) were put in a *L. proxima* mycelial suspension to make paper disc inoculum. The dishes were inoculated with a *L. proxima* paper disc in the centre. The petri dishes then were placed in a 25°C incubator for 12 days from July 12 to July 24, 1989. The diameter of the fungal colonies were measured from two directions at right angles. The petri dishes were randomly mixed among treatments.

Statistical analyses were done with SPSSX software.

Table 38. Treatment combinations of three nutrients and four concentrations of pulp waste supernatant.

No.	Nutrient	Pulp Waste Supernatant Concentration (%)
1	malt 1%	0
2	malt 1%	1
3	malt 1%	10
4	malt 1%	100
5	glucose 1%	0
6	glucose 1%	1
7	glucose 1%	10
8	glucose 1%	100
9	sucrose 1%	0
10	sucrose 1%	1
11	sucrose 1%	10
12	sucrose 1%	100

RESULTS

Nutrients and pulp waste supernatant had significant effects on *L. proxima* growth individually and interactively (Table 39). Malt was a significantly better nutrient than glucose for *L.proxima* growth. There were no significant differences between malt and sucrose in the range of 0% to 10% pulp waste supernatant; however, at 100% concentration of pulp waste supernatant, malt was significantly better than sucrose for *L. proxima* growth. Between sucrose and glucose, there were no significant differences at 0% and 100% pulp waste supernatant while at 1% and 10%

concentration sucrose was significantly better than glucose.

Among 0%, 1% and 10% pulp waste supernatant treatments, there were no significant differences. These three treatments were significantly better than 100% pulp waste supernatant for *L. proxima* growth (Table 39).

L. proxima preferred malt and sucrose rather than glucose as nutrient sources. In general, malt and sucrose were better nutrients for *L. proxima* growth and *L. proxima* inoculum production than glucose.

When the pulp waste supernatant concentration was below 10% *L. proxima* grew unhindered on the three nutrients. Media with 100% pulp waste supernatant impeded *L. proxima* growth. *L. proxima* grown on malt had an apparent greater ability to tolerate the negative effects of high concentration of pulp waste supernatant than did glucose and sucrose.

Table 39. Linear growth rate of *L. proxima* after 12 days on three kinds of nutrients and four concentrations of pulp waste supernatant.

Nutrient	*L. proxima* colony diameter (mm)			
	Pulp Waste Supernatant			
	0%	1%	10%	100%
Malt	72±1 a[1] (a)[2]	74±1 a (a)	74±1 a (a)	48±2 a (b)
Glucose	69±1 b (a)	68±1 b (a)	67±0 b (a)	41±1 b (b)
Sucrose	72±1 ab (a)	74±1 a (a)	71±1 a (a)	40±1 b (b)

[1] All data presented as mean±standard error. Means in the same column with different letters are significantly different ($P = 0.05$) by one-way ANOVA. [2] Means in the same row with different letters are significantly different ($P = 0.05$) by one-way ANOVA.

DISCUSSION

This experiment established that much of the toxic materials in pulp waste can be leached out. Low concentrations of pulp waste supernatant failed to influence the growth of *L. proxima*. This was expected because low concentrations of fibrous pulp waste failed to inhibit and even improved seedling growth.

This work provided strong evidence that if pulp waste were leached to remove some soluble toxic materials prior to use, it would be easier for the growth of *L. proxima* and the formation of mycorrhiza on seedling roots. At the same time, more pulp waste can be utilized each time.

APPENDIX VII

CHEMICAL AND PHYSICAL ATTRIBUTES OF PULP WASTE

Table 40. Chemical attribute of pulp waste.

Sample	N %	P %	K %	Ca %	Mg %	Na ppm	Mn ppm
Pulp Waste	0.15	0.044	0.059	0.753	0.179	3820	190

Table 40. cont'd

Sample	Cu ppm	Zn ppm	Fe ppm	Pb ppm	Ni ppm	Cr ppm	Cd ppm	Al ppm
Pulp Waste	34.0	52	1140	1.2	3.0	<0.1	<0.1	66

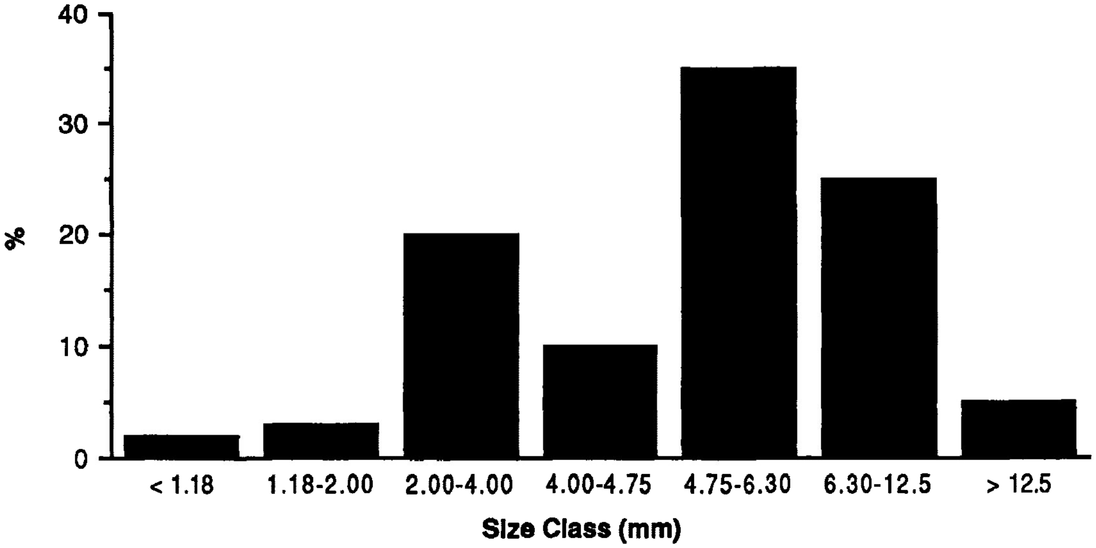

Figure 19. Particle size distribution of pulp waste.

APPENDIX VIII

MICROSCOPIC OBSERVATION OF *L. PROXIMA* ON JACK PINE ROOTS

This experiment was conducted to verify the formation of *L. proxima* as an ectomycorrhiza fungus on jack pine.

MATERIALS AND METHODS

Dichotomized ectomycorrhiza formed on ultimate laterals of jack pine roots were fixed and stored in a FAA solution and later dehydrated and embedded in paraffin (Bakowsky, 1988). Seven µm thick sections were cut with a microtome. Sections were stained with fast green for 5 minutes. Excess staining solution was removed from the root sections with running water. After mounting on slides, sections were observed with a Zeiss photomicroscope and photographed with Kodak Plus-X film.

RESULTS

Laccaria proxima formed an intact fungal mantle and Hartig net on and in jack pine roots (Figure 20).

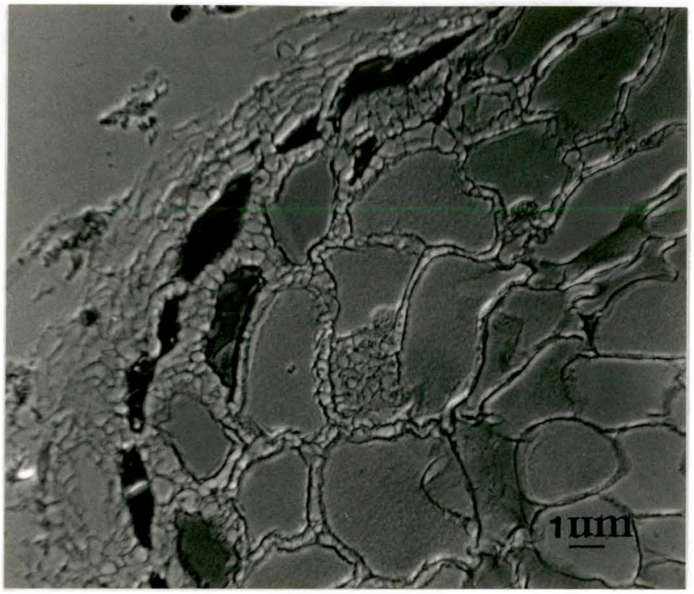

Figure 20. Hartig net and fungal mantle formed by *L. proxima* on the roots of jack pine seedlings.