ALASKA • OREGON • WASHINGTON
PNW RESEARCH NOTE
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Portland, Oregon
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PACIFIC NORTHWEST FOREST AND RANGE EXPERIMENT STATION

PNW-351 March 1980

FORMATION OF ECTOMYCORRHIZAE FOLLOWING INOCULATION OF CONTAINERIZED SITKA SPRUCE SEEDLINGS

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ABSTRACT

Containerized Sitka spruce, [Picea sitchensis (Bong.) Carr.] were inoculated at sowing with pure cultures of either Pisolithus tinctorius (Pers.) Coker & Couch, Laccaria laccata (Scop, ex Fr.) Berk. & Br., Astraeus pteridis (Shear) Feller, Amanita pantherina (D. C. ex Fr.) Schumm., or Cenococcum geophilum Fr. Seedlings were grown in 66-cubic centimeter cells for 6 months in greenhouses at Corvallis, Oregon, and Petersburg, Alaska, and environmental growth chambers at Juneau, Alaska. At Corvallis, Petersburg, and Juneau, respectively, 100, 85, and 100 percent of the seedlings inoculated with L. laccata and 100, 80, and 98 percent, of those inoculated with C. geophilum formed mycorrhizae. Percentage of short roots on colonized seedlings that were mycorrhizal at Corvallis, Juneau, and Petersburg, respectively, were 92, 38, and 77 for L. laccata, and 91, 7, and 12 for C. geophilum. Positive mycorrhizal formation by the other test fungi could not be confirmed at any location.

KEYWORDS: Mycorrhizal inoculation, container nursery stock, Sitka spruce, Picea sitchensis.

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INTRODUCTION

Over 100 000 hectares of old-growth Sitka spruce-western hemlock, Picea sitchensis (Bong.) Carr. - Tsuga heterophylla (Raf.) Sarg., forest have been clearcut on the Tongass National Forest in southeast Alaska. Usually, natural regeneration of both spruce and hemlock has been dense and thrifty on these areas with little need for supplementary planting. With increasingly intensive management, however, areas with inadequate regeneration have become more apparent and the establishment of conifers thereon more desirable.

To supply seedlings for planting on these sites, Region 10 (USDA Forest Service) is developing a greenhouse nursery at Petersburg, Alaska. The facility is scheduled for completion by 1981 and has a projected yearly output of 1,000,000 to 1,500,000 containerized seedlings, largely Sitka spruce. With an abundant supply of planting stock available, some reforestation will also be conducted on routine sites as well as problem sites.

In recent years, production of containerized seedlings has increased rapidly (Tinus et al. 1974). Typically, the potting mixture used to grow these seedlings lacks inoculum of mycorrhizal fungi (Trappe 1977). Mycorrhizae are generally required for woody plants to survive and grow in soil not artificially enriched with nutrients (Marks and Kozlowski 1973, Zak 1977). A lack of mycorrhizae on nursery seedlings may prevent their establishment on mycorrhizae-deficient forest sites or delay establishment until mycorrhizae can form in soils that do not contain natural inocula of mycorrhizal fungi. A cursory examination of containerized Sitka spruce seedlings in the greenhouse nursery at Petersburg showed no development of mycorrhizae.

Techniques are now being refined for inoculating potting mixtures used for growing containerized seedlings with pure cultures of mycorrhizal fungi (Marx and Barnett 1974, Ruehle and Marx 1977, Molina 1979). Such procedures use specific mycorrhizal fungi that may aid establishment and survival of outplanted seedlings on both normal and "problem" reforestation sites (Marx 1977).

Literature dealing with mycorrhizae on Sitka spruce is limited (Trappe 1964, Levisohn 1965, Alexander 1971, Guadray 1973, Thomas and Jackson 1979), and reports no previous attempts to artificially establish mycorrhizal fungi on the roots of container-grown seedlings. The objective of this study was to determine if fungi known to form mycorrhizae could be established on containerized Sitka spruce seedlings through controlled inoculations.

METHODS

Sitka spruce were grown in 66-cm^3 Ray Leach^R Cells[1] in greenhouses at Corvallis, Oregon, and Petersburg, Alaska, and environmental growth chambers at Juneau, Alaska. The cells were inoculated at sowing with pure cultures of either Pisolithus tinctorius (Pers.) Coker & Couch (S-216)[2] Laccaria laccata (Scop, ex Fr.) Berk. & Br. (S-238), Astraeus pteridis (Shear) Feller (S-237), Amanita pantherina (D. C. ex Fr.) Schumm. (S-331), or Cenococcum geophilum Fr. (A-176). Control seedlings received no fungus inoculum. The isolate of C. geophilum was obtained in Alaska while the others were from Oregon. The ability of all fungus species to form ectomycorrhizae with Sitka spruce had been confirmed previously by pure culture synthesis (Molina, unpublished).

Fungus inocula were prepared at the Forestry Sciences Laboratory in Corvallis, Oregon, by procedures similar to those described by Marx and Bryan (1975) and Molina (1979). After leaching, inocula for use in Alaska were placed in plastic bags, cooled to 5°C, air-freighted in an ice chest to Juneau, and stored at 5°C until use (up to 3 weeks).

[1]The use of trade, firm, or corporation names does not constitute an official endorsement by U.S. Department of Agriculture.

[2]Reference numbers for the PNW culture collection maintained at the Forestry Sciences Laboratory, Corvallis.

A nonsterilized peat-vermiculite mix (approximately 1:1) was used as a potting medium. Inoculum was thoroughly mixed with the potting medium at a ratio of 1:7. Planting cells were filled firm with the medium, each sown with three stratified Sitka spruce seed from Juneau, and covered with perlite. Seeds were sown in late May at Corvallis and Petersburg and early June in Juneau. All cells were later thinned to one seedling each. Corvallis and Petersburg seedlings were harvested in late December and Juneau in early January, 6 months after planting.

In Petersburg and Corvallis six treatments (five fungi and control) were planted in a randomized block design, 20 trees per treatment with four replications (480 total seedlings). In Juneau 30 trees were planted per treatment with two replications, one in each environmental chamber (360 total seedlings).

In Petersburg seedlings were grown under natural light supplemented with "grow lites" during three intervals of 6 minutes duration every hour. Air temperature varied from 15° to 27°C. Trees were watered by hand each or every other day. A commercial preparation of soluble 20-20-20 (NPK) fertilizer amended with trace elements was applied every 2d or 3d day after the 1st month at a nominal rate of 0.2 g/liter of water for each 2 m^2 of bench area. This was approximately one-half the standard fertilization rate then in use for Sitka spruce at the Petersburg nursery.

In Corvallis seedlings were reared under a 16-hour photoperiod with air temperatures varying from 15° to 27°C. They were watered with an automatic misting system and fertilized by hand with approximately 3.1 mg of solubilized 20-19-18 (NPK) per seedling, twice monthly.

In Juneau, seedlings were reared under a 16- to 18-hour photoperiod of approximately 1 000 lux with air temperatures varying from 15° to 27°C. The same fertilizer as used in Petersburg was mixed at 0.1 g/liter of water and applied to each 0.28-m^2 chamber area once a week after the 1st month.

At all three locations iron chelate was also applied at roughly one-half the fertilization rate, and water, temperature, and lighting were reduced in late October to induce bud set.

After harvest, roots were gently washed free of the potting mix and each root system was examined microscopically for mycorrhizae. Ten seedlings showing successful inoculation and no contaminant mycorrhizae were selected at random from each treatment-replication. The degree of mycorrhizal formation was calculated by removing three to four large lateral roots and classifying each short root thereon as mycorrhizal or nonmycorrhizal. One hundred or more short roots were examined and classified on each seedling. Height, root collar diameter, and ovendry weights of tops and roots were recorded for each of the 10 seedlings. From within each area, results were examined by analyses of variance and differences between treatment means were compared with Tukey tests. All tests were performed at $P < 0.05$.

RESULTS

At all locations 80 to 100 percent of the seedlings inoculated with Laccaria laccata and Cenococcum geophilum formed mycorrhizae (table 1). These mycorrhizae are distinct and easily separated from contaminant mycorrhizae that were also present. The low percentage of mycorrhizae formed, inconsistency between replications and areas, and the presence of contaminant mycorrhizae—one of which appeared quite similar to Amanita pantherina—precluded confirming successful inoculation by Pisolithus tinctorius, Amanita pantherina, or Astraeus pteridis.

The percentage of short roots that were mycorrhizal on seedlings colonized by L. laccata and C. geophilum was very high (approximately 90 percent) at Corvallis, but dropped markedly for C. geophilum at Juneau and Petersburg and IJ. laccata at Petersburg (table 1). At all locations, some basidiocarp primordia of L. laccata developed on colonized seedlings and mature basidiocarps formed at Corvallis. Mycorrhizal short roots formed by C. geophilum at Juneau and Petersburg were concentrated on the upper few centimeters of the root system.

Table 1—Mycorrhizal formation on inoculated seedlings at Corvallis, Juneau, and Petersburg

Fungus	Corvallis		Juneau		Petersburg	
	Percent of seedlings colonized[1]	Percent of mycorrhizal short roots[2]	Percent of seedlings colonized[1]	Percent of mycorrhizal short roots[2]	Percent of seedlings colonized[1]	Percent of mycorrhizal short roots[2]
Pisolithus tinctorius	10	—	2	—	[3]44	—
Laccaria laccata	100	91	100	77	85	38
Astraeus pteridis	0	—	2	—	[3]25	—
Amanita pantherina	16	—	0	—	18	—
Cenococcurn geophilum	100	90	98	12	80	7
Control	6		0	—	11	—

[1]Mean of all seedlings.

[2]Mean of 10 colonized seedlings per replication.

[3]Appeared to be contaminant mycorrhizae.

Analyses of variance for top height, top weight, root collar diameter, root weight, total weight, and root-shoot ratio were performed within each location for seedlings colonized by *L. laccata*, *C. geophilum*, and uninoculated controls. Significant differences occurred only at Corvallis for root collar diameter, root weight, and total weight. Tukey tests for differences among treatment means for these variables at Corvallis were not significant; however, for all three variables, mean values for control seedlings were greater than those for seedlings colonized by either *L. laccata* or *C. geophilum* (table 2). Seedlings colonized by *L. laccata* also had a markedly larger but marginally non-significant (P=0.065) root shoot ratio (table 2).

Disregarding inoculation, Petersburg seedlings were significantly taller but had significantly less root weight and a smaller root shoot ratio than Corvallis (table 2). Differences in experimental design at Juneau, Corvallis, and Petersburg did not allow statistical comparisons among the three areas; but overall, Juneau seedlings were the smallest (table 2).

Table 2--Growth of Sitka spruce seedlings at Corvallis, Juneau, and Petersburg

Treatment location	Growth measurements[1]					
	Top height (cm)	Top weight (g)	Root col. diameter (mm)	Root weight (g)	Ratio (Root weight/ top weight)	Total weight (g)
Laccaria laccata, Corvallis	6.7	0.29	1.4	0.35	1.26	0.64
Cenococcum geophilum, Corvailis	6.8	.28	1.3	.26	.95	.54
Control, Corvallis	7.6	.38	1.6	.36	.99	.74
Control, Juneau	12.4	.20	1.3	.073	.35	.28
Control, Petersburg	13.3	.39	1.6	.15	.38	.54

[1]Mean of 10 seedlings per replication.

DISCUSSION

Successful inoculation with Laccaria laccata and Cenococcum geophilum indicates the potential for artificial establishment of selected mycorrhizal fungi on containerized Sitka spruce nursery stock. The failure of the remaining isolates to colonize seedling roots consistently, however, indicates that not all mycorrhizal fungi are suitable to use with this inoculation technique. The reasons for these failures are largely unknown and are probably a combination of many factors. For example, the age and original host associate of the fungus isolate may markedly influence its ability to form mycorrhizae (D. H. Marx, personal communication) . Some fungi may not be able to tolerate the rigorous mechanical handling involved in inoculum preparation or the lag period between inoculation and feeder root production. Also, different fertility regimes can affect the performance of fungus inoculum (Marx and Barnett 1974, Trappe and Molina, unpublished data). Clearly, more research is needed to determine if these potentially useful fungi could be introduced onto the roots of container-grown seedlings.

Both L. laccata and C. geophilum are distributed worldwide and are able to form mycorrhizae with most trees that host ectomycorrhizae (Trappe 1962 and 1964, Molina, unpublished data). Both isolates used in this study performed well over a variety of growing conditions, often colonizing entire root systems. Molina recently found similar excellent performance by both fungi on inoculation of containerized Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco, western larch, Larix occidentalis Nutt., western hemlock, ponderosa pine, Pinus ponderosa Laws., and lodgepole pine, Pinus contorta Dougl. Thus, it appears these fungi could be used for wide scale inoculations in container nurseries growing west coast conifers.

Ectomycorrhizal inoculation of containerized seedlings rarely aids their growth in the nursery (Marx and Barnett 1974, Molina 1979 and 1980). With the use of soluble fertilizers, even at much reduced levels, uninoculated control seedlings obtain adequate nutrition to make comparable growth. A more important consideration is how inoculated seedlings will perform upon outplanting in comparison with uninoculated control seedlings. Outplanting studies with containerized Sitka spruce successfully inoculated with L. laccata and C. geophilum are currently in progress.

Finally the Alaskan inoculations show that fungus inoculum can be commercially transported over long distances and still maintain viability. This may be an important consideration for use of inoculum in isolated nurseries or production of inoculum for several nurseries at a single location.

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GPO 990-396