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Ectomycorrhizal Inoculation of Containerized Western Conifer Seedlings

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Abstract

Of 15 ectomycorrhizal fungi inoculated onto five container-grown conifer species (Larix occidentalis, Pinus contorta, P. ponderosa, Pseudotsuga menziesii, and Tsuga heterophylla), only Laccaria laccata and Cenococcum geophilum consistently formed ectomycorrhizae on all conifer hosts. Percents of mycorrhizal feeder roots were generally high, ranging from 86 on L. occidentalis to 94.5 percent on T. heterophylla for L. laccata and from 48.1 to 81.8 percent on these respective hosts for C. geophilum. L. laccata significantly colonized more feeder roots than C. geophilum for most conifer species. Only P. menziesii seedlings inoculated with C. geophilum were significantly larger than controls. There is a need fot further studies with a wider range of fungi.

Keywords: Mycorrhizal inoculation, container nursery stock.

Introduction

Increasing needs to reforest cutover public and private forest lands have generated increasing demand for containerized seedlings. Given these needs as well as the sizable economic investments in container nurseries, use of container seedlings will continue for the immediate future.

Although the tops of seedlings grown in containers often grow luxuriantly, most root systems we have examined lack normal ectomycorrhizal development. This most likely results from the use of artificial (non-soil) potting substrates, restriction of natural fungus inoculation through greenhouse rearing, and, most importantly, the high dosages of regularly applied soluble fertilizers. High levels of fertility have been shown to retard mycorrhizal development of containerized seedlings (Marx and Barnett 1975). A more natural mycorrhizal root system may greatly improve planting success of containerized seedlings especially on hard-to-regenerate sites (Marx and Barnett 1975, Trappe 1977).

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Marx and Bryan (1975) have recently developed techniques to artificially inoculate bareroot seedlings with the ectomycorrhizal fungus Pisolithus tinctorius. Inoculations of containerized loblolly pine (Pinus taeda L.) (Marx and Barnett 1975, Ruehle and Marx 1977), lodgepole pine (Pinus contorta Dougl. ex Loud.) and Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) (Molina 1979) by similar techniques have also been successful. Little data is available, however, on artificial inoculation with other ectomycorrhizal fungi. Trappe (1977) suggests that ectomycorrhizal fungi in the genera Rhizopogon and Suillus, with their ease of isolation and rapid growth in pure culture, offer high potential for artificial inoculation of conifer seedlings. Also, fungi in these genera often fruit only with a particular host genus or species; the more specialized, host-specific fungus may benefit its particular host more than would a non-host-specific fungus (Mikola 1970).

The purpose of this study was to assess the success of inoculating containerized Douglas-fir, western hemlock (Tsuga heterophylla (Raf.) Sarg.), western larch (Larix occidentalis Nutt.), lodgepole pine, and ponderosa pine (Pinus ponderosa Dougl. ex Laws.) seedlings with a variety of host specific and non-host-specific fungi and their effects on seedling growth.

Methods

Fungus Isolates

Table 1 lists the fungus isolates tested for each tree species and their dates of isolation. Except for Cenococcum geophilum, all had originally been isolated from sporocarp tissue; C. geophilum was isolated from a surface-sterilized sclerotium (Trappe 1969). All isolates were previously tested in pure culture synthesis to confirm their mycorrhiza-forming ability.

Inoculations with Laccaria laccata, Cenococcum geophilum, Pisolithus tinctorius, Paxillus involutus, and a control of no fungus addition served as common inoculation treatments for all tested host tree species. These fungi are well known for their broad host ranges (Trappe 1962). The remaining three to four fungi tested per host tree species (see table 1) were selected for their known or inferred specificity to that particular host.

Inoculum Preparation

Inoculum was prepared according to Marx and Bryan (1975) as modified by Molina (1979). Vegetative mycelium of each isolate was grown aseptically in glass-capped 2-liter flasks containing 1 450 ml of vermiculite plus 50 ml of sphagnum peat moss moistened with 750 ml of modified Melin-Norkrans nutrient solution (Marx 1969); dextrose was substituted for sucrose in this solution. Control flasks contained no fungus. After 3 months at room temperature, inoculum was removed from the flasks and leached with cold running tap water to remove unused nutrients. Excess free water was removed by gently squeezing the inoculum wrapped in cheesecloth. Inoculum was placed in plastic bags and stored overnight at 5°C.

Table 1—Ectomycorrhizal fungus isolats used to inoculate containerzed western mite and their dates of isolation

Tree species inoculated	fungs isolate		
	No.	Species	
Douglas-fir and western hemlock	A-153	Rhizupoqon vinicolor Smith	August 1976
	S-311	Rhizopogon parksil Smith	September 1976
	S-242	Suillas ponderosas Smith & Thlers	[illegible] 1976
	S-243	Suillus lakei (Murr) Smith S Thlers	[illegible]
Lodgepole and ponderosa pine	S-297	Rhizopoaon orcidentalis Zeiler & Dodge	November [illegible]
	S-218	Rhizopogon vulgaris Vitt.) M. Iange	[illegible] 1976
	S-223	Suillus brevlpes (Pk.) Yuntze	September 1976
	S-222	Suillus tomentosas Lant. Snell, Sirger & Dick	Sept ember 1976
Western larch	S-255	Suillus grevillei (*K*) Singer	October 1976
	S-281	Fuscoboletinus aerugurascans (Sect.) Pom. & Smit	[illegible]
	S-298	Suillus cavipes (Opat. Smith \ Thiers	October 1976
All of the above	A-145	Cenococcum geophilum Fr.	December 1974
	S-238	Laccaria laccata (Scop, ex Fr.) Bk. and Br.	September 1976
	S-403	Paxillus involutus (Batsch.) Fr.	September 1976
	S-216	Pisolithus tinctorius (Pers.) Coker s Couch.	September 1976

Inoculation and Sowing

A potting substrate, containing equal volumes of vermiculite and sphagnum peat moss, was pasteurized in steam at 80°C for 30 min to kill resident mycorrhizal fungi. For each species of fungus, one part inoculum was added to six parts potting substrate in large plastic bags and was then vigorously shaken to evenly distribute the inoculum particles. Sixty individual "Leach super cell" containers, 165-ml capacity, were then filled with the inoculated potting substrate per tree-fungus combination; of these, groups of 20 cells each were then randomly placed into three replicate blocks per host tree. A randomized block design containing three replicate blocks, each with 20 seedlings per fungus treatment, was used for each tree species. The large number of treatment combinations necessitated keeping each tree species as a separate test. Cells were then sown with three prestratified seeds and misted twice daily until germination was complete. Seedlings were then thinned to one per cell.

Growing Conditions

All seedlings were grown in the greenhouse from late May through November 1978. Supplemental light of approximately 11 000 lx over a 15-h photoperiod was provided by overhead sodium-vapor lamps. Photoperiod was lengthened to 20 h from mid-August through September to offset premature budset. Because high fertility is known to retard mycorrhiza formation of container seedlings (Marx and Barnett 1975), a completely soluble 20-19-18 NPK fertilizer (Peat-lite special)[2] plus Sequestrene Fe 330 iron chelate were applied at approximately one-quarter strength, the dosage suggested by Owston (1975) for growing western conifers. The soluble fertilizer was dissolved in tap water and evenly distributed by hand over all seedlings at the rate of 6 g/m^2 of bench space; Sequestrene was applied at the rate of 3 g/m^2 of bench space. Each seedling thus received approximately 3.1 mg of Peat-lite special fertilizer plus 1.6 mg of Sequestrene in each fertilization. Fertilizations were performed twice monthly from July through October. Seedlings were mist irrigated with tap water as needed.

Data Collection and Analysis

At the end of the experiment, all seedlings were harvested and their roots gently washed free of potting substrate. Each seedling root system was examined by stereomicroscopy for success of inoculation. For those fungus treatments showing successful inoculation, 10 seedlings were randomly selected per treatment replication; and their height, stem diameter, percent of mycorrhizal feeder roots, and ovendry weights of tops and

[2]Trade names used do not imply endorsement by the U.S. Department of Agriculture over similar products.

roots were recorded. Degree of mycorrhiza formation was assessed by randomly removing three to six major lateral roots per seedling from their points of attachment to the tap roots and then counting the total number of mycorrhizal and non-mycorrhizal feeder roots. At least 100 total feeder roots were counted per seedling. Mycorrhiza formation was expressed as percent of total feeder roots examined which had formed mycorrhizae. Control or inoculated seedlings with other, contaminant mycorrhiza types were discarded initially and not included in analyses. All results were subjected to analysis of variance and differences among treatment means were compared with Scheffe' tests. All significant differences are reported at $P \leq 0.05$.

Results

Only 2 of the 15 fungi tested, L. laccata and C. geophilum, formed abundant mycorrhizae on all the conifer species. P. involutus produced mycorrhizae with only a few seedlings from each tree species. No mycorrhizae were produced by any Suillus species, Rhizopogon species, Fuscoboletinus aeruginascens, or P. tinctorius. Control seedlings were mostly free of any mycorrhiza formation . Thelephora terrestris (Ehrh.) Fr. was the most prevalent contaminant mycorrhizal fungus but only colonized about 4 percent of all seedlings.

Inoculation success with both L. laccata and C. geophilum was excellent. With very few exceptions, practically all L. laccata- and C. geophilum-inoculated seedlings formed abundant mycorrhizae. Percent of mycorrhizal feeder roots ranged from 86 on western larch to 94.5 percent on western hemlock for L. laccata inoculations and from 48.1 to 81.8 percent on these respective hosts for C. geophillum inoculations (table 2). Except for ponderosa pine, L. laccata significantly colonized more feeder roots than C. geophilum. Mycorrhizal development was always strongest at the top of seedling plugs for both fungi, but usually the entire plug was colonized.

L. laccata sporocarps fruited prolifically among the different hosts, and various stages of primordia were abundant in the containers. Sclerotia of C. geophilum were also frequently observed on the root systems. Both fungi were easily reisolated from these reproductive structures. The effects of these fungi on seedling growth will be briefly discussed for each tree species.

Douglas-Fir

L. laccata mycorrhizae were most often well developed, pinnately branched structures and averaged 89 percent of the total short roots. C. geophilum mycorrhizal development was also extensive, colonizing 76.7 percent of total short roots. Mycorrhizae were most often short and cylindric to simple pinnate and of typical jet black color.

Table 2—Mean growth and mycorrhiza formation of Douglas-fir, western hemlock, western larch, lodgepole pine, and ponderosa pine seedlings inoculated with *Laccaria laccata* and *Cenococcum geophilum*[1]

Tree specxes	Fungus treatment	Height	Stem diameter	Dry weight		Top: root	Percent mycorrhizal short roots
				Tops	Roots		
		Centimeters	Millimeters	Grams			
Douglas-fir	Control	9.27a	2.00	0.535	0.921	0.585	--
	Laccaria laccata	9.20a	2.00	.549	.853	.656	89.0a
	Cenococcum geophilum	10.51b	2.02	.561	.813	.694	76.7b
Western hemlock	Control	12.23	2.09	.574	.581a	.996	—
	Laccaria laccata	11.44	1.94	.520	.489b	1.090	94.5a
	Cenococcum geophilum	12 56	1.96	.533	.474b	1.168	81.8b
Western larch	Control	9.97	2.17	.576	.679	.868ab	--
	Laccaria laccata	9.61	2.27	.468	.650	.735a	86.2a
	Cenococcum geophilum	12.08	2.03	.573	.626	.923b	48.1b
Lodgepole pine	Control	4.24	1.78a	.243	.670a	.360a	—
	Laccaria laccata	4.30	1.56b	.204	.501b	.418a	9 3.0a
	Cenococcum geophilum	4.42	1.77a	.274	.524ab	.532b	69.7b
Ponderosa pine	Control	5.84a	2,81a	.564a	.994a	.585	—
	Laccaria laccata	5.27a	2.24b	.319b	.607b	.557	89.5
	Cenococcum geophilum	5.81a	2.45ab	.499ali	.740b	.684	77.8

[1,2]When no letters follow a group of means, no significant differences were seen in the analysis of variance. Means within individual tree species not sharing a common letter are significantly different ($P \leq 0.05$) by Scheffe'tests.

No significant differences were found in stem diameter, dry weights of tops or roots, or in top:root ratio between any treatments (table 2). *C. geophilum*-inoculated seedlings, however, were significantly taller than all other seedlings.

Western Hemlock

Although *L. laccata* colonized 94.5 percent of total feeder roots, the mycorrhizae were most often very short, 2-5 mm long, occasionally becoming slightly longer and pinnate. Similarly, *C. geophilum* colonized 81.8 percent of total short roots, but these were also usually short and simple.

Control seedlings had significantLy greater root dry weight than either fungus treatment, possibly as a result of the very short mycorrhizae observed (table 2). No significant differences were found in height, stem diameter and dry weight, or top:root ratio.

Western Larch

L. laccata mycorrhizal development on western larch resembled Douglas-fir inoculations in colonizing 86.2 percent of total short roots and in forming elongate, variously branched to pinnate mycorrhizae. *C. geophilum* development was less extensive, colonizing 48.1 percent of total feeder roots, these often concentrated in the upper third of the plug.

No significant differences between treatments occurred in seedling height, stem diameter, or dry weights of tops and roots (table 2). *C. geophilum*-inoculated seedlings had a significantly higher top:root ratio than *L. laccata*-inoculated seedlings; yet, *L. laccata* colonized significantly more short roots than *C. geophilum*.

Lodgepole Pine

L. laccata formed abundant mycorrhizae with lodgepole pine colonizing on the average 93 percent of total feeder roots. Mycorrhizae were well developed, often forming large coralloid clusters. *C. geophilum* mycorrhizae were also abundant and colonized 69.7 percent of total feeder roots. Mycorrhizae were most often simple cylindric to bifurcate, occasionally compoundly bifurcate.

No significant differences between treatments were seen in height or top dry weight. Significant differences were found, however, for stem diameter, root dry weight, and top:root ratio (table 2). *L. laccata*-inoculated seedlings had smaller stem diameters than either control or *C. geophilum*-inoculated seedlings. Control seedlings had greater root dry weight than *L. laccata*-inoculated seedlings and also a lower top:root ratio than *C. geophilum*-inoculated seedlings.

Ponderosa Pine

Both *L. laccata* and *C. geophilum* formed extensive mycorrhizae on ponderosa pine, colonizing 89.5 and 77.5 percent of total feeder roots, respectively. Mycorrhizae were very similar to those described for lodgepole pine.

Significant differences among fungus treatments were found for seedling height, stem diameter, and dry weight of tops and roots. Control seedlings had significantly greater stem diameters and top dry weight than *L. laccata*-inoculated seedlings and also greater root dry weight than both fungus treatments. Analysis of variance indicated significant differences among treatment means for seedling height, but comparison of means with Scheffé tests (a conservative mean comparison test) failed to isolate the differences.

Discussion

It remains unknown why only two of the many fungi consistently formed abundant mycorrhizae after inoculation. Clearly this inoculation technique may not work for many fungi. The failure with *P. tinctorius*-isolate S-216 is particularly puzzling; this isolate performed well in an inoculation the previous year (Molina 1979). Long-term culture maintenance with repeated transferring may have been a problem; Marx (personal communication 1979) found that this fungus quickly loses its ability to form mycorrhizae as the culture ages and is repeatedly transferred.

Successful inoculation of containerized seedlings with species of *Suillus* or *Rhizopogon* have not been reported; only limited success has been reported for their inoculation onto bareroot nursery seedlings (Moser 1959, Theodorou and Bowen 1970, Vozzo and Hacskaylo 1971). Maybe these particular isolates cannot withstand the disturbance involved in inoculum preparation or survive within the vermiculite particle until young germinants produce feeder roots for mycorrhizal colonization. Also, their growth pattern in inoculum flask culture resembles that of many fungi growing in petri plate culture: young, actively growing mycelium progresses as a colony edge into the peat-vermiculite substrate leaving behind darkened, slower growing mycelium. Whether the older, darkened mycelium remains viable is unknown. Because fungi in these genera are easily isolated, grow quickly in culture, and are often host-specific, their use in mycorrhizal inoculation of seedlings is highly desirable (Trappe 1977).

The successful mycorrhizal inoculation with *L. laccata* and *C. geophilum* still emphasizes the practical use of this inoculation technique. Most seedlings inoculated with these two fungi formed abundant mycorrhizae, at times the entire feeder root system being colonized. The prolific sporocarp formation by *L. laccata* among the various hosts indicates the high activity of this fungus in the container system. Also, for both fungi, the seedling root plugs were usually strongly bound by the fungal mycelium; this may significantly reduce root disturbance during outplanting. Future research will concentrate on ecotypic variation among different isolates of these fungi as it influences the inoculation potential of nursery seedlings and outplanting performance onto various sites.

Mycorrhizal inoculation of containerized seedlings grown under routine nursery conditions utilizing completely soluble fertilizers, even at reduced rates, rarely increases growth (Molina 1979, Marx and Barnett 1975). In this study, only Douglas-fir growth was significantly increased; *C. geophilum*-inoculated Douglas-fir seedlings were significantly taller than both control and *L. laccata*-inoculated seedlings. *L. laccata* seedlings were generally smaller than controls, sometimes significantly so; both *Pinus* species inoculated with *L. laccata* had significantly smaller stem diameters and root dry weights than control seedlings. The prolific mycelial growth of *L. laccata*, including its abundance of mycorrhizal colonizations and sporocarp formation, may have been a considerable drain on host photosynthates.

To fully realize the practical significance of mycorrhizal inoculation of containerized seedlings, further research is needed on the effects of different nutrient levels and fertilizer schedules on inoculation success. Inoculation methods may have to be modified to include a more diverse array of fungi in future experiments. Finally, the performance of inoculated vs. uninoculated seedlings when planted at various sites must be evaluated.

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