Growth Response of Five Rocky Mountain Conifers to Different Ectomycorrhizal Inocula

Raymond C. France and Michael L. Cline

Section leader, regeneration research/nursery operations, and research associate, tree growth and development, International Paper Co., Bainbridge, GA, and Tuxedo Park, NY

Artificial inoculation with the mycorrhizal fungus Pisolithus tinctorius *(Pers.) Coker & Couch significantly increased the growth of Engelmann spruce (*Picea engelmannii *Parry ex. Engelm.) and Douglas-fir (*Pseudotsuga menziesii *(Mirb.) Franco) containerized seedling stock. No significant differences in height, diameter, and weight of limber pine (*Pinus flexilis *James), lodgepole pine (*Pinus contorta *var*. latifolia *Engelm.), and ponderosa pine* (Pinus ponderosa *Dougl. ex Laws.) were noted when artificial and natural inoculation were compared. Root/ shoot ratios were greater with artificial inoculation for lodgepole and ponderosa pines. Pine species readily form* P. tinctorius *mycorrhizae; Englemann spruce and Douglas-fir do not.* Tree Planters' Notes 38(1):18-21; 1987

Successful reforestation of land requires proper site preparation, a quality planting job, proper seedling handling, and high-quality planting stock. Good quality seedlings must be physiologically and morphologically able to withstand transplant shock and the rigors of the planting site environment. Seedling quality is especially important in the reforestation of the central Rocky Mountains, where seedling size, planting time constraints, and environmental and climatic extremes are critical elements to success.

Mycorrhizae have been shown to play a significant role in successful reforestation with a variety of conifers in many parts of North America. A sufficient amount of the proper mycorrhizal symbiont on seedling root system aids in seedling survival and early tree growth. Mycorrhizae provide the seedling with more root surface to absorb soil nutrients and water, better physiological balance, and therefore, a better chance of seedling establishment. Thus, mycorrhizae are a significant component of the quality seedling.

Over the last few years, emphasis has been placed on the commercial development of vegetative mycelial inoculum of *Pisolithus tinctorius* (Pers.) Coker & Couch, a mycorrhizal symbiont. *P. tinctorius* has been reported to form mycorrhizae with many coniferous and hardwood tree species throughout the world *(4)*. Marx and others *(1, 5)* have shown *P. tinctorius* to improve the survival and growth of many southern pine species on a diverse range of reforestation sites.

The purpose of this study was to compare the growth response and mycorrhiza-forming ability of five Rocky Mountain conifers that were inoculated "artificially" with *P. tinctorius* or "naturally" with screened duff.

Methods and Materials

Artificial inoculation. Mycelial cultures of *P. tinctorius* (isolate 166, D.H. Marx) were grown on modified Melin-Norkrans liquid nutrient medium for 30 days. For inoculation, mycelial mats were leached of excess nutrients with distilled water and macerated in distilled water in a Waring Blendor. A 30-milliliter suspension of mycelium was injected into a growth medium of vermiculite and peat moss (1 :1 mixture) in 1-quart paperboard containers.

Natural inoculation. Indigenous mycorrhizal fungi were obtained from localized forest duff samples collected underneath pure stands of the conifer species tested. The duff was screened to remove cone fragments and undecayed pine needles, then mixed with vermiculite (1:1) and placed in the paperboard containers. Growth medium for both inoculation treatments was rinsed with water several times before seed planting.

Seedling management. Seeds of Englemann spruce (*Picea engelmannii* Parry), lodgepole pine (*Pinus contorta* Dougl. ex Loud.), limber pine (P. flexilis James), ponderosa pine (P. ponderosa Dougl. ex Laws.), and Douglas-fir (*Pseudotsuga* *menziesii* (Mirb.) Franco) were germinated in containers of one of the two inoculation treatments. Sixty seedlings per inoculation treatment per tree species were grown in a greenhouse for 6 months. Seedlings were fertilized with full-strength Hoagland's nutrient solution every 30 days; they were watered every 2 days to minimize differential water availability because of drying.

Data collection and statistical analysis. After 6 months, 30 seedlings for each inoculation treatment for each tree species were randomly selected for destructive sampling. Seedlings were removed from the containers and cleaned in tap water. Shoot height, root collar diameter, and shoot and root dry weights were measured for each seedling. Mycorrhizal infection was determined by the counting procedure on fresh root systems *(2).*

Statistical comparisons (one-way analysis of variance) were made between inoculation treatments for each seedling parameter. All testing was performed at P = 0.05.

Table 1—*Seedling growth of five Rocky Mountain conifers in response to natural (duff) and artificial* (Pisolithus tinctorius) *inoculation*[1]

Inoculation treatment	Shoot height (cm)	Root collar diameter (mm)	Total dry weight (g)	Root/ shoot (g/g)	Ectomycorrhizal infection (%)
Picea engelmannii (Engelmann spruce)					
Artificial	10.9 a	2.2 a	0.68 a	0.51 a	1 a
Natural	7.2 b	1.4 b	0.20 b	0.47 a	0 b
Pinus contorta (lodgepole pine)					
Artificial	11.0 a	2.5 a	1.20 a	0.73 a	59 a
Natural	11.0 a	2.4 a	1.07 a	0.47 b	11 b
Pinus flexilis (limber pine)					
Artificial	5.6 a	1.9 a	0.58 a	0.51 a	31 a
Natural	5.8 a	1.9 a	0.58 a	0.50 a	18 b
Pinus ponderosa (ponderosa pine)					
Artificial	10.0 a	2.9 a	1.99 a	0.72 a	35 a
Natural	9.3 a	2.6 a	1.78 a	0.55 b	17 b
Pseudotsuga menziesii (Douglas-fir)					
Artificial	15.8 a	1.7 a	0.63 a	0.56 a	<1 a
Natural	12.4 b	1.4 b	0.38 b	0.52 a	<1 a

[1]Mean values within a species and between inoculation treatments, followed by the same letter are not significantly different at $P \geq 0.05$.

Results and Discussion

Engelmann Spruce. Shoot height, root collar diameter, and seedling dry weight of Englemann spruce seedlings were significantly greater with artificial inoculation with *P. tinctorius* (table 1). Seedling dry weight was more than three times greater in the *P. tinctorius* inoculation treatment, even though mycorrhizal infection was low (1 percent). No mycorrhizae formed with natural inoculum sources. Root/shoot ratio was not significantly different between treatments.

Lodgepole pine. No significant differences occurred between inoculation treatments for shoot height, root collar diameter, or dry weight for lodgepole pine (table 1). However, dry weight was 12 percent greater with artificial inoculation. This differential was further expressed in a significant treatment difference in the root/shoot ratio. The greater ratio with artificial inoculation is partly attributable to the high degree of *P. tinctorius* mycorrhizae formation. Over five times more mycorrhizae formed with artificial inoculation. Lodgepole pine formed the greatest amount of *P. tinctorius* mycorrhizae of all species. Natural mycorrhizae were of two types; one with white hyphae, the other with brown. The brown hyphae are distinctive from the golden brown *P. tinctorius* mycorrhizae.

Limber Pine. Limber pine seedlings were almost identical in all seedling characteristics except mycorrhizal infection between inoculation treatments (table 1). Mycorrhizal infection was significantly greater with artificial inoculation. Natural fungi were white.

Ponderosa pine. As with lodgepole pine, inoculation of ponderosa pine did not result in significant differences in shoot height, root collar diameter, or seedling dry weights (table 1). However, the root/shoot ratio was significantly greater with artificial inoculation. *P. tinctorius* mycorrhizal infection was twice as great as formation with natural white symbionts.

Douglas-fir. Shoot height, root collar diameter, and seedling dry weight were significantly greater for Douglas-fir with artificial inoculation, even though no *P. tinctorius* mycorrhizae were formed (table 1). Root/shoot ratios were not significantly different. Only 7 percent of the seedlings inoculated with forest duff formed mycorrhizae, but the mean value showed less than 1 percent infection. These white mycorrhizae were monopodial, which was unique to Douglas-fir among the five conifer species tested.

Growth response and mycorrhizal infection charateris-tics of these conifer species can be used to separate species into two groups. Englemann spruce and Douglas-fir showed significant treatment differences in morphological characteristics and little or no mycorrhizal infection. The three pine species showed no significant treatment differences in growth; there were high levels of mycorrhizal infection, with artificial inoculation producing significantly higher levels of infection than did natural inoculation.

Lodgepole pine (7,9), ponderosa pine (6,9) , and Douglas-fir (6,9) have previously been shown to form mycorrhizae with *P. tinctorius* in containerized systems. Engelmann spruce and limber pine have not been previously reported to form *P. tinctorius* mycorrhizae.

Improvements in seedling growth due to *P. tinctorius* inoculation have been shown by Marx and others (6). In their 1977 tests, ponderosa pine shoot height was not significantly great in *P. tinctorius* seedlings (3-, 6-, and 12- percent inoculation rates) compared to control seedlings. Higher inoculation rates (6 and 12 percent) resulted in greater shoot height and weight of ponderosa pine seedlings and in greater weight of Douglas-fir seedlings compared to inoculated seedlings.

The results of this study suggest that seedling growth and development are influenced by factors other than inoculation treatment. These factors include differential water-holding capacity of the two growth media, a greater micro-flora population available for competition for nutrient and water absorption, and different nutrient adsorption properties of the growth media. These factors and their interactions may have contributed to treatment differences.

Summary

Artificial inoculation with *P. tinctorius* significantly increase shoot height, root collar diameter, and dry weight of Engelmann spruce and Douglas-fir seedlings. However, little or no mycorrhizae formed in either treatment.

No significant differences occurred in height, diameter, and seedling weight due to inoculation treatment for limber, lodgepole, and ponderosa pines. The root to shoot ratio of lodgepole and ponderosa pines was significantly greater when seedlings were inoculated with *P. tinctorius* than with duff. The three pine species readily form *P. tinctorius* mycorrhizae, and at levels two to five times greater than natural inoculum sources, depending on the pine species.

Care should be taken in the selection of sources of natural

mycorrhizal inoculum. Seedling growth and quality returns as a result of application of natural inoculum sources may not be worth the investment.

Literature Cited

1. Berry, C.R.; Marx, D.H. Effects of *Pisolithus tinctorius* ectomycorrhizae on growth of loblolly and Virginia pines in the Tennessee Copper Basin. Res. Note SE-264. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station; 1978. 6 p.
2. France, R.C.; Cline, M.L.; Reid, C.P.P. Gravimetric determination of ectomycorrhizal infection. Soil Biology and Biochemistry 17:381-382; 1985
3. Marx, D.H. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic fungi and soil bacteria. Phtyopathology 59:153-163; 1969
4. Marx, D.H. Tree nost range and world distribution of the ectomycorrhizal fungus Pisolithus tinctorius. Canadian Journal of Microbiology 23:217-223; 1977.
5. Marx, D.H.; Bryan, W.C.; Cordell, C.E. Survival and growth of pine seedlings with *Pisolithis tinctorius* mycorrhizae after two years on reforestation sites in North Carolina and Florida. Forest Science 23: 363-373;1977.
6. Marx, D.H.; Ruehle, J.L.; Keeney, D.S.; Cordell, C.E.; Riffle, J.W. Molina, R.J.; Pawuk, W.H.; Navratil, S.; Tinus, R.W.; Goodwin, O.C. Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on container-grown tree seedlings. Forest Science 28:373-400; 1982
7. Molina, R. Ectomycorrhizal inoculation of containerized Douglas-fir and lodgepole pine seedlings with six isolates of *Pisolithus tinctorius* . Forest Science 25:585-590; 1979.
8. Molina, R. Ecotomycorrhizal inoculation of containerized western conifer seedlings. Res. Note PNW-357. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station; 1979. 10 p.
9. Molina, R.; Trappe, J.M. Patterns of ectomycorrhizal host specificity and potential among Pacific Northwest conifers and fungi. Forest Science 28: 423-458; 1982