Effect of Various Nutrient Regimes and Ectomycorrhizal Inoculations on Field Survival and Growth of Ponderosa Pine (*Pinus ponderosa* var. *scopulorum* Engelm.) Container Seedlings in Arizona[1]

L. J. Heidmann and Zane J. Cornett

Research plant physiologist, USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Flagstaff, AZ, and borough forester, Matanuska-Susitna Borough, Palmer, AK

Pinus ponderosa *var*. scopulorum *Engelm. container seedlings raised under four nutrient and three mycorrhizal inoculation treatments survived well in the field. Trees inoculated with forest duff survived better than noninoculated seedlings. Generally, seedlings raised under high nitrogen regimes had greater growth.* Tree Planters' Notes 37(3):15-19; 1986.

In the Southwest--Arizona, New Mexico, and southwestern Colorado--reestablishment of Rocky Mountain ponderosa pine (*Pinus ponderosa* var. *scopulorum* Engelm.) is difficult because precipitation is erratic during critical periods of the year. Nursery stock can be planted successfully when proper procedures are followed (6,14). Initial growth of planted seedlings, especially bareroot stock, is very slow (5). Seedlings that grow slowly are subject to predation by a host of biotic agents, primarily browsing mammals and insects. Hastening juvenile growth reduces the time trees are susceptible to damaging agents.

Inoculating the roots of many tree species with mycorrhizal fungi has improved survival and growth, especially when the symbionts are ecologically adapted to the site (9). In North Dakota, Riffle and Tinus (13) found that survival, stem diameter, current height increment, and biomass of ponderosa pine seedlings were increased, after 2 and 3 years, for seedlings inoculated with pine duff, *Rhizopogon roseolus* (Corda) Hollos, or *Suillus granulatus* (L.: Fries) O. Kuntze.

Trees require mycorrhizae to absorb adequate water and nutrients, especially phosphorus (7,8,10,11). Greater absorption is likely due to a larger surface area of the roots resulting from the formation of the fungal mantle and from mycelia that emanate from the mantle and permeate the soil. In addition, mycorrhizal roots remain functioning for longer periods of time, whereas uninfected roots are ephemeral (1).

Root to shoot ratios have long been considered an important factor in determining initial survival of planted ponderosa pine. Cornett (3) has shown that root to shoot ratios (ovendry weight basis) are inversely related to amounts of foliar N, P, and K applied. Thus, if a formula for producing seedlings with short, compact tops and well-developed mycorrhizal root systems could be found, survival and growth in the field should be improved. This field study tested the survival and growth of containerized seedlings raised under four nutrient regimes and inoculated with forest duff or *Pisolithus tinctorius* (Pers.) Coker & Couch (Pt).

Methods

Seedlings. Ponderosa pine container seedlings were raised in the Bureau of Indian Affairs' greenhouse at McNary, AZ, following general guidelines from Tinus and McDonald (15).[2] Trees were raised in Tinus Rootrainers (492 cubic centimeters) manufactured by Spencer-Lemaire, Ltd., Canada. Seedling substrate consisted of equal parts of peat moss and vermiculite inoculated with forest duff, Pt, or no inoculum (3). Duff was collected from an 85-year-old ponderosa pine stand in central Arizona on a 6- to 8-percent slope with a southwest aspect. Recent litterfall layers were discarded, then humus layers (down to mineral soil) were collected. The material was prepared as inoculum by passing it through a 1.3-centimeter screen. All inocula were mixed with the substrate immediately prior to filling and seeding containers at a rate of 8 percent duff by volume.

Basidiospores of Pt were collected in 1979 from a dry site in Jackson County, OR, at an elevation of approximately 600 meters. The spores were stored dry at a temperature of 2 to 4 °C until used as inoculum. At that time, 100 milligrams of Pt spores was suspended in a 2-liter solution of distilled

[1] The research reported here was conducted at the Rocky Mountain Forest and Range Experiment Station's research work unit at Flagstaff in cooperation with Northern Arizona University.

[2] Seedlings were raised by Cornett as part of a Ph.D. research project at the University of Arizona, Tucson. However, this study was not a part of the dissertation.

water to which 0.75 milliliter of the surfactant Tween 80 was added. The mixture was then returned to cold storage. Spores were applied at a rate equivalent to 0.1 milligram per 0.09 square meter of exposed substrate when the containers were filled. This was done by mixing the appropriate amount of suspension in several liters of water, then pouring it over vermiculite. The treated vermiculite was then mixed well with the rest of the substrate and the containers were filled (3).

Seedlings were watered with Peters soluble fertilizer (15-30-15) applied as a foliar spray three times a week for 6 weeks and two times a week for 5 weeks. Fertilizer treatments were calculated to supply 30, 45, 60, and 75 parts per million (ppm) nitrogen. Peters' Stem (containing S, B, Cu, Fe, Mn, Mo, and Zn) was added in equal amounts to each of the four treatment solutions along with equal amounts of iron chelate (sodium ferric diethylenetriamine pentacetate, 10 percent by weight). Fertilization was begun 4 weeks after seed were sown.

Root to shoot ratios and mycorrhizal infection rates at the end of the greenhouse experiment are listed in table 1. After the greenhouse experiments (3), 240 of the seedlings were planted on the Fort Valley Experimental Forest, approximately 24 kilometers northwest of Flagstaff, AZ.

Site. The study site is on level terrain at an elevation of approximately 2,225 meters. Soils are silt loams derived from basalt parent material. Most of the mature ponderosa pine overstory had been removed in the 1960's. Tree cover now consists of pole-sized ponderosa pine originating in 1919. Ground cover is primarily Arizona fescue (*Festuca arizonica* Vasey) and mountain muhly (*Muhlenbergia montana* (Nutt.) Hitchc.).

In 1976, grass openings were sprayed with a mixture of dalapon (6.63 kilograms active ingredient (ai) per hectare) and atrazine 80-W (5.6 kilograms ai per hectare) before bareroot seedlings were planted. The planting was a failure because of poor quality nursery stock. In 1982, a small opening about 15 meters by 15 meters, still free of live grass, was selected for this study.

The study consists of four randomized blocks, each with 12 rows of five trees. Each row was randomly selected for planting with seedlings raised under one of the regimes (combination of nutrient and inoculation treatment) shown in table 2.

Trees were planted with planting bars at a spacing of 0.9 meter by 0.9 meter at the end of July 1982. There was a space of 0.9 meter between blocks. After planting, mesh plastic seedling protectors were placed around each tree and secured with two wire pins.

Measurements. Seedling survival was recorded periodically in 1982 and 1983 with the following notation: 1 = alive-growing, 2 = alive-not growing, and 3 = dead. Seedling heights were measured in the spring of 1983 before growth began and in the fall after growth ceased.

Table 1—*Root to shoot ratios and extent of mycorrhizal infection for container ponderosa pine seedlings raised in the greenhouse under various nitrogen supplementation regimes and inoculation treatments*[1]

Treatment	Root/shoot ratio (ovendry weight) (g/g)	Mycorrhizal infections (percent short roots infected)
N supplementation		
30 ppm	0.916 a	40 a
45 ppm	.926 a	26 b
60 ppm	.770 a	20 b
75 ppm	.758 b	22 b
Inoculation		
Control (none)	.725 a	14 a
Duff	.741 a	16 a
Pt	.707 a	16 a

[1]Means with the same letter in common are not significantly different ($P = 0.05$) as determined by analysis of variance. Differences in means determined by Student-Newman-Keuls multiple range test.

Table 2—*Effect of greenhouse N supplementation and inoculation on growth characteristics and survival of ponderosa pine outplanted in Arizona*

Greenhouse treatment[1]	Total height[2] (cm)	Height growth[2] (cm)	Survival[3] (%)
30 ppm N			
Control	15.5 bc	5.8 d	90 a
Duff	16.5 d	5.2 cd	95 a
Pt	12.9 a	3.3 b	95 a
45 ppm N			
Control	14.6 b	4.4 c	90 a
Duff	17.5 de	5.1 cd	100 a
Pt	15.8 cd	3.1 a	80 a
60 ppm N			
Control	19.2 e	6.1 d	85 a
Duff	15.0 b	3.2 ab	100 a
Pt	15.8 bcd	5.7 d	95 a
75 ppm N			
Control	15.4 bcd	6.0 d	85 a
Duff	18.0 e	5.2 cd	95 a
Pt	19.5 e	5.5 cd	90 a
Mean	16.3	4.9	92

[1]Pt = *Pisolithus tinctorius*.
[2]Treatments followed by the same letter are not significantly different (P = 0.10) as determined by analysis of variance (LSD = 0.05).
[3]Treatments with the same letter in common are not significantly different as determined by Chi square.

Table 3—*Field survival rates of ponderosa pine container seedlings averaged by greenhouse treatments*[1]

Treatment[2]	Survival (%)
N supplementation	
30 ppm	93 a
45 ppm	90 a
60 ppm	93 a
75 ppm	90 a
Inoculation	
Control	88 a
Pt	90 ab
Duff	98 b

[1]Treatment values with the same letter in common are not significantly different (P = 0.1) as determined by Chi square.
[2]Pt = *Pisolithus tinctorius*.

Because of interaction between nutrients and inoculation treatments, and heterogeneous variance among the treatment combinations, total height and height growth for the 12 treatment combinations were analyzed by randomized block analysis of variance, followed by heterogeneous variance LSD pairwise multiple comparisons when a significant overall F test of treatment differences was first observed (2). Survival was analyzed with Chi square techniques.

Results and Discussion

In spite of the fact that seedlings were enclosed by protectors, 28 percent of the trees were browsed by elk (*Cervus elaphus*), which knocked the protectors over. Nevertheless, overall survival (92 percent after one and a half growing seasons) was excellent (table 2). Individual treatment means did not differ significantly (table 2); however, results averaged across nutrient levels showed that seedlings inoculated with forest duff survived significantly better than noninoculated trees (table 3).

Even though significant treatment effects were observed for both total height and height growth (P = 0.05), the interaction of nutrient level and inoculation treatment clouds the analysis of individual effects of added nutrients and inoculation. Generally speaking, height growth was greatest under high N regimes (table 2), although noninoculated seedlings exhibit high growth rates for the lowest N level. Seedlings inoculated with Pt exhibited less growth for the two lowest N levels than for the two highest levels. Seedlings inoculated with duff grew equally at all but 60 ppm N. Patterns of total height response were similar except that total height of noninoculated seedlings was high only at 60 ppm N.

Root to shoot ratios of the seedlings used in this study were inversely related to N application rates but not to mycorrhizal fungi. A similar finding for ponderosa pine inoculated with Pt was reported by Trappe (16). However, results from this experiment tend to minimize the importance of root to shoot ratios as a factor in the establishment of ponderosa pine container seedlings in the Southwest. Seedlings raised under the lowest N regime had a root to shoot ratio, before planting, of 0.916 but did not survive better than seedlings raised under the highest regime, which had the lowest root to shoot ratio (0.758). A similar conclusion was reached by Racey et al. (12), who found top length, stem diameter, top volume, and root volume to be better indicators of nursery stock quality and potential growth performance than top to root ratios for red pine (*Pinus resinosa*) bareroot seedlings. Tinus and Ronco[3] found little correlation between root to shoot ratios of container seedlings and field survival in Arizona and New Mexico.

Root to shoot ratios and mycorrhizae may be more important in improving survival under conditions more adverse than those in this study. During the first year after these seedlings were planted, more than 635 millimeters of precipitation fell on the study site. Almost 102 millimeters of rain fell in the first month after planting. Normal precipitation for the year in this area is about 559 millimeters.

In the same area, Cornett (4) found that significantly more of the ponderosa pine container seedlings inoculated with *Suillus granulatus* survived than did controls during an abnormally dry year.

Although in this experiment there was not a strong effect on survival rates by inoculation treatments, there is an indication that survival is improved by inoculation with forest duff, presumably because of the presence of native mycorrhizal fungi. Growth of seedlings was not improved by inoculation treatments.

[3] Unpublished data on file, Rocky Mountain Forest and Range Experiment Station, Flagstaff, AZ 86001.

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