

# Influence of Mycorrhizal Inoculation on Ponderosa Pine Seedlings Outplanted on Wildfire Sites in Northeast Oregon and Washington

Florian Deisenhofer

*Silviculture Manager, Hancock Forest Management, Vancouver, WA*

## Abstract

Hot-planted, 4-month-old container ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson) seedlings were planted on five sites affected by wildfire in northeast Oregon and Washington. Seedlings were planted with or without mycorrhizal treatments. Survival exceeded 90 percent regardless of site or treatment after two growing seasons. Mycorrhizal inoculation at the nursery or in the field before outplanting did not improve seedling survival or growth. Only one test site, likely the most severely burned site, averaged better seedling growth with mycorrhizal inoculation compared with the noninoculated control treatment. Height and stem diameter growth differed among sites, likely due to differences in vegetation management strategies and subsequent competing vegetation levels. This paper was presented at the Joint annual meeting of the Western Forestry and Conservation Nursery Association and the Pacific Northwest Reforestation Council (Corvallis, OR, October 11–12, 2017).

## Introduction

In the hot and dry summer of 2015, numerous wildfires burned across the Pacific Northwest, affecting several thousand acres of forest land in eastern Washington and Oregon. On land managed by Hancock Forest Management, salvage logging activities started immediately after the wildfires, raising questions of how to best reforest thousands of acres of interior forest land dominated by ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson), western larch (*Larix occidentalis* Nutt.), and interior Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *glauca* [Beissn.] Franco). The scale of reforestation called for innovative ideas and approaches,

offering an opportunity to test both old and new methods during the multiyear effort.

Webster and Fredrickson (2005) provided valuable insights into wildfire reforestation and prioritizing planting units. The need to reforest burned areas quickly to take advantage of the brief period of reduced competing vegetation became one of the guiding principles. Inspired by a nursery visit that indicated that outplanting success can be obtained with small container ponderosa pine seedlings, we approached several nurseries with the idea to grow a 4-month-old seedling started in January for hot-planting in the spring of 2016—less than 10 months after the fires started. In discussions with these nurseries, it became obvious that doing so might be possible but would involve some risk, as no operational experience with the approach was available. In the end, one nursery was confident that they could produce a viable seedling, and two Hancock Forest Management regions ordered approximately 340,000 seedlings for spring 2016 planting.

A second question that we wanted to address was whether we should inoculate these seedlings with mycorrhizae. Reforestation sites typically have an adequate complement of mycorrhizal fungi that quickly colonize outplanted seedlings. Severe forest fires, however, may eliminate soil microorganisms, including mycorrhizal fungi (Landis and Dumroese 2006). Although we were unable to directly test for fire severity, wildfire reforestation sites were generally in areas of lower site productivity, especially in eastern Oregon. Landis and Dumroese (2006) recommend that plants destined for sites potentially lacking mycorrhizal inoculum should receive an appropriate fungal symbiont before outplanting.

For example, Steinfeld et al. (2003) reported 30 to 56 percent higher survival on two harsh, dry sites in southern Oregon for bareroot ponderosa pine seedlings inoculated with mycorrhizal fungi compared with noninoculated seedlings. We thus hypothesized that wildfire-affected soils of lower productivity would benefit from a mycorrhizal treatment.

Timing of inoculation is also an important consideration, as many mycorrhizal fungi may not survive in the high nutrient environment of a nursery (Landis and Dumroese 2006). Furthermore, mycorrhizal inoculation rates at nurseries and subsequent plant performance on the outplanting site are dependent on the type of disease management and fertilization regime used at the nursery (Meikle and Amaranthus 2008). Therefore, comparing nursery and field applications of mycorrhizal fungi to potentially improve survival on these generally harsher sites fit well with the overall experimental approach of hot-planting spring seedlings. Field inoculation may provide another means to mitigate a lack of effective inoculation at the nursery.

Thus, our hypotheses were:

1. A viable seedling could be grown in 4 months for hot-planting in the spring immediately following a wildfire on generally low productivity sites.
2. Mycorrhizal inoculation increases percent survival.
3. Field mycorrhizal inoculation improves survival relative to nursery inoculation.

## Methods

### Site Descriptions

Five sites were selected for this study: two sites in the Cornet-Windy Ridge fire south of Baker City, OR, two sites in the Carpenter Road fire northwest of Deer Park, WA, and one site in the Stickpin fire west of Colville, WA. Elevation ranges from 3,500 to 5,200 ft (1065 to 1585 m), and the estimated soil site productivity (50-year Douglas-fir site index) varies from 69 to 79 ft (21 to 24 m) (table 1).

Site preparation varied by region and site. The Oregon sites were treated with glyphosate and atrazine approximately 1 week before planting. In Washington, two of the sites were treated with atrazine, and one site received no chemical site preparation treatment (table 1).

### Seedlings

Three wild ponderosa pine seed lots were used specific to the geographic location of the test sites. Seedlings were grown at CalForest Nursery in Etna, CA. Seedlings were sown in mid-January 2016 in Styroblock™ containers (310B, 3.3 in<sup>3</sup> [54 ml] cavity volume; Beaver Plastics). Seedlings were lifted in the first week of May with calipers of 2–3 mm and 7–10 cm (3–4 inches) in height (figure 1). Only well-rooted seedlings, or “solid plugs,” were packed for planting in bundles of twenty and stored upright in rigid cardboard boxes. A refrigerated truck was used to transport the seedlings to a central location near the planting sites. Seedlings were planted within 7 days of being shipped.

**Table 1.** Characteristics, site preparation, and seed lots for each of the five sites used to evaluate mycorrhizal inoculation of hot-planted ponderosa pine seedlings.

State	Site name	Elevation (ft)	Soil SI (50)	Fire	Chemical site prep	Herbicides/Surfactants	Seed lot
Oregon	Alder 02	5,200	69	Cornet-Windy Ridge	Yes	Glyphosate, Atrazine, Grounded	Gremlin 853
Oregon	Marsh 02	4,900	72	Cornet-Windy Ridge	Yes	Glyphosate, Atrazine, Grounded	Gremlin 853
Washington	Fruit Top 02	3,800	79	Carpenter Road	Yes	Atrazine	Adams Lot 109
Washington	Spokane Adams 1 01	3,500	79	Carpenter Road	Yes	Atrazine	Adams Lot 109
Washington	Rabbit	4,300	72	Stickpin	No	none	Adams Mt Lot 57



**Figure 1.** Four-month-old seedlings at time of lifting (with and without growing medium). (Photo courtesy of CalForest Nursery, May 2016)

## Mycorrhizae Treatments

Two mycorrhizal products were obtained from Mycorrhizal Applications (Grants Pass, OR): (1) MycoApply<sup>®</sup> Ecto liquid blend, a mixture of seven ectomycorrhizal fungi with 100 billion spores per gallon and (2) MycoApply Soluble MAXX containing 19 endomycorrhizal and ectomycorrhizal fungi, two trichoderma species, and 12 bacterial species, as well as a blend of specially formulated amendments (minor amounts of N, P, and K: 1, 0.5, 1).

Four treatments were field tested—one nursery treatment, two field inoculation treatments, and a noninoculated control. MycoApply Ecto liquid blend was applied at the rate of 1 gal/100 gal (1 L/100 L) water to the seedlings through the nursery irrigation system in late February (approximately 6 weeks after sowing) for the nursery treatment. Several hundred seedlings were excluded from the treatment as control seedlings and for later field inoculation. Seedlings designated for the two field treatments were inoculated at the planting site with either MycoApply Ecto liquid blend (at the rate of 1 gal/100 gal [1 L/100 L] water) or MycoApply Soluble MAXX (at the rate of 8 oz/100 gal [62 ml/L water). The mycorrhizal products were mixed onsite according to the label. Initially, seedlings were dunked into the respective treatment bucket. As this treatment resulted in some loss of growing medium, the remaining field applications were made by leaving the seedlings in their plastic bags and applying the mycorrhizal solution through watering cans just before planting. Care was taken not to contaminate control seedlings with mycorrhizal products and cross-contaminate among mycorrhizal treatments.

## Study Design

Each study site consisted of 15 row plots of 10 seedlings planted at a 10 ft by 10 ft (3 m by 3 m) spacing (figure 2). The first 12 plots were randomly assigned to control or nursery and field treatments of MycoApply Ecto liquid blend (four rows of each treatment per site). The field treatment of MycoApply Soluble MAXX treatment was an add-on treatment after the initial layout had been completed and was applied to 3 rows of 10 seedlings at each site.

## Measurements and Analysis

Initial seedling height was measured right after planting and varied little among seedlings. Due to the homogeneous seedling crop and fragile stem, initial stem diameter was not measured. Seedling height, stem diameter, and survival were measured in October 2016 and September 2017. Seedling stem volumes were calculated assuming the shape of a cone:  $\text{volume} = \pi (\text{diameter}/2)^2(\text{height}/3)$ . During the 2016 fall measurement, one tree per treatment was systematically selected, carefully excavated, and placed on a board for visual comparison of root systems from different treatments. Colonization of roots by mycorrhizal fungi was not quantified.

Data were analyzed using analysis of variance, or ANOVA, with site and mycorrhizal treatment as the two factors in a completely randomized factorial design. Differences among sites and treatments for all response variables were determined at  $\alpha = 0.05$ .



**Figure 2.** Marsh 02 site at the time of planting. (Photo by Florian Deisenhofer, May 2016)

## Results and Discussion

### Survival

Seedlings on all five sites had excellent survival in both years (table 2), ranging from 97 to 99 percent in 2016 and 93 to 99 percent in 2017. Three sites had slightly lower survival in the second growing season than in the first, likely due to the particularly long, dry summer and fall of 2017. The Alder site had the highest overall 2-year mortality (9 percent) despite excellent vegetation control. This site is located on an exposed ridge with the lowest site productivity of the five test sites. The Rabbit site did not receive any site preparation treatments and still had more than 90 percent survival in both growing seasons.

Seedling survival did not differ among mycorrhizal treatments and the nontreated control or between nursery and field mycorrhizal applications. It is likely that these sites did not experience fire disturbance severe enough to significantly affect soil fungal communities and the natural inoculation processes (Certini 2005). For example, we observed morel mushrooms during planting on one of the sites.

Two-year data from operational plantings with the same seedlings indicate survival rates of 72 to 83 percent. Better quality control during seedling handling and planting or the preplant watering may have contributed to higher seedling survival inside the test plots compared with operational deployment. Survival of dormant, spring-planted ponderosa pine seed-

**Table 2.** Survival on each site after the first and second growing seasons.  
Note: Because some trees were destructively sampled after the first growing season, survival data for Year 2 are based on the remaining trees.

Site	Survival (%)	
	Year 1	Year 2
Alder 02	97	93
Marsh 02	98	99
Fruit Top	97	99
Spokane Adams 1 01	99	97
Rabbit	99	93
<b>Overall</b>	<b>98</b>	<b>96</b>

lings is typically expected to be 85 to 95 percent after 2 years based on operational experience.

### Growth

Growth responses differed significantly among sites (figure 3). Marsh is the only test site where all mycorrhizal treatments tended to perform better than the control, although this performance was nonsignificant. Although fire intensity was not assessed, the Marsh site likely had the highest burn intensity, which might explain the better performance of mycorrhizal treatments.

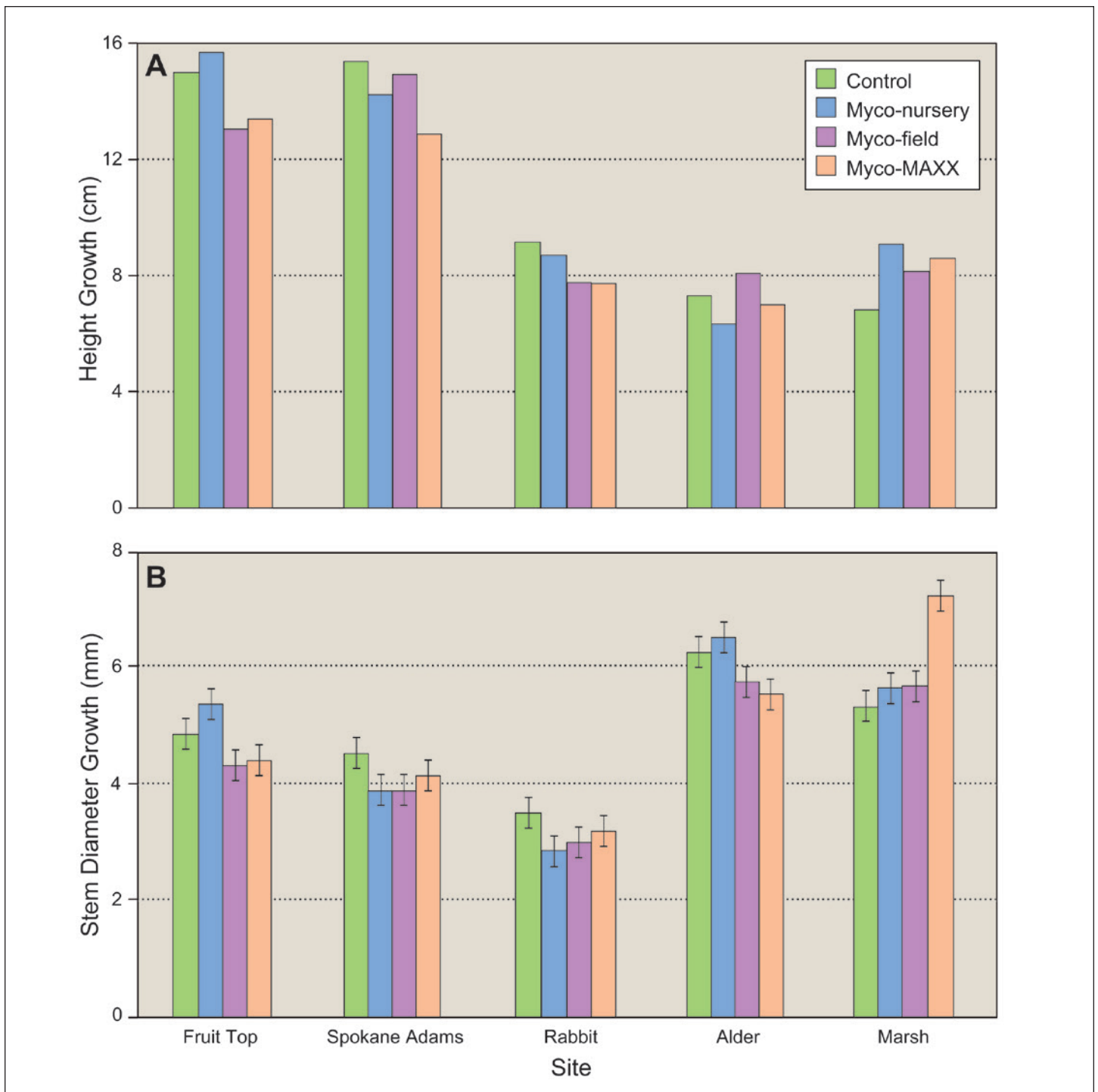
The two sites in Northeast Washington located within the Carpenter Road fire (Fruit Top and Spokane Adams) had the best height growth despite considerable competition (figure 4). Cole and Newton (1987) reported that height growth can increase for a short time under competitive stress. Conversely, stem diameter is reduced in response to competing vegetation and is therefore a good indicator of competitive stress in young trees (Wagner 2000). The two sites in Northeast Oregon (Alder and Marsh) had considerably larger stem diameters after two growing seasons, which could be a reflection of their lower competitive stress compared with the other sites.

Different seed sources (provenances) may also be responsible for the different growth patterns observed on the test sites. Cline and Reid (1982) studying the growth performance of ponderosa pine seed sources with mycorrhizal inoculation found a significant seed source effect on shoot height in a greenhouse environment. In their study, ponderosa pine seedlings exhibited overall low levels of infection in all mycorrhizal treatments and found no correlation between colonization and dry weight.

No significant differences occurred in stem volume among treatments after two growing seasons, although control seedlings tended to be as large as or larger than seedlings in the mycorrhizal treatments (data not shown).

### Root Development

The visual assessment of root systems from seedlings excavated at each test site after one growing season did not reveal any obvious or consistent treatment differences (figure 5). In general, seedling



**Figure 3.** (a) Two-year height and (b) stem diameter growth by treatment and test site.

root development across all sites and treatments was impressive. Grossnickle (2012) concluded that for container-grown seedlings, the amount of root development out of the plug and into the soil in relation to shoot mass best reflects drought avoidance and thus survival potential. The quick spring root development combined with little shoot growth appears to be critical to early seedling survival on

harsh and droughty sites like the Northeast Oregon sites. The active root growth immediately following planting may be the biggest benefit of hot-planted spring seedlings (figure 6). Alternatively, seedling survival may be more related to the longest or deepest root rather than other measures of the root system (Davis 2016). With future assessments, root



**Figure 4.** Fruit Top site during the second growing season (June) with a closeup of a seedling (see inset). (Photo by Florian Deisenhofer, June 2017)



**Figure 5.** Seedlings excavated from the Fruit Top site after one growing season (left to right: control, Myco-field, Myco-nursery, and Myco-Maxx). Seedlings have little height growth; mostly stem diameter and root growth. (Photo by Florian Deisenhofer, October 2016)

growth would be a valuable measure to include for determining possible correlations with survival.

## Conclusions

We will continue to explore hot planting 4-month-old container seedlings in the spring for reforestation sites in the Intermountain West. The short ordering timeline, the relatively low cost of the seedling, and the aggressive spring root growth make this approach an attractive reforestation tool. Additional testing in 2018 will be expanded to other tree species and repeated for ponderosa pine. The use of mycorrhizal inoculation at the nursery is cheap and may provide benefits on some sites. For postharvest or low-to-moderate postfire reforestation sites in the Intermountain West, however, mycorrhizae treatment does not appear to provide any measurable benefits.



**Figure 6.** Seedling root development 2 weeks (left) and 7 weeks (right) after planting on the Marsh 02 site. (Photo by Patrick Marolla, Hancock Silviculture Manager Northeast Oregon, June 2016)

### Address correspondence to—

Florian Deisenhofer, Hancock Forest Management, 17700 SE Mill Plain Blvd., Suite 180, Vancouver, WA 98683; email: FDeisenhofer@hnrg.com; phone: 360-635-5804.

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