# Improving Root Growth and Morphology of Containerized Oregon White Oak Seedlings

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## Abstract

We conducted four trials to determine if we could alter root morphology of containerized Oregon white oak seedlings in order to potentially improve their performance in restoration plantings. Early pruning of the radicle produced a branched taproot, though with fewer branches than reported for other oak species. Pruning the taproot at 15 cm (6 in) promoted greater taproot branching than radicle pruning but did not increase formation of lateral roots. Nontransplanted seedlings grown in tall containers (2.83 L; 0.75 gal) responded to air-pruning with increased lateral root growth and minimal circling of roots. Inoculation with soil containing ectomycorrhizal fungi substantially improved shoot- and root-growth response to fertilization.

## Introduction

The range of Oregon white oak (*Quercus garryana* Dougl. ex Hook.) extends from southern California to southern British Columbia. It is the only species of *Quercus* native to northern Oregon, Washington, and British Columbia. Oregon white oak occupies sites ranging from rock outcrops to riparian zones, although its extent has been reduced significantly during the past 150 yr by agriculture, urbanization, and lack of regular fire. In recent years, interest in restoring oak-associated plant communities and in landscaping with native species has resulted in the planting of Oregon white oak seedlings on a variety of private and public lands. Bareroot and container-grown Oregon white oak seedlings are produced by numerous commercial nurseries in California, at least 30 in Oregon, and several in Washington and British Columbia.

Many native Oregon white oak sites are relatively harsh, with shallow or coarse-textured soils, dense vegetative competition, and limited soil water availability during the dry summers characteristic of its range. Access to soil water on these sites is related to early growth of planted seedlings (Devine and others 2007); thus, development and morphology of seedling roots will likely influence performance. The extra costs associated with containerized seedlings (compared to bareroot seedlings) might be justified on these dry sites, as survival rates may be higher and the window of time for planting may be greater than for bareroot seedlings (Wilson and others 2007). Additionally, restorationists or landowners planting relatively few oak seedlings or planting at low density may be willing to accept the higher per-seedling cost for the benefits of container stock.

Container-grown Oregon white oak seedlings ideally have root systems with many fibrous lateral roots and a straight taproot that ends at the base of the container. The lateral roots increase the total root surface area, which, in turn, is related to greater potential for water and nutrient uptake, reduced planting stress, and improved post-planting survival and growth (Schultz and Thompson 1996, Grossnickle 2005). The taproot grows rapidly following germination in the fall. Given the droughty growing-season soil conditions in much of the species' range, this taproot may be an evolved strategy for acquiring water from deeper soils with relatively more available moisture in summer. In a standard pot, this taproot grows straight to the bottom and circles repeatedly, creating a 'pot-bound' seedling.

Oak seedlings planted with this root morphology perform poorly (McCreary 1996) and may never fully recover. To prevent this and other root deformities, techniques such as air-pruning or chemical pruning often are used for tree seedlings grown in containers. In several oak species, pruning the newly emerged radicle promotes branching of the taproot (Barden and Bowersox 1989, McCreary 1996, Tilki and Alptekin 2006). These branches grow down, with the appearance of multiple taproots of similar size and morphology. We refer to these branches as multiple taproots in this report. If we assume that lateral roots originate from each taproot at a similar frequency regardless of the number of taproots, a seedling with multiple taproots would have a greater root surface area than a seedling with a single taproot.

A wide variety of container types also has been developed to improve seedling root morphology (Owen and Stoven 2008). Various container designs and root treatments have been tested on other western oak species in California (McCreary 2001) and on oaks in eastern North America (Landis 1990). Inoculating container-grown seedlings with mycorrhizal fungi also may significantly increase growth and nutrient uptake (Dixon and others 1984, Mitchell and others 1984). The effects of root manipulation treatments on containerized Oregon white oak, however, have not been documented. In this study, we examined the effects of radicle and root pruning, air pruning, and mycorrhizal inoculation on development of root systems of containerized Oregon white oak seedlings.

## General Methods

Four trials (2003–2006) examined the root- and shoot-growth responses of Oregon white oak seedlings to various treatments. This section describes the general practices used; further details appear in the pertinent sections. Trials were conducted in a lath-walled building under a corrugated, translucent white roof in Olympia, WA. Sunlight was unobstructed except in early morning and late afternoon, when trees blocked direct sunlight. January and July temperatures in Olympia average 3 °C and 17 °C (38 °F and 63 °F), respectively.

**Sowing practices.** Acorns were collected from regional sources and refrigerated until sowing in October or November. Before sowing, acorns were placed in water for 24 h; those that floated were discarded. Sound acorns were sown horizontally at a 1–2 cm (0.4–0.8 in) depth in Tall One 2.83-L (0.75-gal) Treepots$^{TM}$, 10 cm (4.0 in) square at the top and 36 cm (14.2 in) deep (Stuewe & Sons, Inc., Corvallis, OR). Potting medium was a 2:1:1 volumetric ratio of mixed peat moss: coarse perlite: vermiculite. Seedlings were irrigated as needed. Water-soluble fertilizer (20 N-20 $P_2O_5$-20 $K_2O$) with micronutrients (The Scotts Company, Marysville, OH) was added at the manufacturer's recommended rate during the growing season.

**Statistical treatment.** Sample size ranged from approximately 30 to 100 seedlings per treatment, depending on the number of treatments. Randomized block designs were used; blocks were designated based on bench and distance from the lath wall. All data were analyzed with the Mixed Procedure in SAS (SAS Institute 2005). Results are reported with a minimum confidence level of 95 percent.

## Radicle Pruning

We tested the effect of radicle pruning on Oregon white oak acorns that we germinated in November 2003 in a tray of moist vermiculite at 20 °C (68 °F). After the radicle had emerged from each acorn and reached a length of at least 2.5 cm (1 in), the acorn was randomly assigned to the pruned or the unpruned treatment. In the pruned treatment, the radicle was clipped 1 cm (0.4 in) from the acorn; the unpruned treatment was an undisturbed control. Both treatments were then sown in pots and grown until November 2004.

After 1 yr, 47 percent of the seedlings in the pruned treatment had multiple taproots, compared to 3 percent of the seedlings in the unpruned treatment. The number of taproots averaged 1.8 ± 1.0 (standard error) in the pruned treatment and 1.1 ± 0.5 in the unpruned treatment. The multiple taproots originated where the radicle had been clipped. The average combined length of all taproots per seedling was significantly greater in the pruned treatment (92 cm; 36 in) than in the unpruned (63 cm; 25 in).

Seedlings in the pruned treatment formed somewhat fewer taproots than other oak species, which averaged 3 to 4 taproots in response to similar radicle-pruning treatments (Barden and Bowersox 1989, McCreary 1996). Pruning the radicle had no significant effect on seedling shoot weight, root weight, stem diameter, or stem height after 1 yr. Similarly, shoot growth of other oak species has shown little response to radicle pruning (Bonner 1982, Barden and Bowersox 1989, McCreary 1996). On harsh sites where early root-soil contact influences survival, however, the potentially greater root surface area resulting from multiple taproots may increase establishment success.

## Root Pruning

Undercutting oak seedlings in the nursery bed at a 15- to 20-cm (6- to 8-in) soil depth increases frequency of lateral roots, which is positively related to post-planting survival and growth (Schultz and Thompson 1996). We examined the effects of severing the taproot of containerized Oregon white oak seedlings to determine how root morphology was affected. We tested three treatments on 4-mo-old oak seedlings in March 2003: (1) seedling removed from potting medium, taproot severed at a 15-cm (5.9-in) depth, and seedling repotted, (2) seedling removed from potting medium and repotted, and (3) seedling undisturbed. Comparison of treatments 1 and 3 indicated the effect of taproot pruning and repotting, while comparison of treatments 2 and 3 isolated effects due to repotting alone.

Pruning taproots of 4-mo-old seedlings resulted in an average of 2.8 ± 1.2 taproots at a depth of 30 cm (12 in), compared with 1.1 ± 0.3 taproots in the undisturbed treatment, and 1.0 ± 0.3 taproots in the repotted treatment. In all pruned seedlings, the multiple taproots originated from the point at which the original taproot was cut. Average diameter of taproots at a 30-cm depth (pot bottom) varied with the number of taproots (figure 1), indicating that individual taproots were smaller as the number of taproots increased. Pruning the taproot had no effect on seedling total dry weight, shoot dry weight, shoot: root ratio, stem diameter, or stem height after 1 year. Pruning the taproot also did not affect the dry weight of lateral roots above or below the 15-cm depth. While the method of root pruning used in this trial would not be practical on a large scale, our results suggest that changes in morphology, but not dry weight, of Oregon white oak roots may result from treatments that sever the taproot, such as undercutting or pruning at the time of transplanting.

## Air-Pruning

When seedling taproots are pot-bound, the pot-bound portion is typically pruned at planting. This results in the loss of both a substantial portion of taproot biomass and associated starch reserves and the lateral roots that originate from the pruned section. To prevent this taproot deformity, we began using air-pruning (Landis 1990) on our Oregon white oak seedlings and initiated a trial to document its effects on the species. We compared two container types: a Tall One Treepot™ and a Tall One Treepot™ with the base removed. In the air-pruned treatment (i.e., the containers without bases), containers were placed on galvanized hardware cloth [0.25-in (0.6-cm openings] with a layer of newspaper between the pot and the hardware cloth to retain the medium (McCreary 2001). The hardware cloth was suspended on a wood frame 2.5 cm (1.0 in) above the surface of the bench to create an air gap.

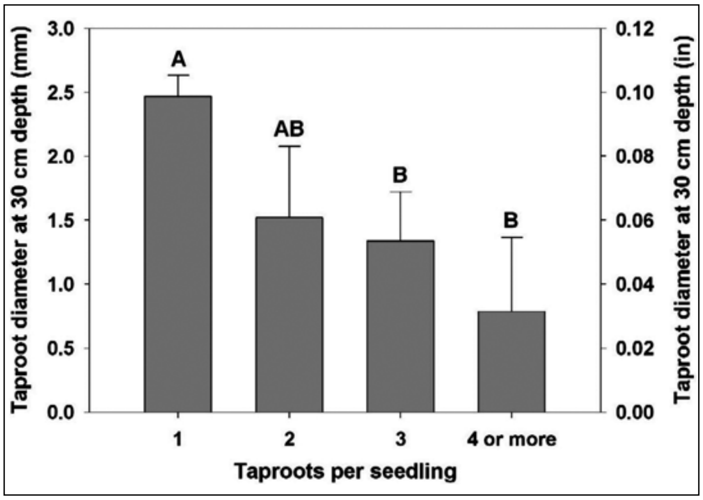

**Figure 1.** Average taproot diameter at a 30-cm (12-in) soil depth for Oregon white oak seedlings with various numbers of taproots. Bars with the same letter do not differ at the 95-percent confidence level.

After the first and second years of growth, seedling height and stem diameter were similar for seedlings in the intact pot and the air-pruned treatment. After the first year, total seedling dry weight and taproot weight did not differ significantly between treatments. Dry weight of lateral roots was 62 percent greater for air-pruned seedlings, however, indicating that air-pruning increased growth of these roots (figure 2). This lateral root growth did not come at the expense of taproot growth, as taproot weight did not differ between treatments. The average total weight of roots circling the pot base was greater in the intact pot (0.29 g; 0.0102 oz) than in the air-pruned treatment (0.01 g; 0.0003 oz) after 1 yr. After 2 yr, there was a visible concentration of lateral roots in the lower portion of the containers in both treatments (figure 3); however, these roots typically circled the pot base in the intact-pot treatment but were more likely to either terminate or grow horizontally for only a short distance in the air-pruned treatment.

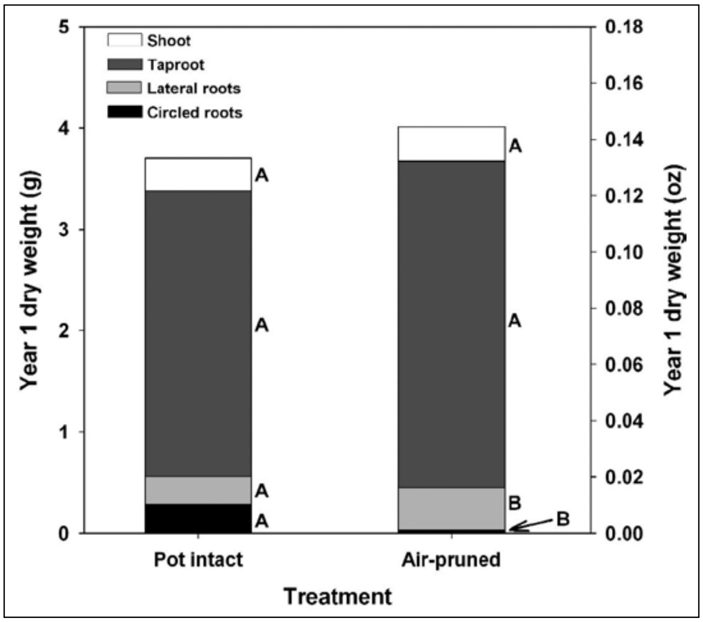

**Figure 2.** Dry weight after 1 yr, by component, of Oregon white oak seedlings grown in pots with the base intact or in pots with the base removed to promote air-pruning. Bars with the same letter and representing the same component do not differ at the 95-percent confidence level.

**Figure 3.** Two-yr-old Oregon white oak seedlings grown in intact pots (two seedlings on left) and in pots with the base removed to promote air-pruning (two seedlings on right). Note the difference in lateral root development. Photos by Diana Livada.

In an ongoing study in which Oregon white oak seedlings were transplanted from air-pruning D16 Deepot™ cells (Stuewe & Sons, Inc.) to Tall One Treepots™, 1-yr-old air-pruned transplants produced greater aboveground biomass growth and a more fibrous root system below the point of air-pruning (personal communication, Timothy Harrington, Forest Service, Olympia, WA). Similarly, in nursery stock of blue oak (*Quercus douglasii* Hook & Arn.) in California, seedlings started as an air-pruned plug had greater shoot height, root weight, and root system fibrosity at the time of planting than did 1+0 container seedlings or 1+0 bareroot stock (McCreary and Lippitt 2000).

## Inoculation With Mycorrhizal Fungi

In December 2004, we started a 2-yr trial to determine whether growth of containerized Oregon white oak seedlings in fertilized potting medium was limited by a lack of mycorrhizal fungi. We used a factorial combination of inoculation (yes/no; abbreviated +IN/-IN) and fertilization (yes/no; abbreviated +FER/-FER).

The inoculum consisted of mineral soil taken from a local stand of Oregon white oak trees. The largest trees in the stand were approximately 80 yr old, and the stand had likely been prairie or savanna before their establishment. The soil was a gravelly sandy loam of the Spanaway series (Soil Survey Staff 2008). We used soil from a 5- to 15-cm (2- to 6-in) depth because density of fine oak roots was high in this interval. Gravel was screened out, and soil (including fine root fragments) was homogenized before inoculation. For the +IN treatment, we removed potting medium to a depth of approximately 6 cm (2 in) from the center of the filled pot and added 120 $cm^3$ (7.3 $in^3$) of the inoculum soil. We then added a 2-cm (0.8 in) layer of potting medium above the inoculum soil and planted the acorn in this medium, at a depth of 1 cm (0.4 in). Acorns in the -IN treatment were planted similarly but with no inoculum soil. This inoculation method was based on the assumption that seedlings would begin to form lateral roots (i.e., roots that are potentially mycorrhizal) while the inoculum was still viable; an alternative approach is to inoculate after lateral roots are present.

The +FER treatment received our standard NPK fertilizer; the -FER treatment received none. All treatments were irrigated equally. After 1 yr of growth, 50 percent of the seedlings in each treatment combination were randomly selected for destructive sampling.

After the first and second years of growth, we compared the -IN-FER and the +IN-FER treatments to determine whether the inoculum affected seedling growth in the absence of fertilization, possibly due to the nutrient content or water-holding capacity of the inoculum soil. There were no significant effects on seedling height, stem diameter, or first-year dry weight between the treatments (figure 4); thus, there was no evidence that the physical or chemical properties of the inoculum soil influenced seedling growth. In all analyses of seedling height and diameter (yr 1 and 2), seedling shoot and root components, and total dry weight, the interaction term for the inoculation and fertilization treatments was significant ($P<0.05$). This interaction can be summarized by three trends: (1) inoculation alone did not produce a growth response; (2) fertilization alone produced a negligible to intermediate growth response; (3) inoculation plus fertilization produced a growth response significantly greater than the other treatments. These growth results suggest that the soil inoculum facilitated the fertilization response. We infer that this was due to an absence of mycorrhizal fungi among seedlings that were grown in potting medium without inoculum soil. Conversely, we would expect a reduced or absent inoculation effect on growth of containerized seedlings planted in medium that had already been “contaminated” by native soil.

Following the second growing season, we identified the mycorrhizas present on the roots of four seedlings from each treatment combination, using a microscope and DNA analysis (see Frank and others 2006). On all of the seedlings examined in the +IN treatment group, we identified the presence of ectomycorrhizas associated with *Cenococcum geophilum*, a common and widespread

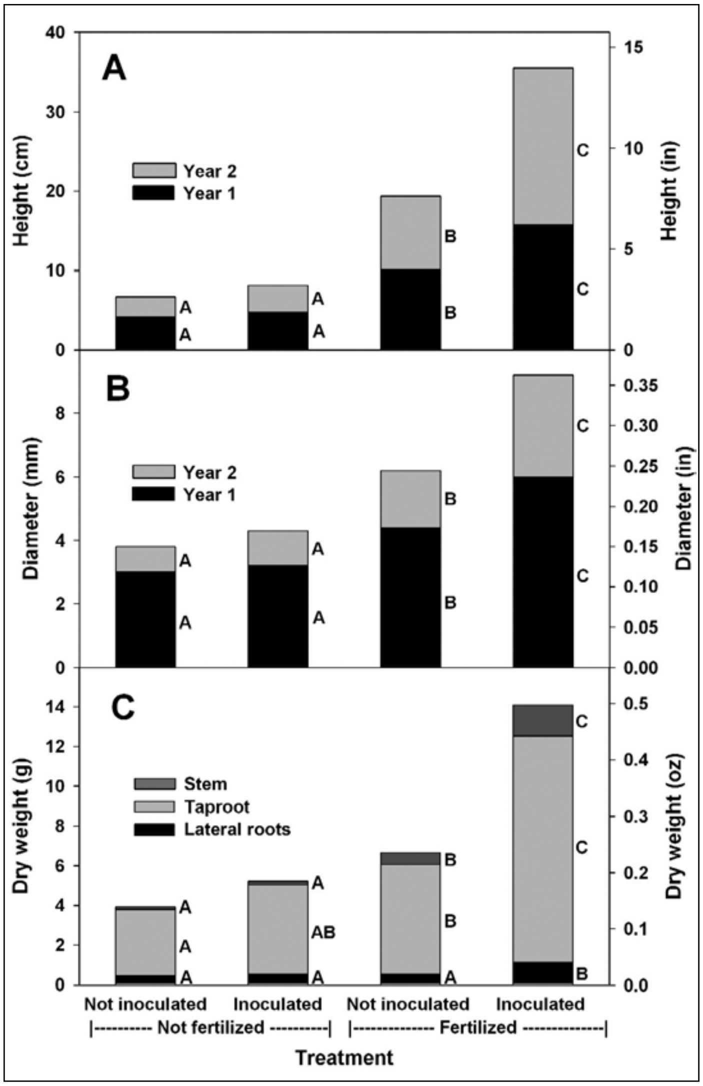

**Figure 4.** Two-year height (A) and stem diameter (B) responses and 1-yr dry weight growth response (C) for Oregon white oak seedlings treated or not treated with fertilization, mycorrhizal inoculation, or both. Bars with the same letter and representing the same year or seedling component do not differ at the 95-percent confidence level.

fungal species previously reported on Oregon white oak in southern Oregon (Valentine and others 2002, 2004). On all of the -FER seedlings, but not on the +FER seedlings, we observed the ectomycorrhizal *Hebeloma* sp. that has been reported previously in association with *Quercus* (Cairney and Chambers 1999). Only one of the four seedlings in the -IN+FER treatment was infected (ectomycorrhizal *Tomentella* sp.).

Mycorrhizal inoculation has been used in production of containerized seedlings for decades, but the mycorrhizal communities associated with Oregon white oak have only been studied in recent years (Valentine and others 2004, Moser and others 2005, Southworth and others 2009). Although research on inoculation with individual species of mycorrhizal fungi would be necessary to better understand their potential influences on Oregon white oak seedlings, we can report that *Cenococcum geophilum* was associated with seedlings that had a significantly improved response to fertilization, compared with seedlings that were not infected with this species. Although soil was used as inoculum in this trial, once the species of mycorrhizal fungi that are most beneficial for containerized Oregon white oak are identified, seedling inoculation might be achieved more efficiently using commercially available isolates, collected spores, or fungal cultures (Castellano and Molina 1989).

## Implications

On the basis of information from other oak species, we assumed that the optimal root morphology for Oregon white oak seedlings is a combination of fibrous lateral roots and one or more straight taproots. Our trials suggest that air-pruning and ectomycorrhizal inoculation (if seedlings are grown in fertilized potting medium) are the most promising methods of improving growth and root morphology of containerized Oregon white oak seedlings. Our nontransplanted seedlings grown in tall (36 cm; 14 in) containers responded to air-pruning with increased lateral root growth and minimal circling of roots. Pruning new radicles or the taproot increased taproot branching but did not increase growth of lateral roots.

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## Acknowledgments

We thank the current and former members of the Silviculture and Forest Models team who assisted with sampling, particularly Diana Livada, Bridget Korman, James Dollins, and Nathan Lubliner. We thank Jonathan Frank for assistance in identification of mycorrhizal fungi. We thank Nabil Khadduri, Timothy Harrington, and Barry Weber for manuscript reviews.

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