

Growth and gas exchange of different ponderosa pine stock types on dry sites

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Abstract

Successful seedling establishment following outplanting relies heavily on a seedling's ability to access soil moisture. In the Inland Northwest USA, the summer season brings drought conditions causing low soil moisture in the upper soil profile—a potentially large barrier to seedling establishment. During the 2007 growing season, three studies examined the survival, growth, and gas exchange attributes of several ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) stock types on two forest sites and on a drought stress experimental plot. These studies examined the advantages of seedling container type and their relationship to overcoming site limiting factors, such as soil moisture. The studies were unique in that they used seedlings cultured specifically to their container types to achieve uniformity in nutrition and water use efficiency, something previous studies failed to address.

The first study evaluated the performance of six different stock types on two sites (mesic and xeric) that varied in volumetric soil moisture content (θ), average temperature, and total precipitation. After two growing seasons, seedlings planted at the mesic site showed high survival (> 99%) and large growth gains; container types exhibited differences in total height, root-collar diameter (RCD), and stem volume with larger containers generally yielding the largest seedlings. Seedlings planted at the xeric site experienced 83% survival and had small growth gains; container types also exhibited differences in height, RCD, and stem volume but the largest containers did not consistently yield the largest seedlings.

In the second study, seedlings from three stock types were planted in a field and subjected to three levels of imposed seasonal drought using winter wheat (*Triticum aestivum* L. em.) as a model soil moisture competitor. Winter wheat was sown in three densities (0, 150, and 300 plants m⁻²; D₀, D₁₅₀, and D₃₀₀, respectively) and was successful at reducing soil moisture 17 cm below the soil surface. High rates of net photosynthesis (A) indicated that seedlings with adequate soil moisture and without vegetative competition were established within three weeks. Conversely, low A , low soil moisture, and low pre-dawn water potential (Ψ_{npw}) measurements indicated that seedlings planted with vegetative competition were moisture stressed ($\Psi_{\text{npw}} < -2.1$ MPa) and not established. As a consequence, mortality rates were severe in both D₁₅₀ and D₃₀₀ with smaller containers reaching 100% mortality, while the largest container experienced 63-75% mortality; seedlings in D₀ suffered no mortality.

Using three stock types, the third study examined temporal and spatial variability in soil moisture and the resultant effects on growth and gas exchange. Soil moisture reached minimum volumetric soil moisture contents (θ) of $0.08 \text{ m}^3 \text{ m}^{-3}$ at a 15 cm depth and $0.15 \text{ m}^3 \text{ m}^{-3}$ at 90 cm by late September. Predicted soil water potential (Ψ_{soil}) reached minima of -1.55 and -0.27 MPa for 15 and 90 cm depths respectively. Seedling net photosynthetic assimilation (A) and transpiration (E) rates followed soil moisture trends, also reaching seasonal lows in September. In early October, gas exchange rates nearly doubled following a replenishment of upper-profile soil moisture by precipitation. Stock types did not differ in gas exchange rates ($P \geq 0.15$), biomass ($P \geq 0.45$), or root penetration depth ($P = 0.60$). Regardless of container size, data suggests upper-profile soil moisture is critical to seedling establishment and growth, and can be drastically compromised by competing vegetation and subsequent rapid changes in soil moisture availability. Essential to successful seedling establishment is to coincide planting with sufficient moisture in the upper soil profile, incorporate practices that enhance soil moisture such as vegetation management, and plant seedlings with sufficient root length to allow root systems ample time to access moisture. These studies enhance our understanding of the tolerance of ponderosa pine seedlings to drought under various reforestation scenarios, thereby providing guidance in the best management practices to improve outplanting performance.

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Dedication

I dedicate this work to my father, Raymond Pinto. Thanks for introducing me to the wonders of the natural world through indigenous and scientific eyes. This work is also dedicated to my mother, Margaret Pinto-Salcido. Thanks for recognizing the fire in my eyes and giving me the support and prayers to keep it alive. This work is also dedicated to my sister Renaye Pinto-Charlet. Thanks for taking care of, and paving the way, for your little brother.

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Chapter 1. Establishment and growth of container seedlings for reforestation: a function of stock type and edaphic conditions

Abstract

Six container types were used to evaluate the performance of ponderosa pine (*Pinus ponderosa* Laws. var. *ponderosa*) seedlings outplanted on two sites that varied in volumetric soil moisture content (θ), average temperature, and total precipitation (mesic and xeric). Seedlings in each container type were cultured specifically to achieve uniformity in nutrition and water use efficiency. After two growing seasons, seedlings planted at the mesic site showed high survival (> 99%) and large growth gains; container types exhibited differences in total height, root-collar diameter (RCD), and stem volume with larger containers generally yielding the largest seedlings. Seedlings planted at the xeric site experienced 83% survival and had small growth gains; container types on this site also exhibited differences in height, RCD, and stem volume, but the largest containers did not consistently yield the largest seedlings. Simple regression analysis revealed that for each site, initial seedling morphological characteristics were better at predicting absolute growth after the first year than after the second year, with initial seedling height offering the best predictive power ($R^2 = 0.66$ on the mesic site, and $R^2 = 0.70$ for the xeric site). Second-year absolute growth prediction was poorest on the mesic site. Regression analysis indicates that initial seedling characteristics lose predictive value with time, especially on a mesic site, as seedlings grow out of their initial, container-induced characteristics and become more limited by current environmental and genetic factors. Conversely, on a xeric site, where absolute growth is reduced, traits determined by the container type persist longer.

Introduction

Reforestation using nursery-produced seedlings is often the most reliable way to ensure successful establishment and rapid growth following outplanting. Plantation establishment success often hinges on decisions and considerations made prior to planting, such as seedling stock type, seedling morphology, genetics, site limiting factors, site preparation, the outplanting window, and planting technique (Scagel et al. 1998). The best seedling for a particular site may differ depending on how these decisions and considerations

are made. To simplify this complex situation, the Target Plant Concept was proposed. This concept provides a means of overcoming the critical variables of forest establishment by focusing on morphological and physiological seedling characteristics that are linked to outplanting success (Rose et al. 1990; Landis and Dumroese 2006).

Over the years, the number of available seedling stock types has increased dramatically, especially for container seedlings. It is well known that varying container size, or density in the tray, modifies seedling phenotype; this occurs even among seedlings of the same seed source grown the same year (Scarratt 1972; Landis et al. 1990; Scagel et al. 1998). The premise behind the Target Plant Concept is that it identifies phenotypic traits that increase outplanting survival and growth under a particular set of site conditions (Rose et al. 1990). Larger phenotypes are often obtained by growing seedlings in larger containers than normal. Whether these larger containers are deeper or wider, they require more medium, more fertilizer, and more growing space than normal, which increases production cost (Bowden 1993). Reforestation projects are often limited by budget constraints, so the cost of a larger, more specialized, seedling may be justified only on sites where they yield a clear benefit (Bowden 1993; Wenny 1995; Scagel et al. 1998).

The Target Plant Concept depends on empirical knowledge of which container types are best for certain site conditions. Studies have examined this question in terms of survival and growth in drought situations (Amidon et al. 1982; Bainbridge 1994; Rose et al. 1997), with vegetative competition (Newton et al. 1993; Hunt 2002), for cost effectiveness (Miller and Schaefer 1985), and by other criteria such as seedling density and supplemental fertilization (Arnott 1975; Sutherland and Newsome 1988; Simpson 1991; Bowden 1993; Paterson 1997; South et al. 2005). These studies suggest that seedlings with larger height and root-collar diameter (RCD) outperform smaller seedlings after outplanting. Likewise, Rose et al. (1997) found that stock types with larger root volumes may have a survival and/or growth advantage after outplanting. But the question remains: how tall, what diameter, how much root volume is needed for a particular species at a particular location?

An abundance of tests have been developed to measure seedling quality and performance potential. Many grading criteria have been proposed and refined to characterize seedling quality and predict performance of outplanted seedlings (Burdett 1987; Örlander and Rosvall-Åhnebrink 1987; Burr et al. 1990; Binder et al. 1996; Templeton and Colombo

1995). These tests are categorized into two areas: morphological and physiological attributes. Although many studies show good correlation between test results and field performance, the tests are often time-consuming and expensive. Consequently, nurseries tend to use morphological attributes as indicators of seedling quality; especially common are height and RCD. While some studies have shown that initial height corresponds well to subsequent growth for conifer and hardwood species (Jacobs et al. 2005; Mullin and Christl 1981 a, b), some are inconsistent (Chavasse 1977). RCD has been correlated to root growth potential, survival, and height growth (Jacobs et al. 2005; South et al. 2005).

With studies available on seedling stock type performance, performance potential, and grading criteria, it is plausible that the best tools for container selection for a specific set of site conditions already exist. Linking these variables together, however, in an all encompassing non-confounded study remains difficult. One primary limitation is the conditions under which the seedlings are propagated. It is well known by container tree seedling growers that seedlings grown in different containers and from varying seed sources have different irrigation and nutritional requirements. By not accounting for these differences in growth pattern, previous studies have made it difficult to make unbiased selections of stock type. In other words, one culturing regime may favor one container type; another culture regime may favor another. In an attempt to reduce this bias, Pinto (2005) showed that fertilizing and irrigating seedlings according to their specific container type—versus multiple container types fertilized and irrigated under a single regime—offered more uniformity in foliar nitrogen concentration and water-use efficiency. This step is necessary for reducing bias and unexplained variation in container comparison studies.

The objective of this study was to employ the culturing technique of Pinto (2005) to (i) quantify mesic and xeric site conditions in northern mountains of the Intermountain region, (ii) compare growth of the different stock types on these sites, and (iii) use initial seedling morphology (height, root-collar diameter, and stem volume) and container size to predict seedling size after one and two growing seasons.

Materials and Methods

Seedling Preparation

In summer 2006, ponderosa pine (*Pinus ponderosa* Laws. var. *ponderosa*) seedlings were grown at the USDA Forest Service, Rocky Mountain Research Station in Moscow, Idaho (46. 7232 °N, 117.0029 °W; 798 m elevation). Seeds were obtained from the Confederated Tribes of the Colville Indian Reservation (Colville Tribal Forestry, Lower Stepstone: stand #1, 610 m elevation) and Potlatch Corporation (Potlatch Corporation, Lot ID#: 92-02, 915 m elevation). Six different Styrofoam[®] container types, differing primarily in cavity depth and volume (Table 1.1), were used to grow seedlings from March until December, when they were harvested and placed into freezer storage (-2 °C). Seedlings were cultured by container type to achieve uniform physiological characteristics, such as foliar nitrogen, water-use efficiency, and cold hardiness (Pinto 2005).

Site Description: Faeber II Planting Unit

Seedlings were outplanted 17 April 2007 on the Potlatch Corporation operational planting unit Faeber II (F2) approximately 18 km east of Orofino, Idaho, (46.4504 °N, 116.0408 °W; 980 m). The clear-cut site was prepared by concentrating leftover slash and burning the previous fall and broadcast applying Atrazine herbicide (Syngenta, Wilmington, DE) in the spring. For each container type, 100 seedlings were hand-planted using a hoedad into 5 replications in a randomized complete block design. Within each replication, 20 seedlings of one container type were planted in a row at 1.5 m spacing, while rows were 3 m apart. Because of browse damage on nearby reforestation sites, yellow mesh Vexar[®] tubes (0.13 m diameter. × 0.76 m height) were installed on each seedling for protection; tubes were removed in June 2008. F2 soils belong to the Grangemont series of Alfisols; they are deep, well-drained, fine-silty soils with a thin mantle of volcanic ash (NRCS 2009).

Site Description: Coyote Creek Planting Unit

The Coyote Creek planting site (CC) is located approximately 13 km northwest of Nespelem, WA (48.2376 °N, 119.1272 °W; 890 m) on the Confederated Tribes of the Colville Indian Reservation. Seedlings were hand-planted using hoedads on 1 May 2007 under the same study design as the F2 planting unit. The open-range, partial-cut site was

prepared by concentrating leftover slash prior to planting. Slash piles were burned in early November of 2007. CC soils belong to the Bearspring series of Mollisols; they are deep, well-drained, loamy-skeletal soils formed in colluvium and residuum from granitic rock and capped with a thin mantle of volcanic ash (NRCS 2009).

Edaphic and Atmospheric Monitoring and Sampling

Soil moisture, air temperature, and precipitation were measured on each site. For soil moisture, three replications of volumetric moisture content (θ)—defined as the ratio of the volume of water in the soil to the total volume of soil ($\text{m}^3 \text{m}^{-3}$)—were measured 15 cm below the soil surface. Hourly measurements were collected using ECH₂O probes and Em50 data loggers (Decagon Devices, Inc., Pullman, WA). A weather station (model 900ET, Spectrum Technologies, Inc., Plainfield, Illinois) was installed in the center of each site to collect hourly temperature and precipitation measurements 1 m above ground.

Soil was sampled where soil moisture probes were installed in order to correct and improve *in situ* soil moisture data (Starr and Paltineanu 2002; Cobos 2007). Raw sensor data from laboratory measurements were plotted against measured θ to generate soil-specific calibration equations. Raw data collected from the field were then inserted into each respective calibration equation for final, corrected θ values. The accuracy for each probe type with calibration is $\pm 0.02 \text{ m}^3 \text{m}^{-3}$ (Decagon Devices, Inc., Pullman, WA).

At both units, initial seedling height (vertical distance from ground-line to terminal leader tip) and root-collar diameter (RCD; diameter of main stem approximately 1 cm above ground-line) were measured one to two weeks after outplanting (24 April 2007 for F2; 16 May 2007 for CC); first season height, RCD, and survival were measured 14 and 24 October 2007 (CC and F2, respectively). The same variables were reassessed 14 and 15 October 2008 (F2 and CC, respectively). Fall measurements for 2007 and 2008 are defined as first- and second-year absolute growth, respectively. Height and RCD measurements were used to calculate seedling stem volume, which was approximated using the formula for an elliptical cone:

$$V = \frac{\pi d^2 h}{6}$$

where d is the stem diameter at ground level (cm), and h is shoot height (cm). Incremental growth was calculated for each season as the difference of spring and fall morphological measurements.

Statistical Analysis

Data were analyzed for each site separately using SAS (v 9.1.3, SAS Institute, Cary, NC, USA). The analysis of variance (PROC GLM) was performed for a randomized complete block design to identify absolute and incremental growth differences among containers for seedling height, RCD, and stem volume. Separate analyses were done for post planting (spring 2007), first growing season (fall 2007), and second growing season (fall 2008). To correct for experiment-wise error rates, multiple comparisons among container types were calculated using Tukey's mean separation test. Single and multiple linear regression analyses were performed on pooled container data by site for seedling height, RCD, and stem volume. Initial analysis plotted spring morphological measurements against their corresponding first and second season measurements (regression model: $y = \beta_0 + \beta_1x + \varepsilon$). Additional regressions looked at how well other variables might work as predictors, e.g. how well RCD might predict height or volume, etc. Once the variables were deemed significant, a full model was developed with height, RCD, and stem volume in an effort to improve model prediction (regression model: $y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_1x_2 + \beta_5x_1x_3 + \beta_6x_2x_3 + \varepsilon$). The Akaike Information Criterion (AIC; Akaike 1974) model selection procedure was then used to select the final model (Burnham and Anderson 2002); the use of AIC best explained the data with a minimum of free parameters and did so without overfitting. When assumptions for equal variances and normality were not met, data were transformed to meet the required assumptions for analysis (noted in the tables and figures where applicable). All results were tested at a significance level of $\alpha = 0.05$.

Results

Faeber II: Survival, Growth, and Site Conditions

The Faeber II site provided mesic growing conditions that were conducive to high survival and absolute growth. Seedling survival exceeded 99% and growth was rapid after two growing seasons. First growing season, height, RCD, and stem volume increased (65,

118, and 702%, respectively) across all container types; second season increases were similar (98, 108, and 781%, respectively). Container type significantly affected absolute height and RCD after the first and second growing seasons ($P \leq 0.0002$; Figure 1.1, A and B). In general, larger container volumes yielded taller seedlings after two growing seasons. However, differences in RCD were less distinct. The largest and smallest mean RCDs tracked the largest and smallest containers throughout the two seasons, while the remaining container types had similar means. Accordingly, because stem volumes were calculated using height and RCDs, mean differences among container types (significant at $P \leq 0.0001$) tracked those of both height and RCD but show larger amplification in the difference between the smallest and largest volume containers; this is especially evident at the end of the second growing season (Figure 1.1, C).

On average, second season incremental growth increased 147, 100, and 794% for height, RCD, and stem volume, respectively (Figure 1.1, D-F). Although mean incremental height growth differences were observed among container types after the first growing season ($P < 0.0001$), the differences were no longer apparent after year two ($P = 0.14$). Conversely, mean incremental RCD growth showed no differences after one season ($P = 0.42$), while a second season of growth yielded differences between the largest and smallest container types ($P = 0.002$). Mean incremental stem volume growth was different among container types for each growing season ($P < 0.0001$) and generally increased with container size.

Temperature, precipitation, and soil moisture data were collected from mid-April through late October 2007. Total growing season precipitation measured 237 mm, and with the exception of a large rainfall event occurring in late July (18 mm), most of the precipitation fell before July and after September (Figure 1.2, A). Volumetric soil moisture content (θ) was highest in early June and lowest in late September. Mean daily temperatures averaged 14.5 °C from mid April to mid October with a low of -3.6 °C (4 April 2007) and a high of 37.9 °C (5 July 2007; Figure 1.2, B).

Coyote Creek: Survival, Growth, and Site Conditions

At CC, survival was lower and growth was slower than at Faerber II. Survival averaged 85% after the first growing season and 83% after the second, with no significant

differences or trends among container types for either year ($P = 0.75$ and $P = 0.94$, respectively). Absolute height, RCD, and stem volume increased 46, 36, and 182% respectively after the first growing season; second-season totals for the same variables were similar, at 40, 39, and 210%, respectively (Figure 1.3, A-C). Container type significantly affected height, RCD, and stem volume for each measurement period ($P \leq 0.0001$). Height generally increased with container volume at the end of the first growing season, but by the end of the growing season, most container types yielded seedlings with similar height, except for the largest and smallest container volumes (Figure 1.3, A). Seedlings grown at the low density in the greenhouse (80, 105, 166 containers; see Table 1.1) tended to have larger RCDs throughout the measurement period (Figure 1.3, E). Stem volume was significantly affected by container type for each measurement period and became increasingly variable by the final measurement.

Second-season, mean incremental growth measurements increased 25, 46, and 220% for height, RCD, and stem volume, respectively (Figure 1.3, D-F). Height growth was significantly different among container types for each growing season ($P \leq 0.0007$), although no discernable trend with container size was observed. The largest incremental height growth increase was observed by the second largest container in year two (C80). Similar results were seen with RCD and stem volume. Differences were detected among container types for each growing season ($P \leq 0.0001$), but the largest percentage increases were observed in C166 and C80 container types (Figure 1.3, E and F). For RCD and stem volume growth, the smallest container exhibited the smallest amount of growth; however, for first and second year height growth, the C90 and C120 containers grew the least.

Temperature, precipitation, and soil moisture data were collected from early May through mid-October 2007. Total precipitation measured 135 mm and mostly occurred before July and after late September; one large precipitation event occurred during mid-July and measured 10 mm (Figure 1.2, E). Soil moisture at 15 cm decreased from 0.34 to 0.08 $\text{m}^3 \text{m}^{-3}$ from early May (shortly after planting) to the end of September before recovering to 0.25 $\text{m}^3 \text{m}^{-3}$ (after several precipitation events; Figure 1.2, E). Mean temperature was 16.2 °C with a low of 0.1 °C (4 May, 29 September, and 4 October 2007) and a high of 35.8 °C (12 July 2007; Figure 1.2, F).

Predicting Outplanting Performance

Initial height, RCD, and stem volume were investigated as simple predictor variables for one- and two-year absolute field growth measures. Because seedling mortality was low on both sites, predictions of survival were not performed. Instead, analyses were done solely to understand the relationship of the initial outplanting measurement and subsequent morphology. All regression analyses, for both sites, showed positive relationships and were significant ($P \leq 0.0001$). In most cases, we were able to predict more of the variation (coefficient of determination, R^2) in the first year than in the second year, although nearly all values tended to be low (Table 1.2). For both sites, initial outplanting height alone explained 66 and 70% of the variation for first-year absolute height growth. AIC analysis showed that adding initial stem volume to the model explained 8% more variation at F2 (74%), while only improving CC by 1%. All second-year single- and multi-parameter height models yielded low R^2 values for both sites ($R^2 \leq 0.27$). At F2, no suitable RCD prediction models were observed with single- or multi-parameter models ($R^2 \leq 0.08$). Results for CC were similar, although R^2 values were higher ($R^2 \leq 0.34$). The best first-year volume predictions came from single-parameter models; initial volume explained 54% of the variability at the CC site, while only 28% was explained at the F2 site. Second-year models explained 27% and 10% of the variability at CC and F2 respectively. Adding more parameters did not improve the models' predictive capacity.

Discussion

Although the literature includes numerous studies that examine the effect of stock type choice on outplanting survival and growth, most fail to include any information about nursery culturing methods related to specific container types (e.g. Amidon et al. 1982; Paterson 1997; Hunt 2002; South et al. 2005; Dominguez-Lerena et al. 2006). This major flaw of most container type selection studies (Burdett 1990) can be avoided by growing seedlings with container-specific fertilizer and irrigation regimes (Pinto 2005). Seedlings produced this way have better uniformity in foliar nitrogen status and water use efficiency than seedlings of different container volumes grown under a single culture regime (Pinto 2005). This study made use of seedlings that were cultured specifically to their container

type to reduce the physiological variation among the seedling types and thereby yield a truer reflection of container volume influence on outplanting performance.

Few studies investigate the difference in stock type growth in differing site conditions (Amidon et al. 1982; Racey et al. 1989; South et al. 2005). Because water availability is one of the most important variables that contribute to successful establishment and growth of seedlings (Burdett 1990; Margolis and Brand 1990), a study objective was to identify two sites that differed in this characteristic. However, our goal was not to statistically compare the two sites as they were expected to be different in soil texture, soil nutrition, and precipitation, but rather to quantify soil moisture differences and analyze the differences in container seedling growth for these sites as two independent case-studies. This is of additional importance in considering that the use of different seed sources lends to inherent differences between the seedling growth expected at each site (McMillin and Wagner 1995; Cregg et al. 2000; Cregg and Zhang 2001).

Data collected from F2 and CC indicate the sites were distinctly different in their precipitation and soil moisture regimes (Figure 1.1, A and E). F2 had fairly mesic conditions as a result of periodic precipitation and a lack of competing vegetation from site preparation, which kept θ high. Conversely, CC had more xeric conditions because of higher mean temperature, lower precipitation inputs, and no site preparation. The presence of transpiring mature trees, shrubs, and grasses in addition to well-drained, loamy-skeletal soils also contributed to soil moisture loss observed at the CC site (Warren et al. 2005). The large soil moisture reductions and reduced precipitation inputs observed at CC are typical of those seen in coniferous forests of the Pacific Northwest and Intermountain West, including the seasonal drought from July through the end of September. Despite the differences in θ between F2 and CC, the seedlings at both sites were planted during a period of favorable soil moisture (Rietveld 1989). With the exception of a short warming event at the first part of June, early-season site conditions remained favorable for establishment until the onset of the seasonal drought in July.

On the mesic F2 site, initial seedling characteristics obtained from the nursery—in this case a true reflection of container type because of container specific culture—were poor predictors of long-term absolute growth (Table 1.2). Seedlings had little difficulty establishing and growing because the combination of ash cap soils, exemplary site

preparation, and periodic rain inputs—55mm during the typical drought period—kept soil moisture conditions high and allowed the seedlings to grow vigorously. While seedling morphology remained stratified for height, RCD, and stem volume, with seedling size increasing with container size, incremental height growth appeared to equilibrate among container types (Figure 1.1, D). These height results are contrary to two-year results observed for bareroot ponderosa pine and Douglas-fir (*Pseudotsuga menziesii*) seedlings by Rose et al. (1991a, b). Two factors may contribute to difference seen in our results. First, variability in seedling size increased with each season making differences among container types difficult to distinguish. Secondly, seedlings that are well established within the soil profile are faced only with limitations in exploiting their environment and are less dependent on the attributes provide by initial nursery growth (Burdett et al. 1984; Nambiar and Sands 1993). In this case, second season height growth may have been more limited by the presence of vegetative competition (in the form of weeds) and the compound effects of limited soil moisture during the shoot elongation phase—typically April through July. (Newton and Preest 1988). However, RCD and stem volume, normally not limited to growth during the shoot elongation phase, were allowed further growth under the resumption of favorable growth conditions when precipitation resumed in the fall.

CC seedlings experienced soil moisture depletion, low precipitation inputs, high temperatures, and competing vegetation while trying to establish and grow after planting—all of which are known to reduce seedling growth (Larson 1967; Rose et al. 1993; Anderson et al. 2001; Zhang et al. 1997). Under these difficult establishment conditions, differences among container types would be expected, as seen by Amidon et al. (1982) with longleaf pine (*Pinus palustris*) in drought conditions. After the first growing season, container-type differences in height, RCD, and stem volume remained relatively unchanged from planting. The second growing season illustrates, however, two interesting trends. Most of the mid-sized containers (90, 105, and 120) became similar in height, RCD, and volume, while the 80 container caught up in height and exceeded them in RCD and volume (Figure 1.3, A – C). Because all container types were similar in physiology, having a smaller container volume outperform larger container types, especially in relatively dry conditions, is uncommon and may show the benefit of growing seedlings with specific regimes for stock type trials. In one study, small and medium containers showed better performance than the largest stock type

under differing watering regimes, but results were confounded with reduced seedling quality (Lamhamedi et al. 1996).

One- and two-year growth results were clear in the differences among container types (Figure 1.3, D-F), but they did not show any consistent differences by container size. Because the site was not prepared with herbicide or burning, it may be that variation in planting microsites contributed to the large variation in growth (Gray and Spies 1997). However, the large number of seedlings used and their distribution over five blocks should have decreased some of this variation. Despite this, these results, in combination with uniform culturing techniques, show the potential for a small containerized seedling to outperform larger container types.

RCD and stem volume R^2 values were too low for the prediction of subsequent CC seedling growth. These results are not consistent with those observed with white spruce (*Picea glauca*; Mullin and Christl 1981, b), white pine (*Pinus strobus*; Mullin and Christl 1981, a), or loblolly pine (*Pinus taeda*; McGilvray and Barnett 1982), where initial seedling morphological characteristics correlated well with seedling growth for more than three years after outplanting. However, the best predictor values within each variable were higher than those seen at F2, especially after the second season's growth. The harsh site and slower growth of CC seedlings may be responsible. Because the seedlings grew considerably less, the attributes attained in the nursery remained—small seedlings remained small and large seedlings remained large.

This study offers unique perspective in seedling stock type selection because it used differing stock types cultured to their specific container type and outplanted on divergent sites. By planting trees of common physiology, but different size, on different sites, we obtained a clearer understanding of a container type's true gains in performance in the field. As seen at F2, when quality seedlings are planted carefully, at the correct time, and under favorable (mesic) growing conditions, container selection is not important so much for seedling survival as they might be for overall growth. When considering the CC site, with more potential site limiting factors, container selection might be more important (Landis and Dumroese 2006).

A larger seedling with a longer root plug might have the greatest total growth, and may do better in dry conditions (Chirino et al. 2008), but the risk of improper planting might

offset any gains (Robert and Lindgren 2006). Deep planting—placing the cotyledon scar below the soil surface—a shorter container of similar volume having equal or better growth potential might prove just as beneficial (Stroempl 1990). This may have been the case for the results seen at CC where the long, flimsy root system of C120 was likely poorly planted and the firm stout root system of the C80 was easily and optimally planted. Studies have shown that deep planting bareroot of seedlings increases survival and shows no differences in growth (South and Mitchell 1999; VanderSchaaf and South 2003).

Using simple morphological variables to predict absolute growth remains difficult and inconclusive. Simple, standard grading criteria fail to explain large proportions of variation in seedling growth in the field. However, differences in absolute growth on mesic versus drier sites illustrate how morphological traits acquired in the nursery might persist over time and how sites might influence predictability. Puttonen (1997) suggests one test cannot do it all, but provides a matrix in which morphological measurements alone provide the best test (combining research, operational feasibility, timeliness, and cost). With this in mind, research that ties the understanding of seedling physiology, ecophysiology, and environmental biophysics to morphology will provide the best seedling growth and prediction models relating to field performance.

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Chapter 1 - Tables

Table 1.1. Styroblock® (Beaver Plastics Ltd., Acheson, Alberta) container specifications used to produce *Pinus ponderosa* seedlings.

Container ^a	Cavities				
	Cavities per Container	Volume (cm ³)	Top Diameter (cm)	Depth (cm)	Cavities per m ²
60	160	54	3.0	10.3	756
80	112	80	3.6	10.3	530
90	160	90	3.0	15.1	756
105	112	103	3.6	15.2	530
120	160	120	3.0	22.7	756
166	112	164	3.6	20.3	530

^a 60, 90, 120 manufactured by Beaver Plastics Ltd. (Acheson Alberta). 80, 105, and 166 manufactured by Mansonville Plastics Ltd. (Surrey, British Columbia).

Table 1.2. Coefficient of determination (R^2) for linear regression models of *Pinus ponderosa* seedlings over two field seasons (n = 530–600). Root-collar diameter (RCD) is the diameter of the main seedling stem approximately 1 cm above ground-line.

Independent Variables	Dependent Variables (R^2) ^a					
	Fall 2007			Fall 2008		
	Height	RCD	Stem Volume ^b	Height	RCD	Stem Volume ^b
Spring 2007						
Faeber II						
Height	0.66***	0.02***	0.20***	0.17***	0.04***	0.07***
RCD	0.28***	0.08***	0.19***	0.12***	0.03***	0.06***
Stem Volume ^b	0.63***	0.07***	0.28***	0.21***	0.05***	0.10***
Container Volume	0.47***	0.05***	0.20***	0.13***	0.03***	0.06***
Height + Stem Volume	0.74***	—	—	0.22***	—	—
Coyote Creek						
Height	0.70***	0.04***	0.29***	0.27***	0.03***	0.27***
RCD	0.06***	0.34***	0.32***	0.07***	0.14***	0.07***
Stem Volume ^b	0.46***	0.28***	0.54***	0.26***	0.14***	0.27***
Container Volume	0.36***	0.07***	0.23***	0.16***	0.05***	0.16***
Height + Stem Volume	0.71***	—	—	0.31***	—	—

^aRegression significance: *** $p < 0.0001$. Regression slopes were positive and linear.

^bStem volumes were log transformed.

Chapter 1 - Figures

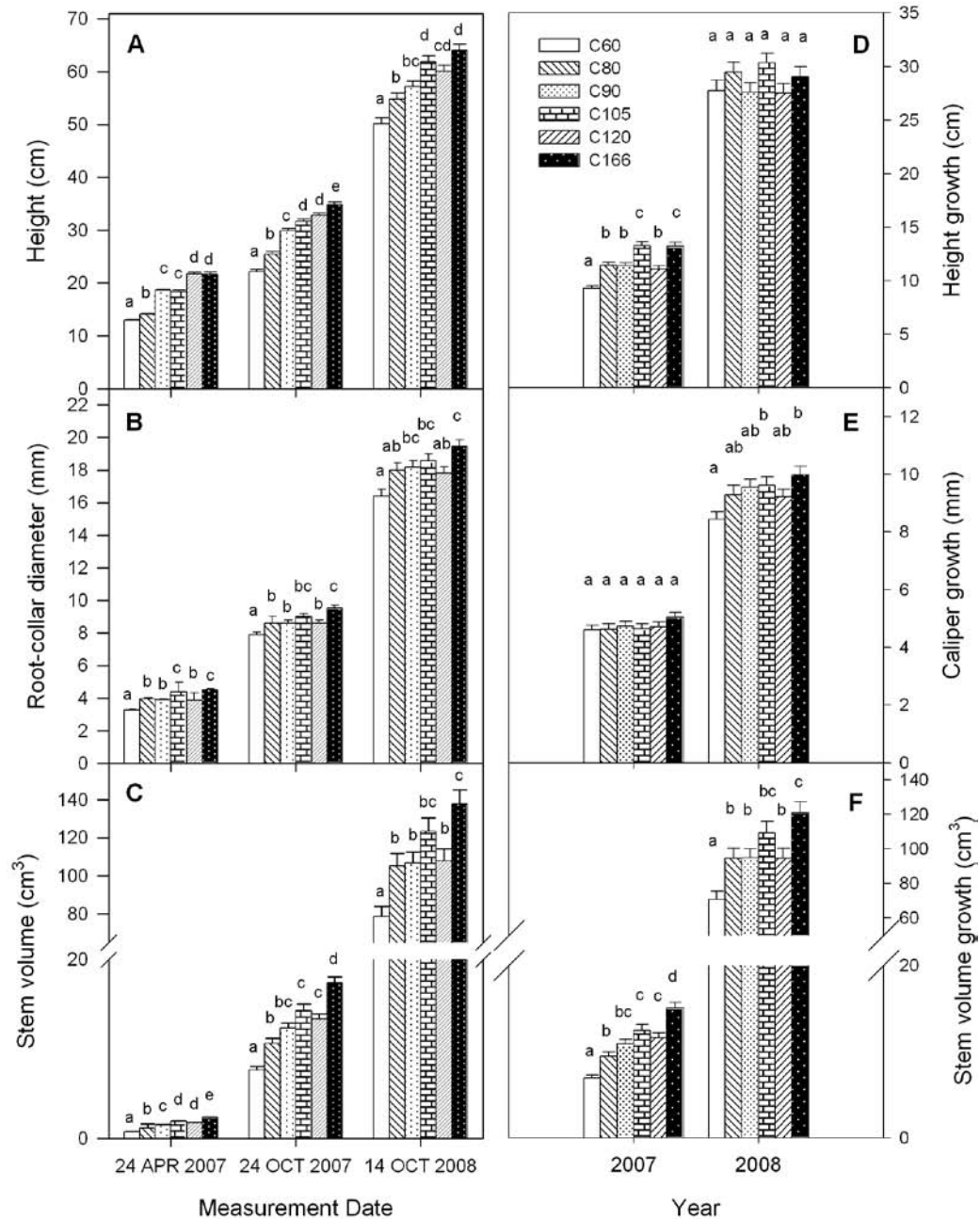


Figure 1.1. (A-C) Absolute and incremental seasonal growth of *Pinus ponderosa* seedlings on a mesic site in Idaho (Faerber II). Morphological measurements were done after planting and after first and second growing seasons ($n = 476 - 600$). (D-F) Incremental growth for height, root-collar diameter, and stem volume were calculated for the first growing season and second year's growth; the first growing season (2007) was 6.25 months ($n = 477 - 507$). Bars show least square means + SE. For each measurement period, bars with the same letter are not significantly different for each measurement date, Tukey adjusted ($P < 0.05$). Stem volume was log transformed for spring and fall 2007 analyses. Container volumes were 60, 80, 90, 105, 120 and 166 cm³.

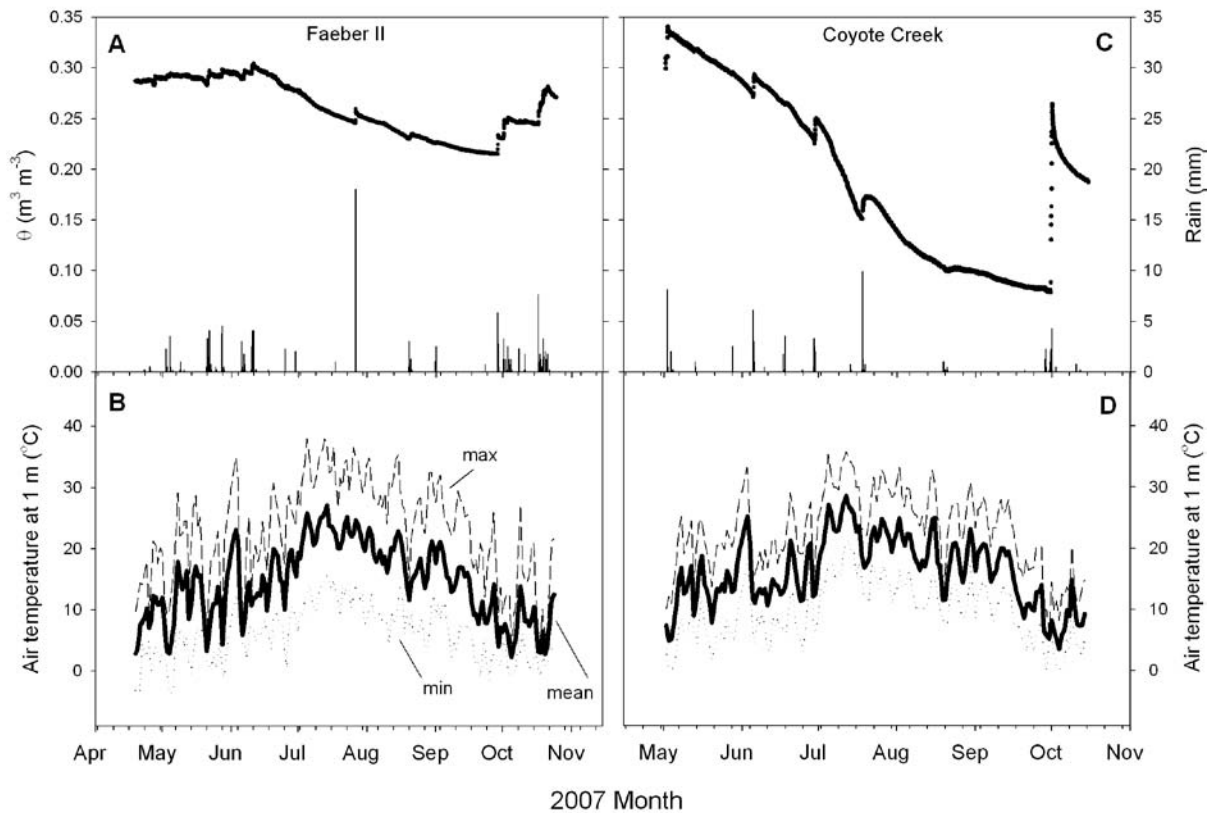


Figure 1.2. Site conditions for reforestation planting units in Idaho (Faerber II; **A**, **B**) and Washington (Coyote Creek; **C**, **D**). Variables measured include θ (soil volumetric water content 15 cm below the soil surface), precipitation (represented by bars; **A** and **C**), and daily air temperatures (mean, maximum, and minimum at 1 m; **B** and **D**).

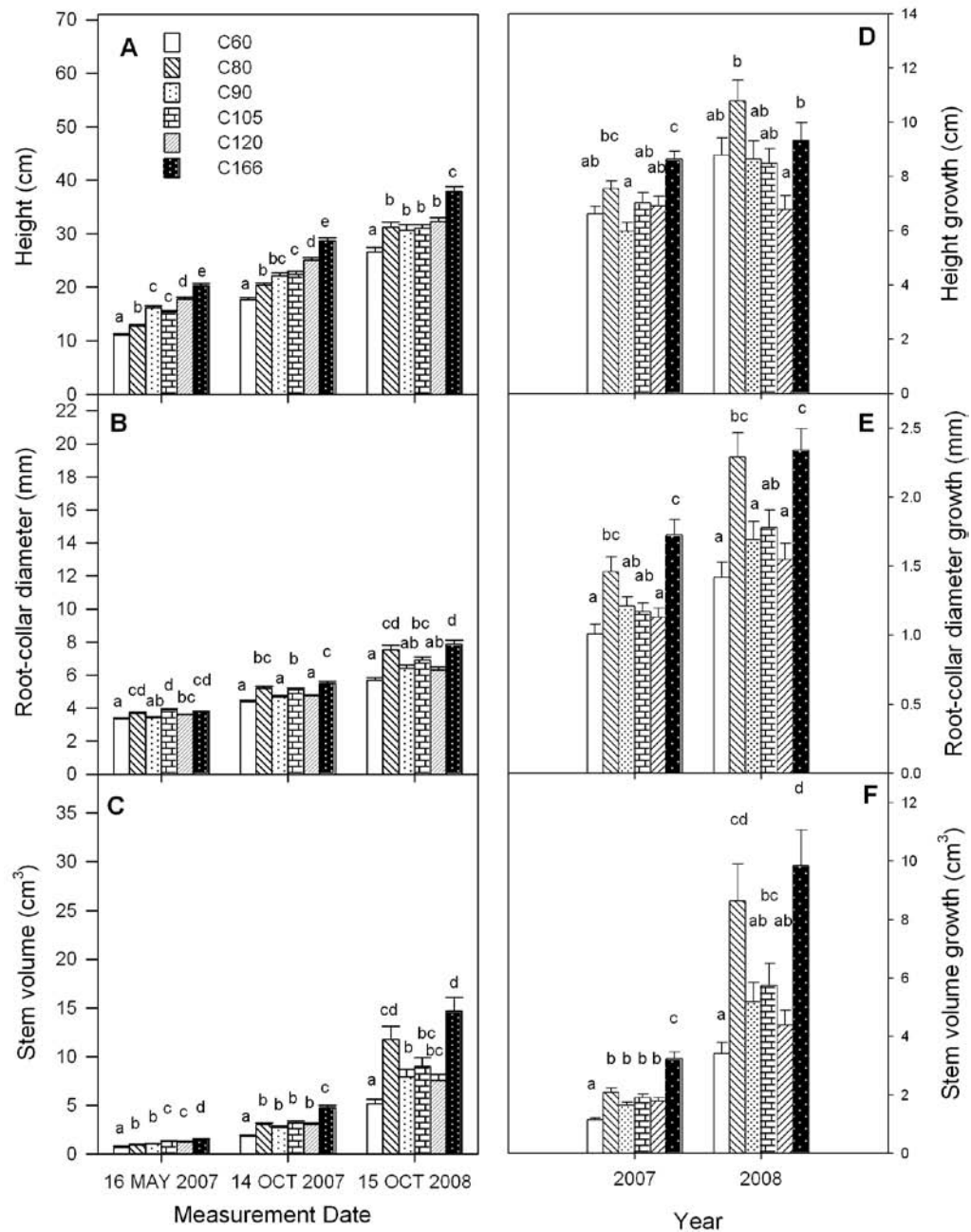


Figure 1.3. (A-C) Absolute and incremental growth measurements of *Pinus ponderosa* seedlings on a xeric site in Washington (Coyote Creek). Morphological measurements were done after planting and after first and second growing seasons ($n = 476 - 600$). (D-F) Incremental growth for height, root-collar diameter, and stem volume were calculated for the first growing season and second year's growth; the first growing season (2007) was 5.5 months ($n = 477 - 507$). Bars show least square means + SE ($n = 477 - 507$). For each measurement period, bars with the same letter are not significantly different for each date, Tukey adjusted ($P < 0.05$). Stem volume was log transformed for all analyses. Container volumes were 60, 80, 90, 105, 120 and 166 cm³

Chapter 2. Photosynthetic response, survival, and growth of three ponderosa pine stock types under water stress enhanced by vegetative competition

Abstract

Three ponderosa pine (*Pinus ponderosa* Laws. var. *ponderosa*) seedling stock types, varying in container size and grown in the greenhouse, were planted in the field and subjected to three levels of competition. Winter wheat (*Triticum aestivum* L. em.) was used as a model competitor. Wheat was sown in three densities (0, 150, and 300 plants m⁻²; D₀, D₁₅₀, and D₃₀₀, respectively); to limit wheat's growth cycle and solar interference with seedlings, it was sown in the spring. Over the course of the study, the wheat planting reduced soil moisture 17 cm below the soil surface. Predicted seasonal minimum soil water potential (Ψ_{soil}) was -0.2, -1.1, and -1.4 MPa for each of the competition treatments. High rates of net photosynthesis (*A*) indicated that seedlings with adequate soil moisture and without vegetative competition were established within three weeks. Conversely, low *A*, low soil moisture, and low predawn water potential (Ψ_{nwp}) measurements indicated that seedlings planted with vegetative competition were moisture stressed ($\Psi_{\text{nwp}} < -2.1$ MPa) and not established. As a consequence, mortality rates were severe in D₁₅₀ and D₃₀₀ with smaller containers reaching 100% mortality, while the largest container experienced 63-75% mortality. In the absence of competition, D₀ seedlings suffered no mortality. These data suggest that, regardless of container size, moisture in the upper soil profile is critical to seedling establishment and growth, and can be drastically compromised by competing vegetation. Successful seedling establishment requires planting when there is still sufficient moisture in the upper soil profile, practices that enhance soil moisture such as vegetation management, and planting seedlings with sufficient root length to allow the root systems ample time to access moisture.

Introduction

The challenges of dry site reforestation are not new, but they continue to, and may increasingly in light of global climate change, limit reforestation success. Normal climate patterns in the Northwestern USA include a pronounced dry season, which causes soil moisture to decrease from spring into summer (Meinzer et al. 2004; Warren et al. 2005).

Successful seedling establishment and growth thus depend on stored soil moisture to ensure survival into the next growing season. For this to happen, seedlings must either have little competition for existing water or must establish root systems at depths where water is available.

Ponderosa pine (*Pinus ponderosa* Laws. var. *ponderosa*) natural regeneration can be low (Keyes et al. 2007) owing to mortality due to lethal temperatures or drought stress (Harrington and Kelsey 1979; Kolb and Robberecht 1996). Seedlings that survive heat or drought conditions are often in microsites that protect them from high temperatures, or they have developed root systems that access soil moisture reserves not depleted by competing vegetation (Pearson 1930; Van Haverbeke 1963). The resulting ability of a seedling to take up water allows the initiation of a positive feedback loop; water uptake increases leaf conductance, which increases photosynthesis, which increases new root growth into wetter soil, which allows for still more water uptake. This positive feedback model of seedling establishment (Burdett 1990) will be referred to throughout this manuscript. Planted seedlings can initiate this process, but their roots are initially confined to a planting hole; they must grow roots into the surrounding environment. Seedlings with short root plugs will have to grow roots deeper into the soil profile to access moisture as opposed to seedlings with longer root plugs that have roots placed deeper in the soil profile to begin with (Chirino et al. 2008).

Seedlings exposed to dry soils exhibit reduced stomatal conductance and growth (Zhang et al. 1997; Olivas-Garcia et al. 2000; Panek and Goldstein 2001). Depending on timing of the drought stress, biomass partitioning to roots and shoots may also be altered. McMillin and Wagner (1995) showed that drought stress during shoot growth decreased current-year foliage biomass more than stress during root growth. Cregg (1994) suggests that an optimum shoot-to-root ratio (S:R) may be most beneficial for drought tolerance in seedlings. Too low a ratio may limit photosynthesis and carbohydrate reserve production (for survival of an extended drought) and too high a ratio may exceed the water absorbing capacity of the roots. In either case, seedlings that survive into the next growing season must somehow balance transpirational demand with root water uptake.

Dry soils result not only from low seasonal precipitation inputs but also from water use by competing vegetation (Elliot and White 1987; Newton and Preest 1988; Caldwell et

al. 1995; Nilsson and Örlander 1999). Research by Pearson (1942) suggests that grasses growing on burned forest sites are effective at extracting moisture from the upper 20 cm of soil, thereby causing reduced growth of seedlings. Additional research has shown that grass competition effectively lowered seedling water potential (Elliott and White 1987; Caldwell et al. 1995) and seedling biomass (Anderson et al. 2001). Grass has also been shown to decrease survival of establishing ponderosa pine by up to 80% (Baron 1962).

Artificial regeneration of ponderosa pine is more effective at stand establishment than natural regeneration (McDonald et al. 2009). Current reforestation options offer a plethora of seedling stock type choices, but studies offering solid data on the advantages of certain stock types are often confounded—planting in the field may not offer drought conditions on a particular year, or seedlings may not be cultured uniformly or to their specific container type (Pinto 2005). To date, only two studies have compared the performance of seedling stock types, and their physiology, under drought conditions using vegetative cover. Both studies failed to create sufficient drought differences with herbaceous vegetation (Lamhamedi et al. 1998; Mohammed et al. 1998), and one failed to use seedlings from uniform seed sources and nursery culturing techniques (Lamhamedi et al. 1998). The objectives of this study were to use a holistic approach to (i) simulate and intensify seasonal drought using plant competition treatments, (ii) quantify competition effects on soil moisture and availability, and (iii) and evaluate survival, gas exchange, and growth responses among three ponderosa pine stock types.

Materials and Methods

Seedling Preparation

During 2006, ponderosa pine seedlings were grown from seed collected on the Confederated Tribes of the Colville Indian Reservation (Colville Tribal Forestry, Lower Stepstone: stand #1, 610 m elevation). The seedlings were grown at the USDA Forest Service, Rocky Mountain Research Station in Moscow, Idaho (46°43' N, 117°00' W; 798 m elevation). Three different Styroblock[®] containers, differing only in cavity depth, and therefore volume (Table 2.1), were used. Seedlings were grown from March until December, when they were harvested and placed into freezer storage (-2 °C). Seedlings were cultured with container-specific regimes to achieve uniform physiological characteristics (Pinto

2005); this ensured that results reflected container type and were not confounded by nursery practices.

Field Preparation

In late spring 2007, seedlings were outplanted at the USDA Forest Service nursery in Coeur d'Alene, Idaho USA (47°43' N, 116°49' W; 688 m elevation). The nursery soil is a sandy loam with an average 1-m depth to gravel. Seedlings were challenged by exposure to three levels of water competition using a cover crop of winter wheat (*Triticum aestivum* L. em.). Wheat was chosen because, when sown in the spring, the plants do not receive sufficient exposure to cold temperatures to satisfy the vernalization requirement, so they do not form seed heads and instead remain relatively short (Amasino 2005). This allowed competition for water in the root zone with little competition for solar radiation in the canopy. Wheat was sown April 6 at three densities (0, 150, 300 plants m⁻²; D₀, D₁₅₀, and D₃₀₀, respectively) to induce drought effects; it was irrigated periodically to aid germination and establishment. After four weeks, D₀, D₁₅₀, and D₃₀₀ were fertilized at 0, 22.4, and 44.8 kg N ha⁻¹, respectively. The low rate was recommended solely for wheat establishment, but expected to be depleted by the time the seedlings were planted (Eramian 2007). Winter wheat leaf area index (LAI) was measured, using a sunfleck ceptometer (model SF-80, Decagon Devices, Inc., Pullman WA), in early fall to quantify the above ground biomass relative to the sowing rate. D₃₀₀ measured 0.64 m² m⁻² while D₁₅₀ measured 0.32 m² m⁻².

Seedling Installation

On May 29, seedlings were moved from freezer storage to cooler storage (5 °C) for thawing. Seedlings were planted on June 5 when wheat was approximately 7.5 cm tall. Competition treatments were replicated four times as whole plots. Each whole plot was divided into 12 planting areas for individual trees, each 1 × 1.25 m. Four seedlings from each of three container-types (an experimental unit) were planted into each level of competition within each replication (a randomized complete block split-plot design).

Weather and Soil Measurements

Instrumentation and data loggers were used to collect weather and soil moisture data, while laboratory analysis was used to calibrate equipment and construct moisture characteristic curves. A weather station (model 2900ET, Spectrum Technologies, Inc., Plainfield, IL) collected relative humidity (%), and rainfall (mm) measurements hourly and stored them on a data logger. Vapor Pressure Deficit (VPD) was calculated from ambient temperature (°C) and relative humidity (%):

$$VPD = \left(ae^{\left(\frac{bT}{T+c} \right)} \right) (1 - h_r)$$

where a , b , and c are constants ($a = 0.611$ kPa, $b = 17.502$, and $c = 240.97$ °C), T is temperature (°C), and h_r is relative humidity (%/100; Campbell and Norman 1998).

Volumetric soil moisture (θ) and temperature measurements were collected hourly *in situ* at 3 depths (17, 50, and 80 cm from soil surface) using ECH₂O-TE soil moisture probes and Em50 data loggers (Decagon Devices, Inc., Pullman, WA). Three soil core samples were taken at each depth where soil moisture probes were installed. Each soil sample was then split vertically into two otherwise undisturbed subsamples. One subsample was used to generate a moisture characteristic curve at 5 pressures (0.03, 0.10, 0.30, 0.50, and 1.50 MPa) using a high-range pressure system and ceramic plates (Klute 1986). The pressures were plotted against θ calculated from the conversion of gravimetric soil moisture using measured bulk density. Soil bulk density was measured using the technique of Blake and Hartge (1986). Regression equations were fit to the curve and subsequently used to predict soil water potential (Ψ_{soil}) in the field. The second subsample was used to generate soil specific calibration equations for the soil moisture probes (Starr and Paltineanu 2002; Cobos 2007). Raw sensor data from laboratory measurements on soil samples were plotted against measured θ to generate the equations. Raw data collected from the field were then inserted into each respective calibration equation for final, corrected θ values. The normal accuracy for each probe type with calibration is $\pm 0.02 \text{ m}^3 \text{ m}^{-3}$ (Decagon Devices, Inc., Pullman, WA, USA).

Survival, Growth, and Biomass Allocation

Morphology, biomass, and survival assessments were performed periodically over the course of the study. At the end of greenhouse production in December 2006, height, root-collar diameter (RCD), and post harvest biomass were measured on three seedlings from four replications of each container type (36 total seedlings). For biomass, rooting medium was gently washed from roots, and shoots and roots were separated and dried to a stable mass at 60° C. Shoot-to-root ratio (S:R) was calculated from dry mass. In June 2007, seedling heights and RCDs were measured one week after outplanting. Seedling survival was assessed nine times throughout the growing season. Seedlings were declared dead when ocular assessment indicated $\geq 95\%$ of the needles were brown. In November 2007, height, RCD, and needle length were measured on all remaining live seedlings. Two seedlings from each container type in the D₀ treatment were sampled for biomass after careful excavation with a shovel. Root egress was measured as the longest length of root growth beyond the bottom of the root plug. After gently removing soil and medium, biomass was determined after partitioning seedlings into sections (roots, stems, and needles produced after outplanting) and drying the sections to a stable mass at 60° C.

Seedling Gas Exchange and Pre-dawn Needle Water Potential

Using a portable photosynthesis system (model LI-6400, Li-Cor, Lincoln, NE) equipped with a blue/red LED light source and CO₂ injector, seedling gas exchange was measured eight times over the course of the first growing season (June to October). Measurements began two weeks after planting. The first four occurred at one-week intervals; measurements five and six occurred at two-week intervals; the last two measured at three to four weeks intervals (June 19, 26; July 3, 11, 23; August 6, 28; October 9, 2007). One seedling from each container type and treatment was randomly chosen from each replication for gas exchange measurements. On the selected seedling, two secondary needle fascicles from the previous year were placed in the chamber while still attached to the stem. The chamber was set at 1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, 25° C, 400 $\mu\text{mol mol}^{-1}$ CO₂, and a flow rate of 400 $\mu\text{mol s}^{-1}$.

Leaf area calculation was adapted from the methods of Svenson and Davies (1992). Fascicle diameters were measured in the center of each fascicle segment that was placed

inside the LI-6400 light chamber. Using the assumption that three needles of a fascicle form a cylinder (Johnson 1984), we could calculate the abaxial leaf area by multiplying the circumference (of the cylinder) by the length of the needle inside the chamber. Total adaxial leaf area is simply calculated by multiplying the radius of the fascicle by the length of needle in the chamber and multiplying the result by six (six is the total number of adaxial surface areas of one needle fascicle). We used the following equation to calculate leaf area for one needle fascicle:

$$LA = (\pi dl) + \left(6 \frac{d}{2} l\right)$$

where d is the diameter of the three-needle fascicle, and l is the length of needle inside the chamber. The length of the needle segments in the chamber was always 30 mm, so the equation simplifies to $LA = 184.2d$.

Three weeks after the seedlings were outplanted, when seedlings should have been established and growing, needle water potential (Ψ_{nwp}) was measured to assess competition effects on plant moisture stress. On June 29, needle water potential (Ψ_{nwp}) was measured on one seedling from each competition treatment $\times$ container $\times$ replication with a pressure chamber (PMS Instrument Company, Corvallis, OR). Between 0200 and 0400 hours, one needle from a fascicle was randomly chosen for measurement.

Experimental Design and Statistical Analysis

Analysis of variance (SAS Inc., v 9.1.3, Cary, NC, USA) PROC GLM ($\alpha = 0.05$) was used to examine the effects of container type on seedling morphology after greenhouse harvest; container types were compared using the Tukey adjustment. The outplanting experiment used 3 competition levels $\times$ 3 container types $\times$ 4 replications in a randomized complete block (RCB) split-plot design, with competition as the whole plot factor and container type as the split-plot factor. This design was chosen because the wheat-planting required the use of a tractor-drawn seeder that yielded uniform densities, but only over large areas. The design contained 4 seedlings per competition $\times$ container $\times$ replication combination (144 total seedlings). Periodic observations of survival showed all seedlings of

the C60 and C90 container types had died in D₁₅₀ and D₃₀₀ before the experiment ended. This precluded *a priori* RCB split-plot analysis of net photosynthesis and growth for all competition $\times$ container combinations on 2007 measurements. *Post hoc* analyses included net photosynthesis on all competition $\times$ container combinations for the first measurement period only, growth analysis on C120 $\times$ competition combinations at the end of the 2007 growing season, and repeated measures net photosynthesis analysis on C120 $\times$ competition combinations (a randomized complete block design analysis) for all eight measurement periods. Seedling survival was analyzed with October 9 data. Because of high mortality, survival data violated assumptions for normality and was consequently analyzed using the non-parametric Kruskal-Wallis and Friedman tests.

Results

Site Conditions

During the 2007 growing season, both competition treatments (D₁₅₀ and D₃₀₀) created drier conditions in the upper 17 cm of the soil profile than did D₀ (Figure 2.1). Between the time of planting (June 5) and the last photosynthesis measurement (October 9), the average θ for D₀ was 0.22 m³ m⁻³ compared to 0.14 and 0.12 m³ m⁻³ for D₁₅₀ and D₃₀₀, respectively. At planting, predicted Ψ_{soil} was -0.04 MPa for D₀, -0.06 MPa for D₁₅₀, and -0.12 MPa for D₃₀₀ (Figure 2.2). θ briefly rose due to precipitation inputs shortly after planting, then proceeded to decline (rapidly for D₁₅₀ and D₃₀₀) until reaching minimum values at the end of September. During this period, on site precipitation measured 68 mm—most of which occurred either near planting or at the end of the growing season, in late September or October. Three small rainfall events (> 5 mm) occurred between July and early September (the typical dry period), but they did not significantly impact θ at 17 cm.

At time of planting, the air temperature and VPD were 13.4 °C and 0.03 kPa. On July 5, maximum air temperature and VPD were reached; values were 37.8 °C and 6.1 kPa, respectively. From June 5 to October 9, overall air temperature averaged 18.5 °C, while VPD averaged 1.4 kPa. Mean maximum daily air temperature and VPD for the growing season were 25.9 °C and 3.0 kPa respectively. Soils at the 15 cm depth were warmest in the D₀ treatments averaging 23.2°C with a seasonal maximum of 34.2 °C. Wheat cover seemed

to reduce surface soil heat flux for D₁₅₀ and D₃₀₀; where seasonal average soil temperatures were only 21.9°C and 21.4 °C, respectively.

Greenhouse Harvest Data, Field Survival, and Growth

Seedlings harvested from different container volumes were morphologically distinct. In general, and as expected, larger container volumes yielded larger seedlings (Endean and Carlson 1975; Pinto 2009). Significant differences were observed in height, root-collar diameter (RCD), shoot dry mass, root dry mass, and S:R (Table 2.2). Pairwise analyses indicated C90 and C120 containers were the most similar.

All seedlings in the D₀ treatment, regardless of container type, survived the duration of the experiment. Conversely, just five weeks after planting, mortality became evident for the C60 seedlings in the D₁₅₀ and D₃₀₀ treatments. Seven weeks after planting, all C60 seedlings in D₃₀₀ were dead, and mortality was nearly 50% for the C90 seedlings in both D₁₅₀ and D₃₀₀ (Figure 2.3). Twelve weeks after planting, all C90 seedlings in D₁₅₀ and D₃₀₀ were dead. Analysis of mortality 14 weeks after planting yielded differences among competition treatments ($P < 0.0001$) and container types ($P = 0.003$). The C120 seedlings that survived showed different morphology among drought treatments (Table 2.3). D₁₅₀ and D₃₀₀ seedlings had reduced RCDs ($P < 0.001$), needle biomass (data not presented), and needle length (> 74 % reduction; $P < 0.0001$) relative to seedlings in D₀. C120 seedlings growing in D₀ remained the tallest of the container types ($P < 0.0001$) and had more total shoot biomass than C60 ($P = 0.002$) but not C90 ($P = 0.67$). RCD, root biomass, and 2007 needle biomass were not significantly different ($P \geq 0.06$) among container types in D₀ (Table 2.4). Likewise, differences in root egress from the bottom of the plug were not significantly different among container types ($P = 0.81$); average maximum egress measured 36.7 cm.

Photosynthesis

No significant differences in net photosynthetic rates were detected among competition treatments two weeks after planting ($P = 0.38$). Conversely, differences were evident among container types. While seedlings in C90 were not significantly different from C60 or C120 seedlings ($P \geq 0.07$), the larger C120 seedlings were photosynthesizing at higher rates than those in C60 (3.77 vs. 2.25 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf area s}^{-1}$; $P = 0.006$). For the

subsequent five measurements, net photosynthesis (A) of seedlings in D_{150} and D_{300} treatments dropped precipitously (Figure 2.4), corresponding to decreasing soil moisture and seasonal increases in temperatures and VPDs. Seven weeks after planting, A reached minimum values ($0.14 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), which presaged the complete mortality of C60 and C90 seedlings. Conversely, all seedlings in D_0 , regardless of container type, followed an upward trend in photosynthetic capacity as the growing season progressed. Repeated measures analysis showed D_0 -C120 seedlings had higher photosynthetic rates than C120 seedlings in either D_{150} or D_{300} ($P < 0.01$; Figure 2.5). A was not significantly different ($P > 0.6$) between D_{150} and D_{300} and no date $\times$ treatment interactions were detected ($P = 0.4$).

Seventeen days after planting, both competition and container type treatment effects showed significantly different Ψ_{nwp} ($P \leq 0.0002$; Figure 2.6). Seedlings in D_{150} and D_{300} already exhibited low Ψ_{nwp} (-2.18 and -2.23 MPa respectively) while D_0 seedlings were under considerably less stress (-0.77 MPa). Among container types, and averaged across all competition treatments, the C60 and C90 seedlings measured -1.85 and -1.86 MPa, while C120 seedlings measured slightly less negative at -1.44 MPa.

Discussion

Evidence, anecdotal along with limited empirical, suggest that planting of deep containers on droughty sites enhances survival and growth of seedlings (Stroempl 1990; Bainbridge 1994; Chirino et al. 2008), while some forest practices advocate proper soil moisture management for the same effects (Nambiar 1990; Nambiar and Sands 1993; Powers and Reynolds 1999; Fredrickson 2003; Balandier et al. 2006). Previous studies have attempted to show that larger stock types are advantageous, not only in size but also in physiology, on sites with competing vegetation and limited moisture availability (Lamhamedi et al. 1996; 1997; Mohammed et al. 1998). However, these studies have not been conclusive due to inconsistencies in nursery culture and failure to create sufficient moisture stress. This study is the first of its kind to capture differences in stock type and physiology using vegetative competition to enhance natural seasonal drought conditions: it does so using uniformly cultured nursery seedlings to avoid the confounding of size differences with inherent differences in seedling physiological status (Pinto 2005).

Seedlings in the D₀ treatment had little problem establishing and growing, and all survived the three-month summer drought of the Northwestern U.S. This success occurred despite the limited soil moisture storage in a well-drained, sandy loam soil and the complete lack of significant precipitation inputs between July and September. The absence of vegetative competition conserved 41 % more soil moisture in the profile when compared to D₁₅₀ and D₃₀₀ treatments. Pinto (2009) showed similar results in a field study where seedlings were grown on a site with extensive site preparation (burning and herbicide vegetation control). These seedlings experienced high soil moisture, low rates of mortality, and rapid growth rates compared to another location with little site preparation. Similar results have been seen with the control of woody and herbaceous vegetation in ponderosa pine, Douglas-fir (*Pseudotsuga menziesii*), and spruce (*Abies spp.*; Ross et al. 1986; Newton and Preest 1988; Sutton 1995; Nilsson and Örlander 1999; Powers and Reynolds 1999; Rose et al. 1999; Rose and Ketchum. 2002). Despite planting later than normal (June vs. April), the conservation of soil moisture in D₀ allowed seedlings to grow well (76 % increase in shoot biomass) perhaps by increasing rates of net photosynthesis during the growing season.

Access to stored soil moisture allowed seedlings to engage the positive feedback model of seedling establishment (Burdett 1990). In this model, water uptake allows leaf conductance and photosynthesis, which then contributes to new root growth. Van den Driessche (1987) supports this model by illustrating the importance of current photosynthate for new root growth in planted conifer seedlings. New growth can place roots deeper in the soil profile, where soil moisture may be more available during the summer drought (Warren et al. 2005) limiting water stress. Van Haverbeke (1963) reports that naturally regenerated ponderosa pine seedlings produce an undisturbed tap root with the capacity to grow deep in the soil profile thereby avoiding moisture stress. Kolb and Robberecht (1996) also show ponderosa pine seedlings grown from seeds were able to grow taproots up to 1 m in length in just three weeks. If seedling quality were adequate, we would expect planted ponderosa pine to also have this ability. We argue that, the growth and establishment of seedlings in D₀, despite the short growing season, indicate that seedling quality was more than adequate. Root egress of D₀ seedlings averaged 0.31 m. These measurements are underestimates because roots were severed during the excavation process. It is therefore likely that root egress reached at least 0.5 m below the soil surface.

Consequently, with sufficient root egress and control of soil moisture, seedlings of differing container sizes in D₀ were all able to maintain similar and sufficient rates of net photosynthesis throughout the growing season (Figure 2.4). Net photosynthesis increased from 3.8 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at planting to 5.6 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, three weeks later, where it remained for the rest of the growing season. These values are similar to (Zhang et al. 1997; Panek and Goldstein 2001) or slightly higher than (Olivas-Garcia et al. 2000) those reported in other studies measuring gas exchange of ponderosa pine seedlings in differing water regimes. Given the lack of differences in carbon allocation rates among container types in D₀, it follows that the larger stock type remained larger at the end of the season, but no advantages in survival were conferred by container size or depth in this competition-free treatment.

The enhancement of seasonal drought using vegetative competition was readily apparent from the soil moisture data. Precipitous drops in soil moisture (from 0.23 to 0.09 and 0.19 to 0.08 $\text{m}^3 \text{ m}^{-3}$ for D₁₅₀ and D₃₀₀, respectively) were observed after spring precipitation diminished. But immediately after planting, and before the rapid drop in soil moisture, net photosynthetic rates were similar among treatments (Figure 2.4, B). This also follows from the similarity in seedling quality at planting and that each of the seedlings would have started photosynthesizing at similar rates on similar dates. Despite high seedling quality, declines in soil moisture and consequently net photosynthesis led to high mortality rates among all container types in both D₁₅₀ and D₃₀₀.

Recalling the model of seedling establishment (Burdett 1990), lack of soil moisture and water uptake in the competition treatments likely caused the breakdown of the positive feedback loop and resulted in death. Theoretically, if initial rates of net photosynthesis had persisted longer, new roots might have been produced; these roots might have occurred deeper into the soil profile and thereby might have mitigated the impending seasonal drought. In addition, the effects of the drought may have been limited by reductions in leaf area via leaf abscission or via limiting new needle expansion (Kozlowski and Pallardy 1997; Taiz and Zeiger 2006). The abrupt change in soil moisture may also have limited opportunities for drought preconditioning. Cregg (1994) showed that ponderosa pine seedlings experiencing several dry-down periods after planting are preconditioned to photosynthesize at higher rates despite decreasing soil moisture—something seedlings in D₁₅₀ or D₃₀₀ failed to experience.

Nursery drought hardening has also been shown to affect seedling survival in drought conditions (van den Driessche 1991; Villar-Salvador et al. 2004), although not for all species (van den Driessche 1991).

In this study, with harsh conditions limiting root growth, and the lack of preconditioning, a preformed long root length offered the best chance for survival. C120 container seedlings survived the dry soil conditions in the upper soil profile despite having similar morphology, including root dry weight, and a larger S:R than C90 seedlings.

In light of changing climate conditions and the anticipated increase of heat and drought stress to seedlings, it will remain increasingly important to identify the limitations to seedling establishment. Among the most common and most important limitations are root growth and soil moisture (Stone 1955; Burdett 1990; Grossnickle 2005); however, the length of time it takes seedlings to establish before the onset of drought is less clear. In this study, seedlings in D₀ were able to increase net photosynthetic rates within three weeks of planting. Between the second and third week, seedlings in D₁₅₀ began to see the abrupt decline in soil moisture, while seedlings in D₃₀₀ were already 28 % lower in soil moisture than D₀; concomitantly, D₁₅₀ and D₃₀₀ net photosynthetic rates also plummeted. No matter the container depth, a large portion of the root system is in the upper soil profile. In the absence of surface soil moisture, large reductions in photosynthesis and consequently no new root growth will prevail. This evidence highlights the importance of upper-profile soil moisture, and how vital it is to capture the opportunity to plant while it remains. Had the seedlings been planted earlier, would there have been sufficient moisture and time to establish root systems deeper into the soil profile? While the length of the establishment period may vary with seedling quality or growing temperature, it seems critical to afford more than just two weeks before drastic declines in soil moisture occur.

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Chapter 2 - Tables

Table 2.1. Styroblock® (Beaver Plastics Ltd., Acheson, Alberta) container specifications used to produce *Pinus ponderosa* seedlings.

Container Designation	US Model (#)	Canadian Model (#)	Top Diameter (cm)	Cavities per Container	Cavity Depth (cm)	Volume (cm ³)	Cavities per m ²
C60	160/60	310B	3	160	10.4	60	756
C90	160/90	315B	3	160	15.1	90	756
C120	160/120	323A	3	160	22.7	120	756

Table 2.2. *Pinus ponderosa* morphological characteristics after 2006 harvest from the greenhouse ($N = 36$). Root-collar diameter (RCD) is the diameter of the main seedling stem approximately 1 cm above ground-line.

Container ^a	Height (cm)	RCD (mm)	Shoot dry Mass (g)	Root dry Mass (g)	Shoot:Root
C60	12.5 a ^b	3.2 a	1.6 a	1.1 a	1.5 ab
C90	16.3 b	3.8 b	2.4 b	1.6 b	1.4 a
C120	19.4 c	3.8 b	2.8 b	1.7 b	1.7 b

^aSee Table 2.1 for container descriptions and codes.

^bColumns with the same letter are not significantly different, Tukey adjusted ($P < 0.05$)

Table 2.3. Morphological characteristics of C120 *Pinus ponderosa* seedlings planted in winter wheat (*Triticum aestivum*) competition treatments ($N = 18$). Measurements were recorded after the 2007 growing season (fall 2007). Root-collar diameter (RCD) is the diameter of the main seedling stem approximately 1 cm above ground-line.

Treatment ^a	Height (cm)	RCD (mm)	Needle Length (mm)
D ₀	28.3 a ^b	6.6 a	95.3 a
D ₁₅₀	24.4 a	4.0 b	16.0 b
D ₃₀₀	23.9 a	4.3 b	24.5 b

^aD₀, D₁₅₀, and D₃₀₀ = 0, 150, and 300 wheat plants per m²

^bColumns with the same letter are not significantly different, Tukey adjusted ($P < 0.05$)

Table 2.4. Morphological characteristics of *Pinus ponderosa* seedlings planted in the D₀ winter wheat (*Triticum aestivum*) competition treatment (0 plants m⁻²; $N = 48$). Measurements were recorded after the 2007 growing season (fall 2007). Root-collar diameter (RCD) is the diameter of the main seedling stem approximately 1 cm above ground-line.

Container ^a	Height (cm)	RCD (mm)	Shoot dry Mass (g)	Root dry Mass (g)	Current Year Needle Dry Mass (g)	Root Egress (cm)
C60	20.0 a ^b	6.2 a	7.5 a	6.4 a	3.7 a	31.4 a
C90	25.0 b	6.5 a	9.8 b	7.2 a	4.9 a	30.8 a
C120	28.3 c	6.6 a	10.5 b	7.2 a	5.0 a	30.3 a

^aSee Table 2.1 for container descriptions and codes.

^bColumns with the same letter are not significantly different, Tukey adjusted ($P < 0.05$)

Chapter 2 - Figures

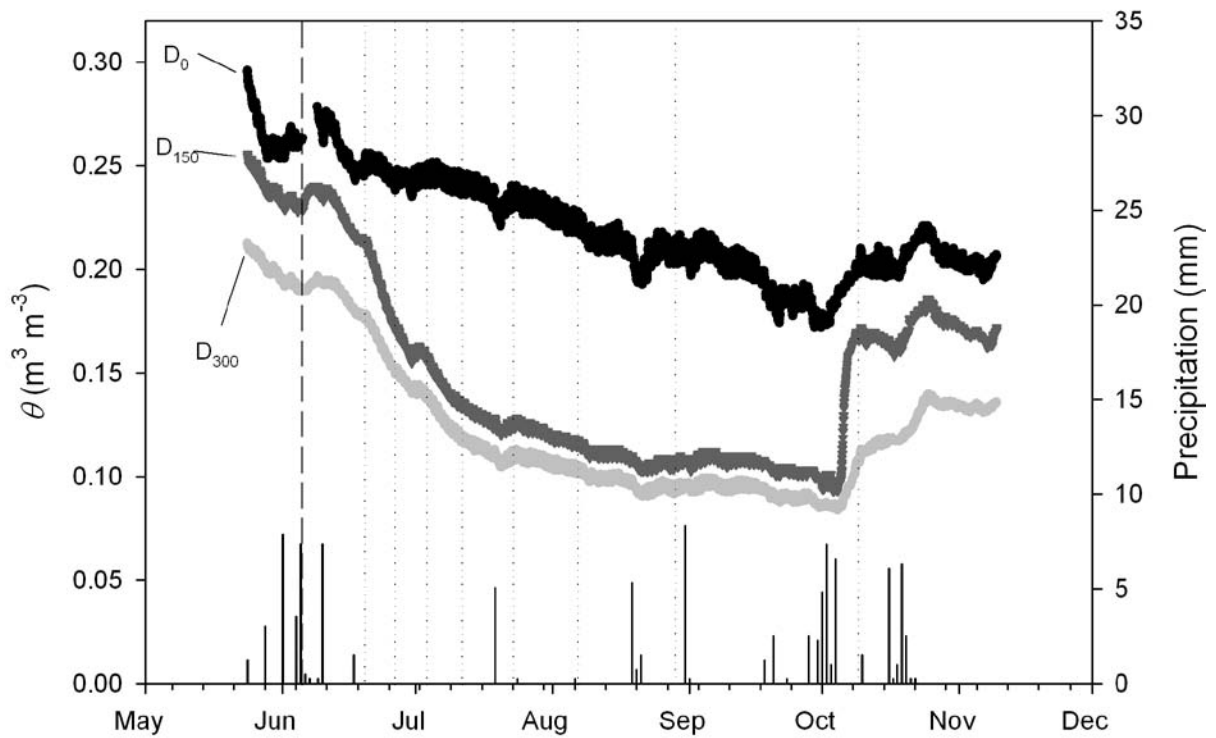


Figure 2.1. Vegetation competition treatment volumetric soil moisture (black and grey lines) and precipitation measurements (bars) during the 2007 growing season. Soil moisture measurements were recorded hourly and are from 17 cm below the soil surface. Heavy vertical dashed line indicates seedling planting (June 5); light vertical dashed lines indicate dates of photosynthesis measurements. D_0 , D_{150} , and D_{300} represent winter wheat (*Triticum aestivum*) vegetation competition treatment planting densities of 0, 150, and 300 plants m^{-2} , respectively.

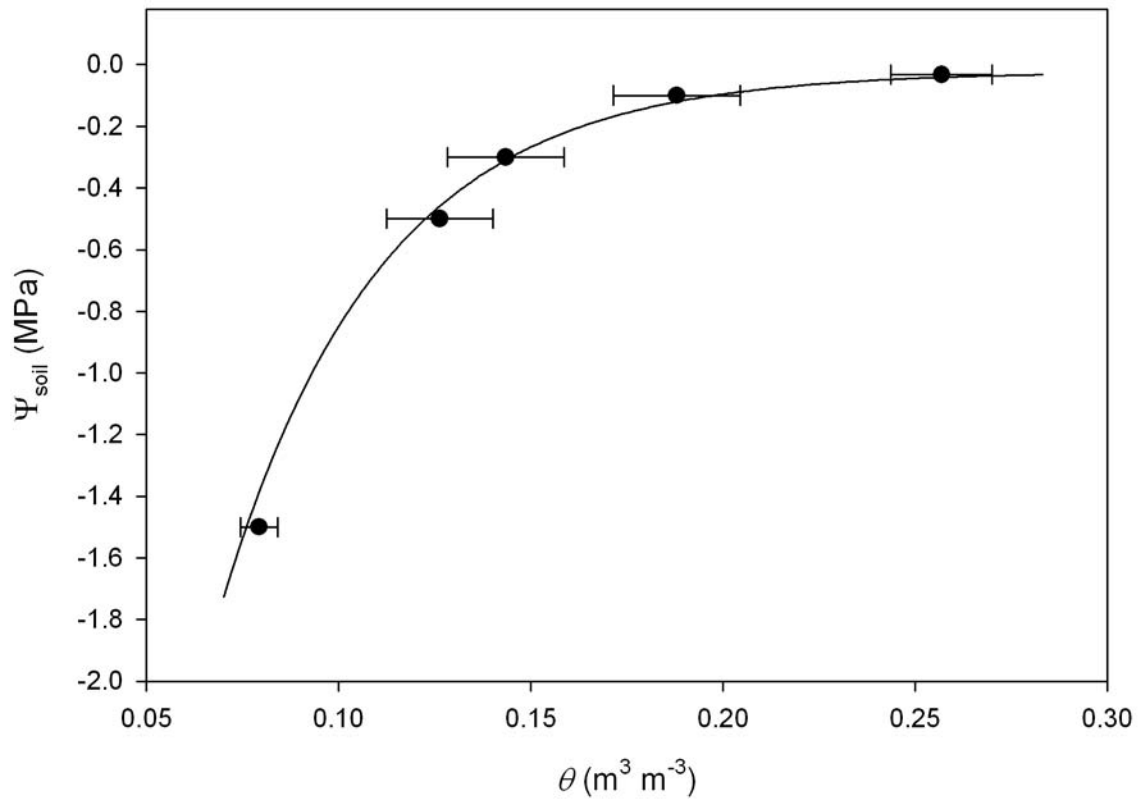


Figure 2.2. Volumetric soil moisture (θ) and water potential (Ψ_{soil}) regression for sandy loam soils within experimental plots at the USDA Forest Service nursery in Coeur d'Alene, Idaho. Points represent means and standard errors of 3 replications tested at 5 pressures. A three parameter, single exponential rise to maximum model ($\Psi_{soil} = -9.39 + 9.37(1 - e^{-24.3\theta})$; $R^2 = 0.90$; $se = 0.17$) was fit to the data.

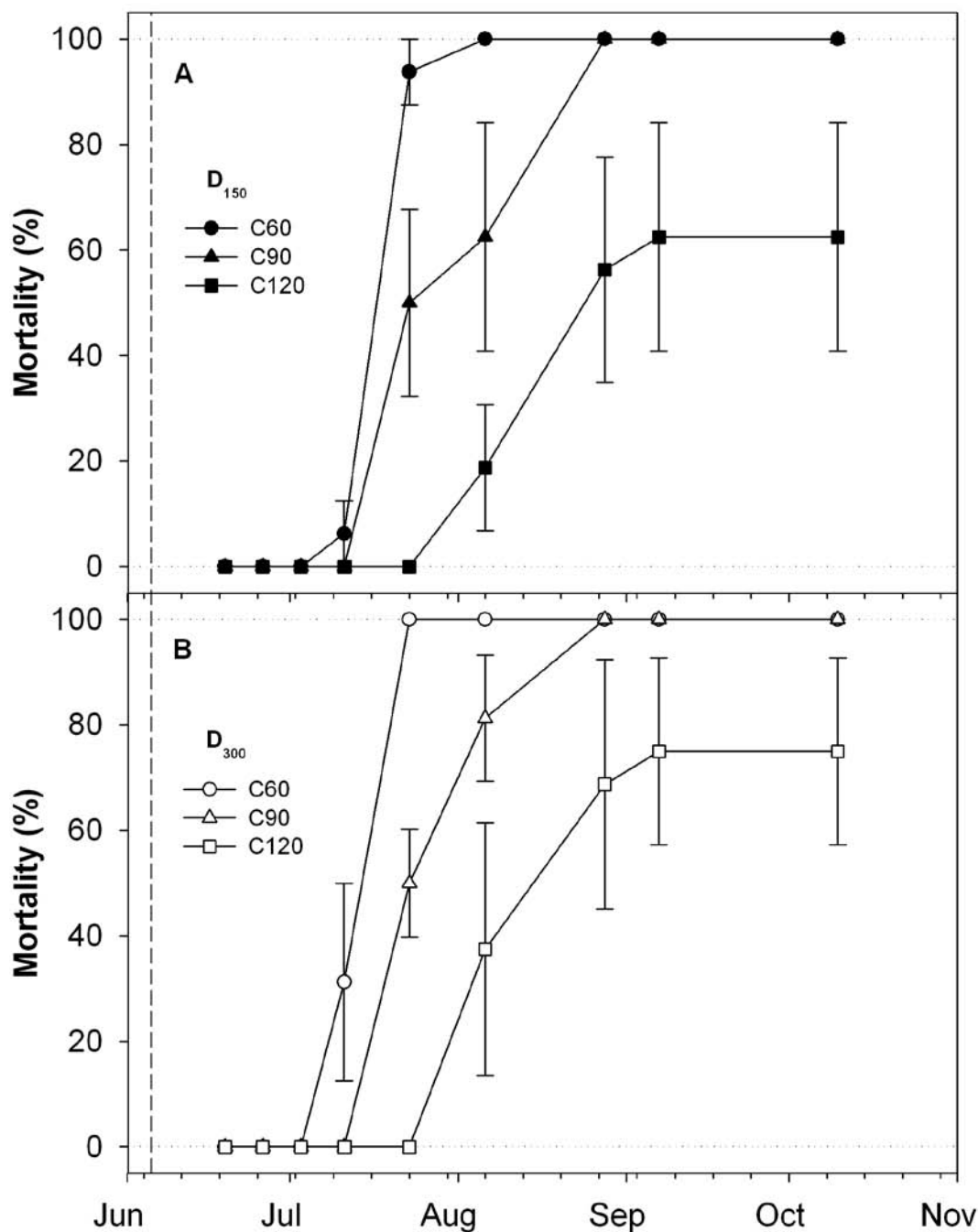


Figure 2.3. Mortality levels for C60 (circles), C90 (triangles), and C120 (squares) seedlings in D₁₅₀ (filled symbols, **A**) and D₃₀₀ (open symbols, **B**) competition treatments over the course of the 2007 growing season. Heavy vertical dashed line indicates time of seedling planting (June 5). No mortality was observed in D₀. Container volumes of 60, 90, and 120 cm³ are represented by C60, C90, and C120, respectively. Competition treatments of 0, 150, and 300 winter wheat (*Triticum aestivum*) plants m⁻² are represented by D₀, D₁₅₀, and D₃₀₀, respectively.

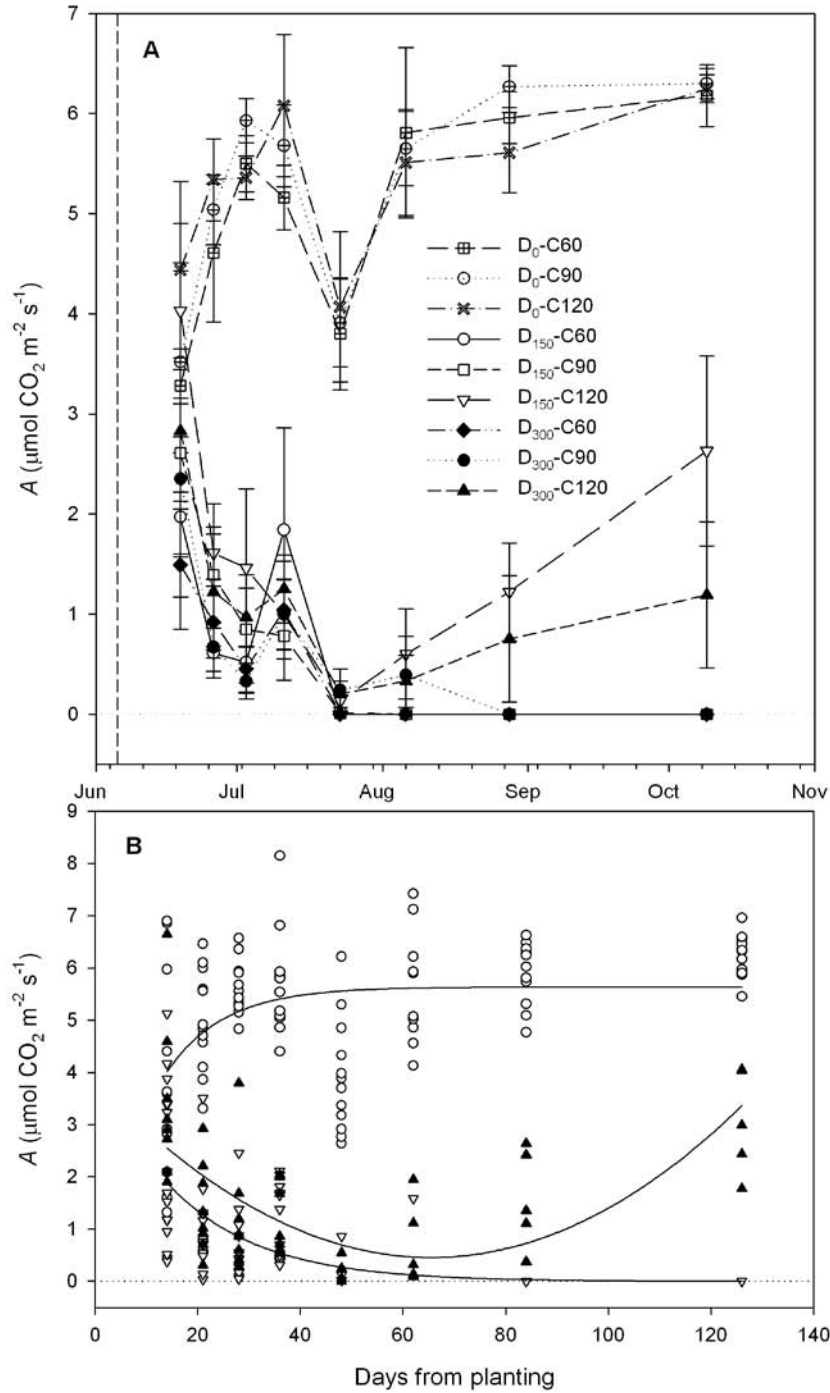


Figure 2.4. (A) Net photosynthesis for all containers in all competition treatments. (B) Net photosynthesis for all D_0 seedlings (open diamonds; $A = 5.6374(1 - e^{(-0.0887x)})$; $R^2 = 0.17$; $se = 1.22$), D_{150} and D_{300} C60 and C90 seedlings (open inverted triangles; $A = 4.2168(e^{(-0.0571x)})$; $R^2 = 0.17$; $se = 1.04$), and D_{150} and D_{300} C120 seedlings (closed circles; $A = 3.8533 - 0.1037x + 0.0008x^2$; $R^2 = 0.39$; $se = 1.09$). Regressions show general seasonal trends. Container volumes of 60, 90, and 120 cm^3 are represented by C60, C90, and C120, respectively. Competition treatments of 0, 150, and 300 winter wheat (*Triticum aestivum*) plants m^{-2} are represented by D_0 , D_{150} , and D_{300} , respectively.

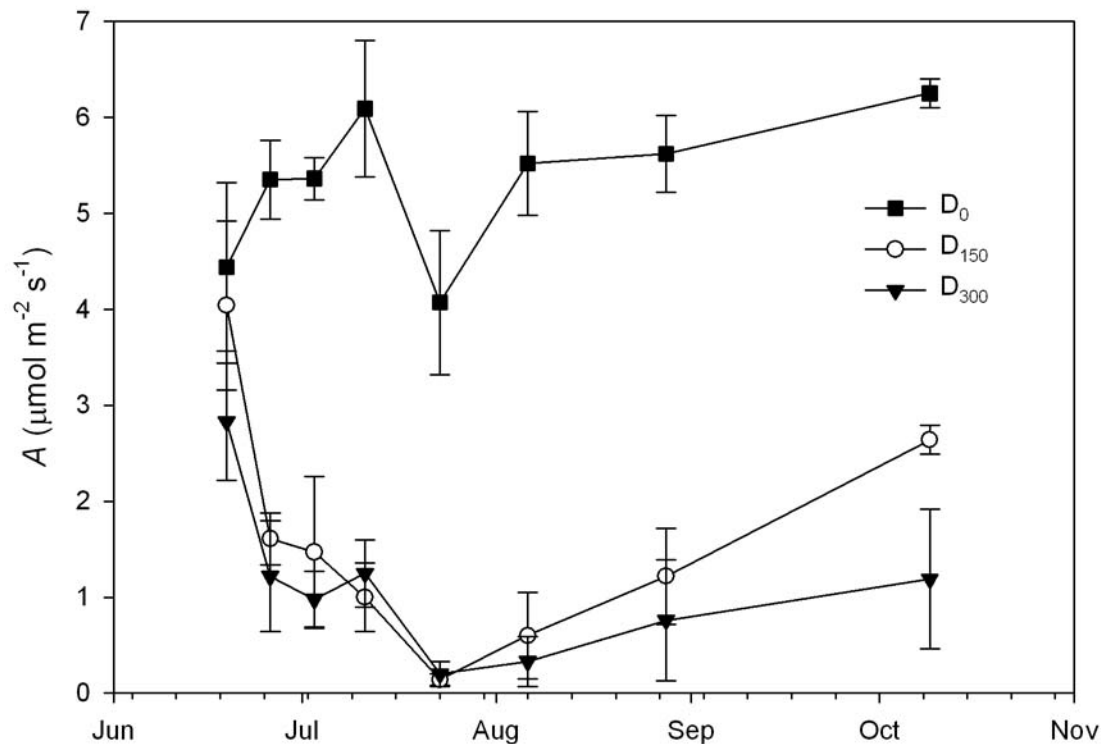


Figure 2.5. Net photosynthesis for C120 containers in competition treatments (D₀, D₁₅₀, and D₃₀₀) during the 2007 growing season. Each point represents a mean $\pm$ SE; $n = 4$ except on the last measurement period where D₁₅₀ had $n = 3$ and D₃₀₀ had $n = 2$. Competition treatments of 0, 150, and 300 winter wheat (*Triticum aestivum*) plants m⁻² are represented by D₀, D₁₅₀, and D₃₀₀, respectively.

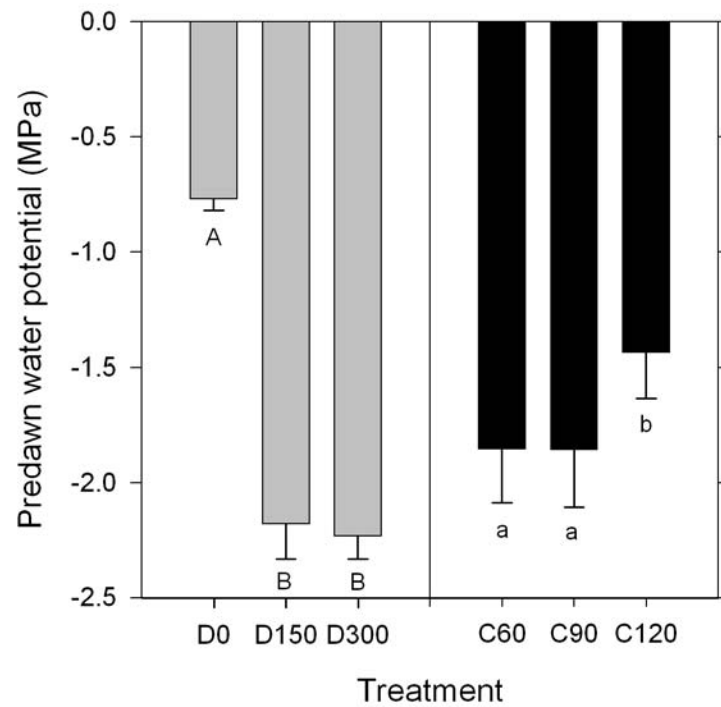


Figure 2.6. Predawn water potential measurements (Ψ_{nwp}) taken on 29 June 2007. Grey bars indicate whole-plot competition treatment effects; black bars indicate split-plot container treatment effects. Bars show least squares mean + SE. For each treatment effect, bars with the same letter are not significantly different, Tukey adjusted ($P < 0.05$). Container volumes of 60, 90, and 120 cm³ are represented by C60, C90, and C120, respectively. Competition treatments of 0, 150, and 300 winter wheat (*Triticum aestivum*) plants m⁻² are represented by D₀, D₁₅₀, and D₃₀₀, respectively.

Chapter 3. Soil moisture limitations to seedling establishment in the Inland Northwest

Abstract

Seedling establishment following outplanting relies heavily on soil moisture and a seedling's ability to access it. In the Inland Northwest, the summer season brings drought conditions causing low soil moisture in the upper soil profile—a potentially large barrier to seedling establishment. Temporal and spatial variability in soil moisture, and their effects on growth and gas exchange, were studied on three ponderosa pine (*Pinus ponderosa* Laws. var. *ponderosa*) stock types in northeastern Washington. The stock types were grown in the greenhouse and varied in container size, primarily depth. Soil moisture reached minimum volumetric soil moisture contents (θ) of $0.08 \text{ m}^3 \text{ m}^{-3}$ at a 15 cm depth and $0.15 \text{ m}^3 \text{ m}^{-3}$ at 90 cm by late September. Using soil water retention curves generated in the laboratory, we estimated that soil water potential (Ψ_{soil}) reached minima of -1.55 and -0.27 MPa for 15 and 90 cm depths, respectively. Seedling photosynthesis (A) and transpiration (E) rates followed soil moisture trends, also reaching seasonal lows in September. In early October, gas exchange rates nearly doubled following a replenishment of upper-profile soil moisture by precipitation. Over the course of the growing season, stock types did not differ in gas exchange rates ($P \geq 0.15$), biomass ($P \geq 0.45$), or root penetration depth ($P = 0.60$). For all seedlings, current-year needles showed greater capacity for A than previous-year needles ($P \leq 0.01$). Stock type showed no differences on this site in this year, suggesting that seedling access to soil moisture was not different among them. However, the temporal and spatial variation in soil moisture availability helps explain why it is critical for early seedling growth to occur while soil moisture is still available.

Introduction

Limited soil moisture, and a planted seedling's limited access to it, are considered two of the main limitations to plantation establishment (Rietveld 1989; Burdett 1990; Grossnickle 2005). In the Inland Northwest USA, the transition from spring to summer brings the onset of the dry season (Waring and Franklin 1979) and drying soil for seedlings. Reduced precipitation combined with plant transpiration commonly decrease soil moisture below -1.5 MPa in the upper soil profile, which is too low for seedlings to easily access

(Meinzer et al. 2004; Warren et al. 2005). These reduced soil moisture conditions may be viewed as a significant barrier to artificial reforestation success, especially east of the Cascade mountain range and in lower elevations.

Seedlings have some capacity to deal with summer drought by changing their root system architecture. Naturally regenerated seedlings can grow a deep taproot and long laterals (Burdett et al. 1984; Zwieniecki and Newton 1994; Kolb and Robberecht 1996a), thereby exploiting any soil moisture not used by competing vegetation or depleted by evaporation (Pearson 1930; Van Haverbeke 1963). Conversely, when seedlings are first planted, their root systems are confined to a planting hole and are limited in soil moisture uptake by their initial root size. To survive summer drought, planted seedlings must either close their stomata to conserve water or grow new roots out of the planting hole into areas where soil moisture is more available (Burdett 1990). In situations where new root growth is insufficient to mitigate moisture stress, stomatal closure can persist, leading to reduced photosynthesis, further reducing root growth (van den Driessche 1987; Burdett 1990). For planted ponderosa pine seedlings, seasonal effects of drought may be seen for years after planting (Irvine et al. 2002).

Understanding and estimating soil moisture patterns during the first year of seedling establishment is valuable. Just knowing the seasonal moisture patterns and soil texture can offer insight on soil water capacity, soil water availability, and therefore, how quickly it can be depleted (Campbell and Norman 1998; Dingman 2002; Gardiner and Miller 2004). This knowledge not only applies to surface soil characteristics, where a newly planted seedlings roots are placed and where new root elongation is needed to overcome transplant shock, but also to vertically within the soil profile, where seedlings will be potentially growing roots throughout the first season of establishment. The importance of this vertical change in soil moisture within the soil profile can be seen in data from Warren et al. (2005). In that study, it was demonstrated that soil water potential and volumetric soil water content (θ) increased with soil depth; as a result, water potential values 60-100 cm below the soil surface remained high and plant-available throughout the seasonal drought. To an establishing seedling, this means that root penetration to this depth can reduce drought effects.

The availability of water deep in the soil profile has led to the anecdotal advocacy of planting longer rooted seedlings to increase survival and growth. Only recently has scientific

evidence supported this idea (Chirino et al. 2008), especially in rapidly drying soil profiles with competing vegetation (Pinto 2009b). The theory behind planting longer-rooted seedlings is that earlier placement of roots deep in the soil profile allows seedlings to engage the Burdett (1990) model of establishment, growing out of the developing drought by rapid root elongation. This theory may be further extended to include growing out of the pressures of competing vegetation (Elliott and White 1987; Anderson et al. 2001; Pinto 2009b). Once the roots have escaped the pending drought, or vegetation pressure, the normal adverse effects of reduced soil moisture on gas exchange and biomass production in young seedlings (McMillin and Wagner 1995; Kolb and Robberecht 1996b; Zhang et al. 1997; Olivas-Garcia et al. 2000; Panek and Goldstein 2001; Pinto 2009b) may be reduced.

Placing a longer-rooted container on a site in the Inland Northwest may not always be the best choice to mitigate drought because of the planting problems associated with larger stock (Robert and Lindgren 2006). It is therefore crucial to understand all of the site limiting factors, especially soil moisture, and their effects on seedling establishment before committing to a unique stock type. To date, few if any studies have integrated soil moisture availability, both spatially and temporally, with its effects on seedling physiology and biomass allocation. Furthermore, these effects have yet to be related to the available container technology in overcoming forest site limitations. Using ponderosa pine (*Pinus ponderosa* Laws. var. *ponderosa*), the objectives of this study aim to (i) quantify temporal and spatial attributes of soil moisture on a planting site, (ii) track seasonal changes in seedling gas exchange, and (iii) relate changes in soil moisture to resultant changes in growth.

Materials and Methods

Seedling Preparation

In 2006, ponderosa pine seedlings from a Confederated Tribes of the Colville Indian Reservation seed source (Colville Tribal Forestry, Lower Stepstone: stand #1, 610 m elevation) were grown in a greenhouse using three types of Styroblock[®] containers. These container types differed only in cavity depth (not in diameter or density); consequently, depth differences converted directly into volume differences (Table 3.1). Seedlings were grown with container-specific regimes to achieve uniform physiological characteristics (Pinto

2005). This was done to ensure that results would reflect container type, as nearly as possible, and not be confounded by an interaction with nursery practices.

Site Description

The Coyote Creek planting site (CC) is an operational planting unit located approximately 13 km northwest of Nespelem, WA (48.2376 °N, 119.1272 °W; 890 m) on the Confederated Tribes of the Colville Indian Reservation. On 1 May 2007, the greenhouse grown seedlings were hand-planted on the Coyote Creek outplanting site. The three container types used in this study were part of a larger stock type study using three additional container types and were intermixed within a complete randomized block design (Pinto 2009a). Within this design, 20 seedlings from each container type were planted into five blocks (100 total seedlings per container type). Seedlings were planted 1.5 m apart within rows, while rows had 3 m spacing. The open-range, partial-cut site received minimal site preparation (concentrated slash piling) prior to planting.

CC soils are classified in the Bearspring series of Mollisols; they are deep, well-drained, loamy-skeletal soils formed in colluvium and residuum from granitic rock and capped with a thin mantle of volcanic ash. Texture of the upper profile (0.3 m) is mostly loam, it becomes coarser with depth, shifting to sandy loam, gravelly, and finally extremely gravelly (0.3 to 1.5 m). These soils are classified as moderate in available water holding capacity, averaging about $0.12 \text{ m}^3 \text{ m}^{-3}$ to a depth of 1.3 m (NRCS 2009).

Edaphic and Atmospheric Monitoring and Sampling

An on-site weather station (model 900ET, Spectrum Technologies, Inc., Plainfield, IL) collected hourly measurements of temperature (°C), relative humidity (%), and rainfall (mm) 1 m above the ground via data logger. Vapor Pressure Deficit (VPD) was determined from calculated saturation vapor pressure using known ambient temperature and relative humidity (Campbell and Norman 1998). Volumetric soil moisture (θ) and soil temperature measurements were collected hourly *in situ* at 5 depths (15, 30, 50, 70, and 90 cm) from the soil surface using ECH₂O EC-5 soil moisture sensors connected to Em50 data loggers (Decagon Devices, Inc., Pullman, WA) and iButtons® (Maxim Integrated Products, Inc., Sunnyvale, CA). Edaphic and atmospheric measurements are summarized in Table 3.2.

Seasonal trends in soil moisture are graphed in Figure 3.1 while temperature and VPD trends are shown in Figure 3.2.

Soil samples were taken at each depth where soil moisture probes were installed. Each soil sample was split into two subsamples. The first subsample was used to generate soil specific calibration equations to improve *in situ* soil moisture data (Starr and Paltineanu 2002; Cobos 2007). Raw sensor data from laboratory measurements on soil samples were plotted against measured θ to generate the equations. Raw data collected from the field were then inserted into each respective calibration equation for final, corrected θ values. The accuracy for each probe type with calibration is $\pm 0.02 \text{ m}^3 \text{ m}^{-3}$ (Decagon Devices, Inc., Pullman, WA). Soil sample bulk density was measured using the technique of Blake and Hartge (1986), and θ was calculated by multiplying bulk density by the gravimetric soil moisture content. The second subsample was used to generate a soil water retention curve (SWRC) using a WP4 Dewpoint PotentiaMeter (Decagon Devices 2006; Decagon Devices, Inc., Pullman, WA). The measured water potentials were plotted against θ , and regression equations were fitted to predict soil water potential (Ψ_{soil}) in the field.

Seedling Gas Exchange and Biomass Allocation

Using a portable photosynthesis system (model LI-6400, Li-Cor, Lincoln, NE) equipped with a blue/red LED light source and CO_2 injector, seedling gas exchange was measured five times from July to mid-October during the 2007 growing season. For each gas exchange measurement, one to three seedlings from each container type and treatment were randomly chosen from each block. On the selected seedling, two secondary needle fascicles were placed in the chamber for *in situ* measurements. For each measurement during the entire growing season, the portable photosynthesis system chamber was set at $1400 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (photosynthetic active radiation), 25° C , $400 \mu\text{mol mol}^{-1} \text{ CO}_2$, and an air flow rate of $400 \mu\text{mol}$. Leaf area calculation was adapted from the methods of Svenson and Davies (1992). Fascicle diameters were measured in the center of each fascicle segment that was placed inside the LI-6400 light chamber. Using the assumption that three needles of a fascicle form a cylinder (Johnson 1984), we could calculate the abaxial leaf area by multiplying the cylinder circumference by the length of the needle inside the chamber. Total adaxial leaf area is calculated by multiplying the radius of the fascicle by the length of needle in the

chamber and multiplying the result by six (six is the total number of adaxial surface areas of one needle fascicle). We used the following equation to calculate leaf area for one needle fascicle:

$$LA = (\pi dl) + \left(6 \frac{d}{2} l\right)$$

where d is the diameter of the three-needle fascicle, and l is the length of needle inside the chamber. The length of the needle segments in the chamber was always 30 mm, so the equation simplifies to $LA = 184.2d$.

To mitigate changes in VPD inside the LI-6400 chamber during gas exchange measurements, and the subsequent effects on stomatal conductance (Running 1976; Meinzer 1982), transpiration fluxes were calculated using ambient VPD. We assumed seedling leaves (outside the chamber) would be well coupled to the atmosphere; transpiration was estimated using stomatal conductance measurement from the LI-6400 and the calculated VPD measurement from the on-site weather station ($E = g_s(D/p_a)$; adapted from Hubbard et al. 1999).

Gas exchange measurements were performed on previous-year needles (2006) until new needles were long enough to measure inside the LI-6400 chamber. On two dates, both new and old needles were measured for comparison. On 16 and 26 October 2007, three seedlings from each replication $\times$ container combination were sampled for biomass measurement after careful excavation with a shovel. After gently removing soil and medium, biomass was determined after partitioning seedlings into sections (roots, stems, and needles) and drying to a stable mass at 60° C. Whole seedling gas-exchange estimates of photosynthesis (A) and transpiration (E) were calculated for the last measurement period after leaf biomass was measured in the laboratory. Total leaf biomass was converted to total leaf area using the specific leaf area (SLA) value of 51.6 cm² g⁻¹ from a nearby population of ponderosa pine (Zhang and Marshall 1995); because this SLA represents a one-sided projected leaf area, the value was multiplied by 2.5, based on trigonometric analysis (Marshall 2009), to match the three-dimensional value of leaf area measured in this study (adapted from Hultine and Marshall 2001 and Wykoff 2002).

Statistical Analysis

The analysis of variance, using PROC MIXED in SAS (v 9.1.3, SAS Institute, Cary, NC), was performed for a randomized complete block design to identify differences among containers for seedling biomass (roots, shoots, needle dry weight, and root length) and total seedling gas exchange. Correcting for experiment-wise error rates, multiple comparisons among container type biomass were analyzed using Tukey's mean separation test. Container type gas exchange data were analyzed using the repeated measures function in PROC MIXED; t-test analysis was used to detect differences in gas exchange between needle years. Linear and non-linear regression analyses were performed on water potential data using SigmaPlot 10 (Systat Software, Inc., Point Richmond, CA). Natural log transformations on water potential data were found to improve model prediction, and model selection was based on the highest value for the best-fit adjusted R^2 . Pearson correlation coefficients were produced for each parameter generated by the models. All results were tested at a significance level of $\alpha = 0.05$.

Results

Edaphic Sampling

Soil water retention curves (SWRCs) varied with soil depth (Figure 3.3). Best-fit regression models were either quadratic or linear depending on depth; selected models explained 72 to 98 % of the variation in Ψ_{soil} (Table 3.3). Soils were driest in September, at the end of the summer drought. At this time, Ψ_{soil} predictions at 15 cm reached a low of -1.33 MPa, while Ψ_{soil} at 30, 50, and 70 cm were -0.25, -0.50, and -0.27 MPa, respectively (Table 3.3). Due to the parabolic shape of the curves, extrapolation into wetter soil conditions yielded improbable Ψ_{soil} predictions. The parabolas reversed direction under the wetter soil conditions, yielding more negative Ψ_{soil} . Therefore all extrapolations were based on linear models, which still yielded high R^2 values (≥ 0.89). For comparison, linear model Ψ_{soil} predictions for 90 cm were -0.02 MPa compared to quadratic predictions of -1.20 MPa.

Seedling Gas Exchange and Biomass Allocation

Gas exchange values changed over time in the old needles, making the date effect significant for both A and E ($P \leq 0.0001$). Over the first three measurement periods, net photosynthetic rate (A) decreased by 61% on average, while corresponding soil moisture at 15 cm decreased by 34%. In October, A showed signs of recovery after several small pulses of precipitation recharged surface soil moisture (Figure 3.4, A and Figure 3.1). Seedling transpiration rates (E) followed a similar pattern from July through October, with a 55% reduction observed over the first three measurements (Figure 3.4, B). As both A and E were declining in old needles, new needles elongated and became long enough for gas exchange measurements. As seen with old needles, significant differences were detected among dates for both new needle A and E ($P \leq 0.0001$). On the second and third measurements date, gas exchange was measured on old and new needles. For these dates, new needle A was 47 and 66% greater, respectively, in new than in old needles ($P \leq 0.01$); new needle E was 48% greater on the first date ($P = 0.001$) and was not significant on the second ($P = 0.17$; Figure 5).

Repeated measures analysis of A and E on old needles showed no container effect ($P = 0.17$ and $P = 0.15$, respectively) and no container $\times$ date interaction ($P = 0.72$ and $P = 0.94$, respectively). No new needle differences were observed among containers from early August to mid-October for either A or E ($P = 0.11$ and $P = 0.06$, respectively) and the container $\times$ date interaction was absent ($P = 0.90$ and $P = 0.43$, respectively). In October, whole-seedling gas exchange calculations showed no differences in A or E ($P = 0.92$ and $P = 0.19$, respectively; data not presented). Container types were not significantly different in biomass or root egress after one season of field growth ($P \geq 0.45$).

Discussion

When seedlings were planted in May, θ was at its highest seasonal value at all measured depths. This is typical for spring conditions in the Northwest USA (Warren et al. 2005), where snowmelt typically recharges the entire soil profile. Shortly thereafter, large and continual decreases in θ were seen at the 15 and 30 cm soil depths (Figure 3.1). The pronounced decreases are probably due to the well-drained, loamy-skeletal soils, low precipitation inputs, and the lack of site preparation to control competing vegetation. Deeper

in the soil profile, the change in θ was less pronounced, which is likely attributable to the limited distribution of roots at these depths (Jackson et al. 1996).

In general, seasonal trends in A followed those of upper-profile soil moisture, decreasing until it reached a minimum in September. Similarly, and as expected, E followed A reaching seasonal minima at the same time as surface-soil moisture reached its minimum. According to Burdett's (1990) model of seedling establishment, when water uptake is insufficient to allow leaf conductance and therefore photosynthesis, root growth and water uptake has been compromised. The reductions in gas exchange, coupled with the reduction in soil moisture, indicate that seedling root systems may have not yet expanded enough to sustain the transpiration levels seen in older, established trees (Weltzin and McPherson 1997; Irvine et al. 2002). Ponderosa pine is considered a drought-tolerant species; one mechanism by which it accomplishes this is highly sensitive stomatal control of water loss (Maherali and DeLucia 2000; Pinol and Sala 2000). Recently planted seedlings with restricted root distributions may have to exercise increased stomatal control of water loss to maintain internal water balance, and avoid permanent losses of hydraulic transport capacity in stems (Kavanagh and Zaerr 1997). Although this means immediate reductions in carbon gain, the long-term protection of functional xylem allows the seedling to resume active growth once favorable soil moisture conditions return, as observed here in the October data.

The 140% increase in root biomass (Table 3.4) and initial gas exchange measurements (Figure 3.2) support the assumption of adequate root-soil contact and root egress into the surrounding soil were initially adequate. Because rocky soils made root harvesting difficult, it is probable that the root systems were insufficiently harvested and almost certain that their depths were underestimated. Despite this, root depths were measured at least 26 cm beyond the shortest root plug (Table 3.4). As a consequence of root growth, levels of A recorded early in July were similar to the levels seen before the onset of moisture stress by establishing ponderosa pine seedlings (Pinto 2009b). Further evidence of good initial root-soil contact is the elongation of new needles (Burdett et al. 1984; Pinto 2009b). As seen in several European pine species (e.g. Escudero and Mediavilla 2003), A measured for new needles was significantly higher than for old needles. Although the increase in needle surface area represents greater potential for moisture loss, the potential for more photosynthesis, and increased rates of carbon gain, might have allowed the seedlings to

successfully engage and sustain the Burdett model of seedling establishment through root growth. However, in our study, root growth was apparently not sufficient for this and the seedlings gradually lost contact with the moister soils at depth. Nevertheless, the extra needles still provided some benefit with the return of soil moisture in October and recovery of photosynthesis (Figure 3.4). The new needles may have allowed the seedlings to have more root growth into late fall and early winter before gas exchange and soil temperatures decline to seasonal lows (Burr 1990). This additional root growth could, in turn, give seedlings even greater opportunity for growth the following season as ponderosa pine seedlings have the potential for late and early season photosynthesis (Marshall et al. 2001).

Our estimates of Ψ_{soil} on the CC site supported the impression of limited free water available to plants, especially near the soil surface. In general, Ψ_{soil} increased with soil depth. This data paralleled earlier observations, for example by Warren et al. (2005) for an old-growth ponderosa pine site. Predicted minimum Ψ_{soil} values of -1.55 MPa were observed at 15 cm. It is possible that θ above this point was lower due to both water uptake by vegetation as well as evaporation from the soil surface (Pearson 1930). The shape of the SWRCs generated for CC soils show that small decreases in soil moisture in dry conditions cause large changes in Ψ_{soil} (Figure 3.3). Using the fitted model parameters (Table 3.3), a 2% reduction in θ yields a Ψ_{soil} of -4.17 MPa, similar to values seen by Kolb and Robberecht (1996b) at depths above 25 cm. Considering that a large portion of a newly planted seedling's root system lies above this point, it is reasonable to assume roots have little access to free available water and the seedling is therefore under moisture stress. One route for mitigation of this water limitation is the growth of seedling roots deeper into the soil profile. Seedlings growing on the CC site showed root penetration to at least 36 cm (Table 3.4) where predicted Ψ_{soil} was -0.25 MPa. From this level downward, the lowest Ψ_{soil} reached only -0.50 MPa—representing considerably more favorable environments for root water uptake.

Pinto (2009b) measured similar upper profile Ψ_{soil} minima and analogous trends in gas exchange in an experiment that tested the survival and growth responses of ponderosa pine seedlings grown in containers of various sizes. That experiment found that seedlings grown in deeper containers, with longer root systems, showed better survival than seedlings grown in small containers with shorter root systems. Likewise, longer root systems have

improved survival and growth of oak (*Quercus suber*) seedlings planted in Spain (Chirino et al. 2008). However, in the current experiment, the seedlings showed no survival or gas exchange benefits due to the longer roots from deeper containers. Limitations to a planted seedling's establishment include not only soil moisture, but also air and soil temperature. After planting, a seedling's shoots may be exposed to unfavorable temperatures (Larson 1967) including those well below 0° C (Burr et al. 1989). Conversely, temperatures may rise to lethally high values, especially near the soil surface (Kolb and Robberecht 1996a). Similarly, seedling roots may be faced with high or low temperatures that inhibit growth (Larson 1967; Lopushinsky and Max 1990) and interfere with a seedling's ability to make root-soil contact (Grossnickle 2005). The temperatures observed after planting in this study indicate that seedlings were not exposed to extreme above- or below-ground temperatures (Figure 3.2, A and B). Although temperatures were low in the upper profile at planting time, temperatures had risen to non-limiting values (Lopushinsky and Max 1990) by the time seedlings came out of planting shock and were ready to grow roots (Burr et al. 1989).

Although the explicit consequences of vapor pressure deficit (VPD) on seedling establishment were not measured in this experiment, observed VPDs were high enough to cause stress. That stress might be expressed as reduction in stomatal conductance (Running 1976; Marshall and Waring 1984; Kavanagh and Zaerr 1997), or a reduction of *A* (Hubbard et al. 1999). Elevated nighttime VPD may also increase resistance to seedling growth and establishment. Evidence suggests that elevated nighttime VPDs can drive nocturnal transpiration and maintain low leaf water potential throughout the night (Kavanagh et al. 2007). Such low water potential might be particularly stressful for an establishing seedling in already dry conditions. The stress might also appear as xylem cavitation or heat damage. Current data shows evidence of elevated nighttime VPDs (Figure 3.2, C), but its impact on the seedlings is not known.

Artificially regenerated seedlings planted in forest environments are faced with a myriad of barriers before they become coupled with their new surroundings. In this study, soil moisture was seen to vary with space and time and have an impact on carbon gain by seedlings. The synchronicity of these critical factors can mean the difference between plantation success or failure. In the Inland Northwest, the seasonal drought is expected, but it can be ameliorated by moisture stored in the soil. This soil moisture reserve is key to

seedling establishment, especially on harsh sites. Such a reserve will become increasingly important in light of global climate change, where seasonal drought is expected to be longer or more intense, creating even higher barriers to seedling establishment and growth.

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Chapter 3 - Tables

Table 3.1. Styroblock[®] (Beaver Plastics Ltd., Acheson, Alberta) container specifications used to produce *Pinus ponderosa* seedlings.

Container Designation	US Model (#)	Canadian Model (#)	Top Diameter (cm)	Cavities per Container	Cavity Depth (cm)	Volume (cm ³)	Density per m ²
C60	160/60	310B	3	160	10.4	60	756
C90	160/90	315B	3	160	15.1	90	756
C120	160/120	323A	3	160	22.7	120	756

Table 3.2. Edaphic and atmospheric monitoring for Coyote Creek planting unit May 1 through October 14, 2007.

	Mean	Maximum	Minimum
Precipitation (mm)	135 ^a	10	0
Air Temperature (°C)	16.2	35.8	0.1
Vapor Pressure Deficit (kPa)	1.4	5.3	0
Soil Temperature (°C)			
15 cm	16.1	24.4	6.4
30 cm	15.1	19.9	7.6
50 cm	14.4	18.2	7.2
70 cm	13.7	17.1	7.1
90 cm	13.7	16.6	7.6
Volumetric Soil Moisture (m ³ m ⁻³)			
15 cm	0.19	0.34	0.08
30 cm	0.19	0.30	0.10
50 cm	0.15	0.24	0.07
70 cm	0.14	0.19	0.09
90 cm	0.18	0.22	0.15

^aTotal precipitation for measurement period

Table 3.3. Soil water retention curve model parameters for 5 depths on the Coyote Creek planting unit. Linear models are presented for all depths; quadratic models are presented only when they yielded better fits.

Soil Depth (cm)	n	Minimum Measured Site θ	Linear Model ^a				Quadratic Model ^b				Model Parameters		
			Ψ_{soil}	P	R^2	SE	Ψ_{soil}	P	R^2	SE	y_o	a	b
15	10	0.08	-2.10	≤ 0.0001	0.90	0.48	-1.33	≤ 0.0001	0.98	0.18	7.7	-138.1	574.7
30	14	0.10	-0.25	≤ 0.0001	0.87	0.49					4.9	-62.9	
50	9	0.07	-0.50	0.0024	0.72	0.84					2.7	-48.1	
70	9	0.09	-0.27	0.0009	0.79	0.65					3.0	-46.9	
90	11	0.15	-0.02	≤ 0.0001	0.82	0.54	-1.2	≤ 0.0001	0.89	0.42	4.6	-112.6	555.5

$$^a\Psi_{\text{soil}} = y_o + a*\theta$$

$$^b\Psi_{\text{soil}} = y_o + a*\theta + b*\theta^2$$

Table 3.4. *Pinus ponderosa* morphological and biomass characteristics after 2006 and 2007 growing season ($N = 45$). Seedlings were field planted in spring 2007. Root egress is defined as the length of root growth beyond the bottom of the root plug. Standard errors are in parentheses.

Container	Height (cm)	Height Growth (cm)	RCD (mm)	RCD Growth (mm)	Shoot Dry Mass (g)	Root Dry Mass (g)	Needle Dry Mass (g)	Root Egress (cm)
2006 ^a								
C60	12.5 (0.44) a ^b	—	3.2 (0.09) a	—	1.6 (0.08) a	1.1 (0.06) a	—	—
C90	16.3 (0.64) b	—	3.8 (0.11) b	—	2.4 (0.14) b	1.6 (0.09) b	—	—
C120	19.4 (0.84) c	—	3.8 (0.12) b	—	2.8 (.016) b	1.7 (0.10) b	—	—
2007								
C60	17.7 (0.40) a	6.6 (0.27) a	4.4 (0.08) a	1.0 (0.07) a	4.4 (0.43) a	3.3 (0.26) a	3.1 (0.32) a	26.4 (1.1) a
C90	22.2 (0.57) b	6.0 (0.32) a	4.7 (0.09) b	1.2 (0.07) a	5.0 (0.39) a	3.4 (0.29) a	3.3 (0.27) a	25.8 (8.8) a
C120	25.1 (0.49) c	6.9 (0.36) a	4.7 (0.09) b	1.1 (0.07) a	5.2 (0.59) a	3.8 (0.27) a	3.4 (0.43) a	29.1 (1.5) a

^aPinto 2009b

^bColumns with the same letter are not significantly different, Tukey adjusted ($P < 0.05$)

Chapter 3 - Figures

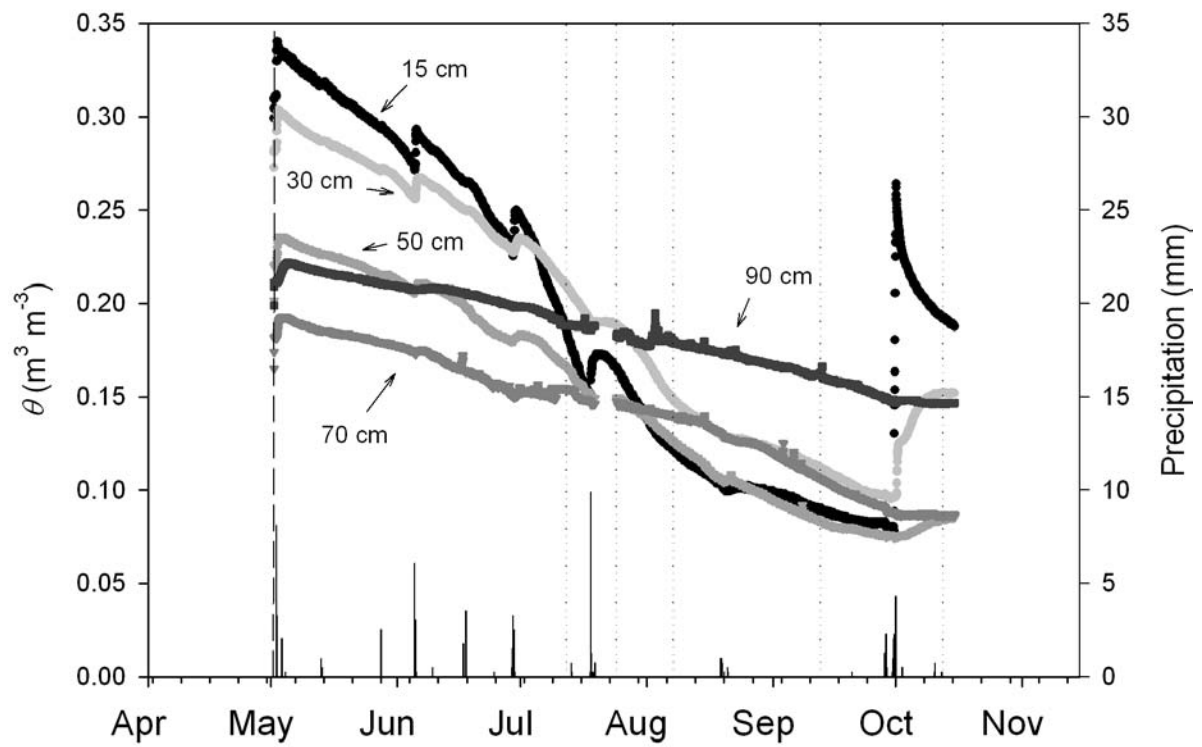


Figure 3.1. Volumetric soil moisture θ (black and grey lines) and precipitation measurements (bars) during the 2007 growing season. Heavy vertical line indicates seedling planting (1 May 2007); light vertical dashed lines indicate dates of gas exchange measurements.

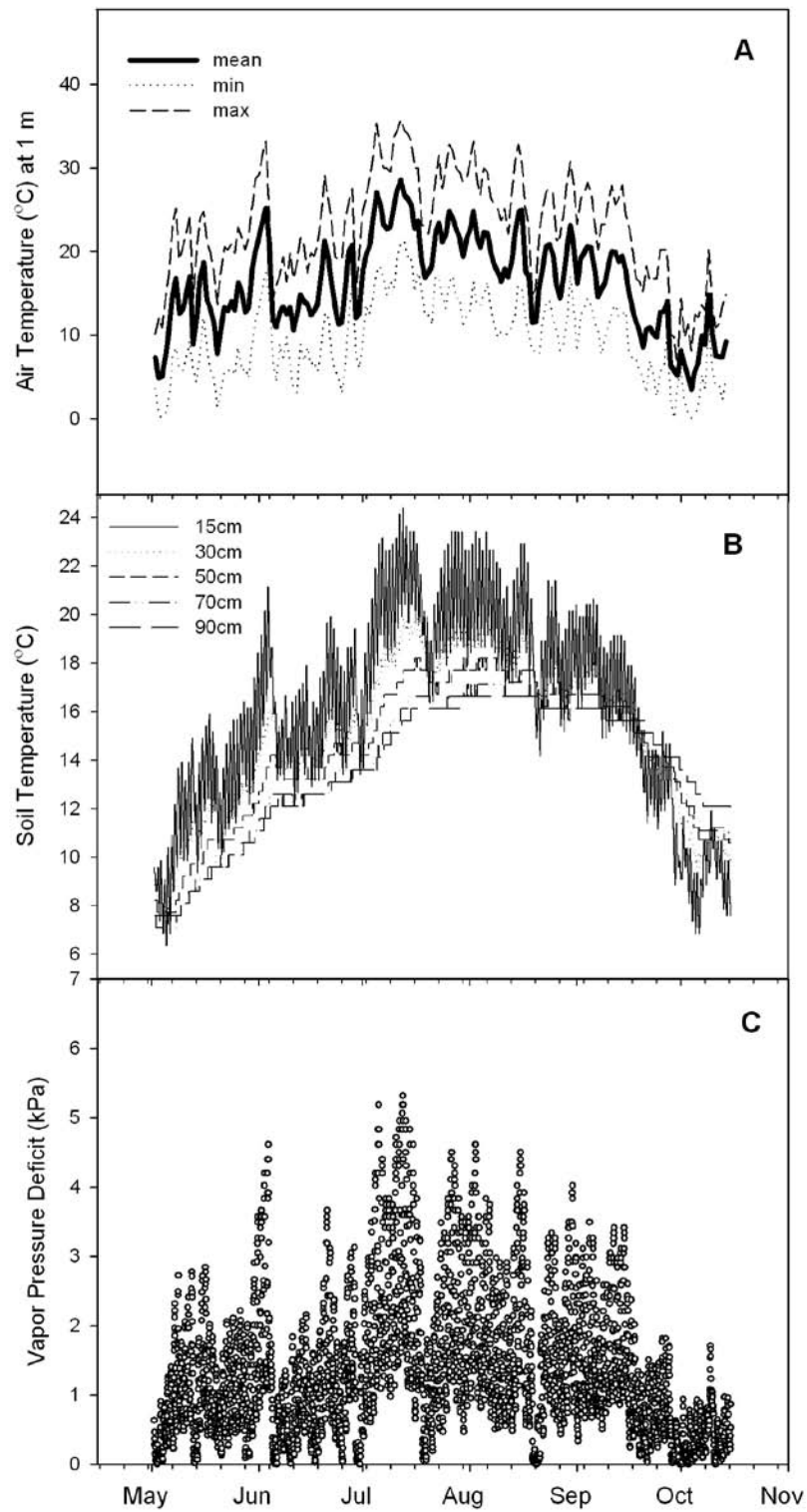


Figure 3.2. 2007 growing season (A) air temperature, (B) soil temperature, and (C) vapor pressure deficit conditions at the Coyote Creek planting unit.

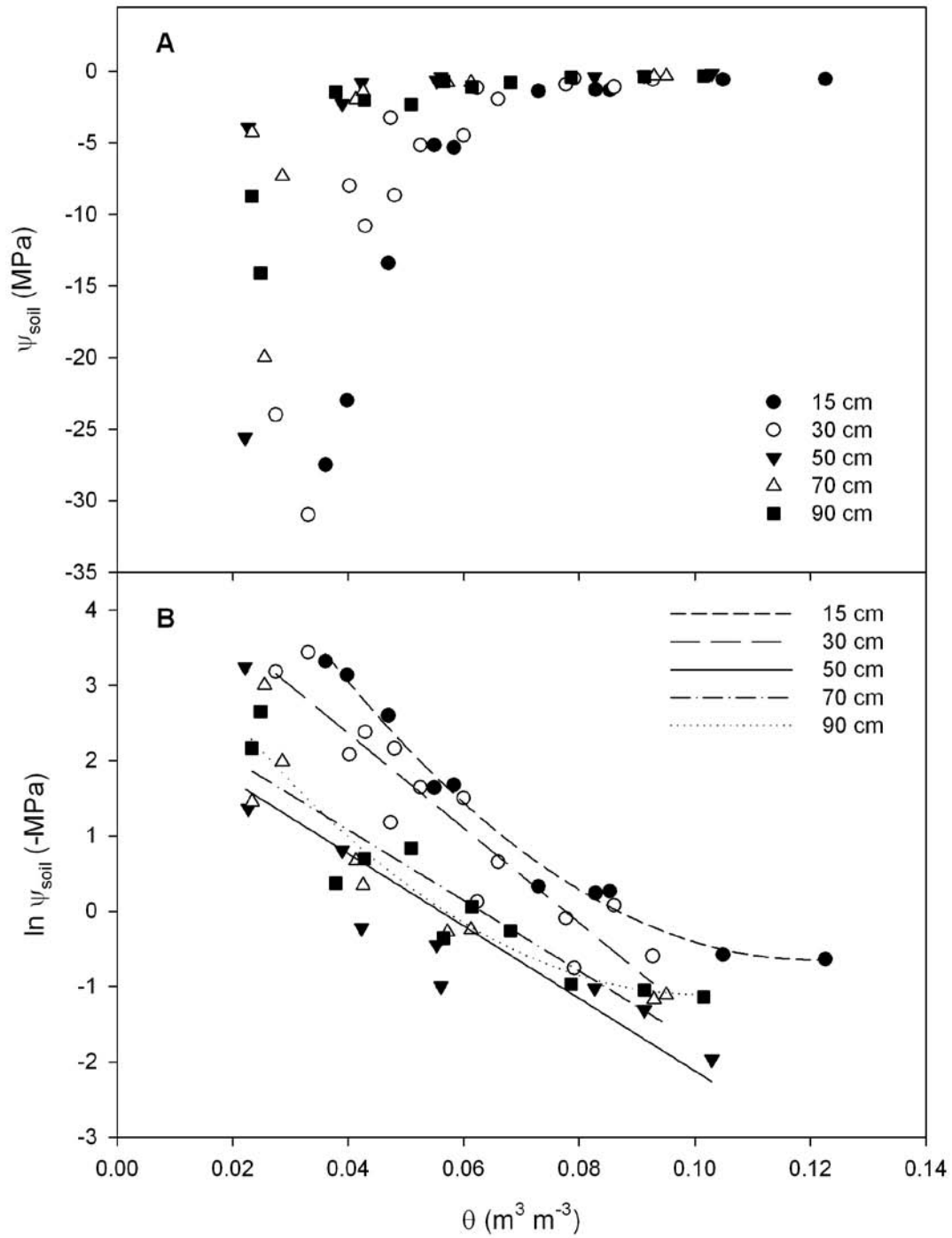


Figure 3.3. Soil water retention curves generated using the WP4 Dewpoint PotentialMeter. Laboratory generated values of θ and Ψ_{soil} (A), and θ and log transformed values of Ψ_{soil} for model prediction purposes (B). Model results are presented in Table 3.2.

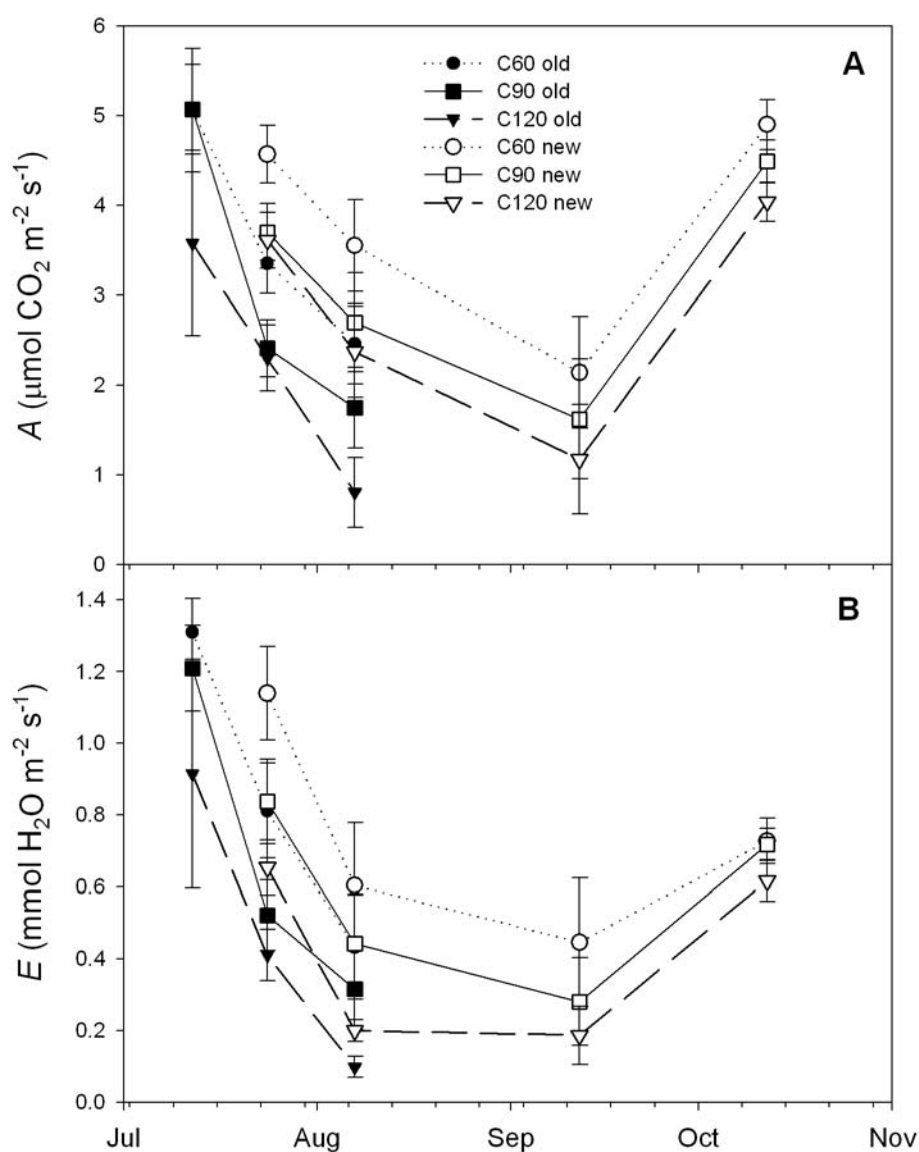


Figure 3.4. (A) Measured net photosynthesis (A) and (B) transpiration (E) for planted *Pinus ponderosa* seedlings on the Coyote Creek planting unit. Closed symbols represent measurements on previous-year needles (old); open symbols represent measurements on current-year needles (new).

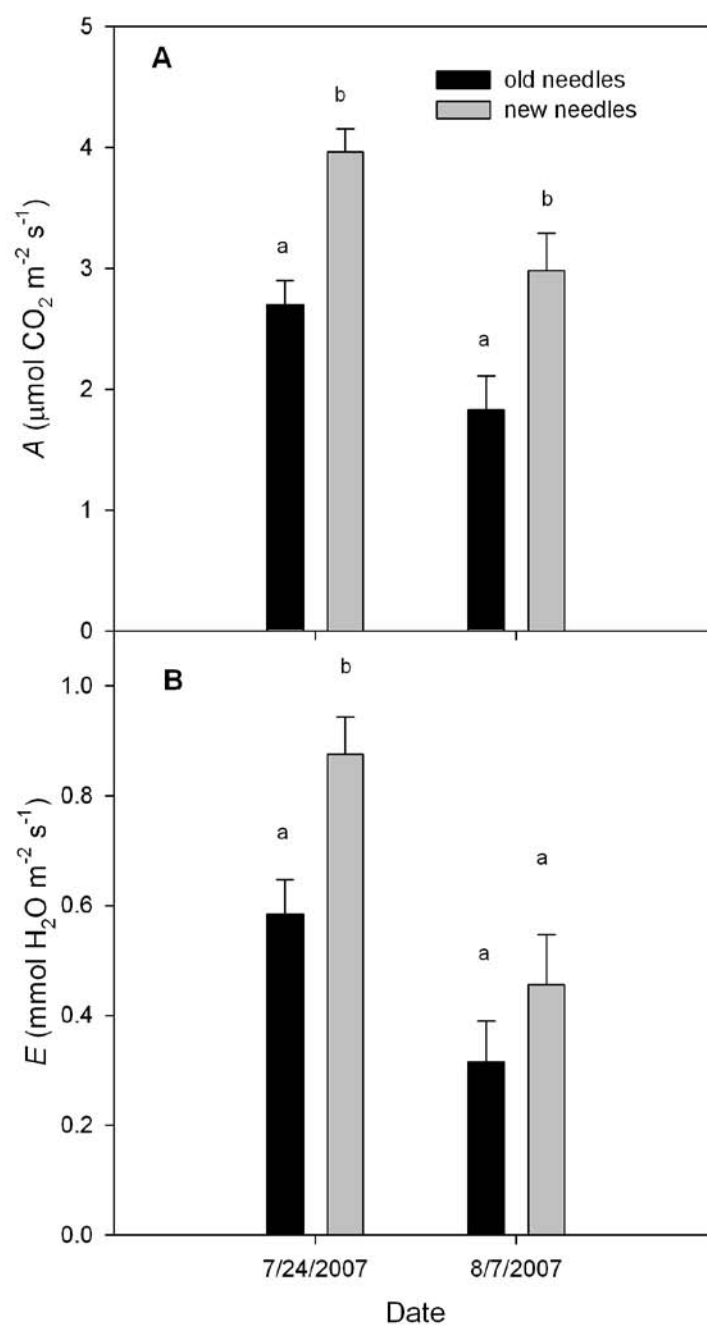


Figure 3.5. (A) Net photosynthesis (A) and (B) transpiration (E) for previous-year (old) and current-year (new) needles measured on two dates at the Coyote Creek planting unit. Bars show least square means + SE.