Photosynthetic response, carbon isotopic composition, survival, and growth of three stock types under water stress enhanced by vegetative competition

Jeremiah R. Pinto, John D. Marshall, R. Kasten Dumroese, Anthony S. Davis, and Douglas R. Cobos

Abstract: Selecting the proper stock type for reforestation on dry sites can be critical for the long-term survival and growth of seedlings. In this study, we use a novel approach to understand stock type selection on a site where drought was induced with vegetative competition. Three ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *ponderosa* C. Lawson) seedling stock types were planted in the field and subjected to three levels of competition. Winter wheat (*Triticum aestivum* L. em.) was sown in three densities (0, 150, and 300 plants·m^{-2}) and was successfully used as a model competitor to create drought conditions. 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 measurements indicated that seedlings planted with vegetative competition were moisture-stressed and not established. Drought conditions created by the wheat caused 100% mortality among smaller stock types, whereas the largest stock type had a 63%–75% mortality rate. Measures of stable carbon isotopes showed stratification based on water availability, with significant δ^{13}C enrichment in competition treatments. Soil moisture is critical for seedlings to establish quickly after planting. Our data suggest that proper stock type selection on drought- or vegetation-prone sites can confer survival and growth benefits.

Résumé : Le choix du type de stock approprié pour le reboisement des stations sèches peut être déterminant pour la survie et la croissance à long terme des semis. Dans cette étude, nous avons utilisé une nouvelle approche pour comprendre comment choisir le type de stock dans une station où la sécheresse a été provoquée par la végétation compétitrice. Trois types de stock de semis de pin ponderosa typique (*Pinus ponderosa* Lawson & C. Lawson var. *ponderosa* C. Lawson) ont été plantés au champ et soumis à trois niveaux de compétition. Le blé d'hiver (*Triticum aestivum* L. em.) a été semé à trois densités (0, 150 et 300 plants·m^{-2}) et a été utilisé avec succès comme compétiteur modèle pour créer des conditions de sécheresse. Des taux élevés de photosynthèse nette (*A*) indiquaient que les semis s'étaient établis après moins de trois semaines si l'humidité du sol était adéquate et qu'il n'y avait pas de végétation compétitrice. À l'inverse, de faibles valeurs de *A*, d'humidité du sol et de potentiel hydrique de base indiquaient que les semis plantés en présence de végétation compétitrice subissaient un stress hydrique et ne s'étaient pas établis. Les conditions de sécheresse engendrée par le blé ont causé 100 % de mortalité chez les types de stock plus petits tandis que les types de stock les plus gros avaient un taux de mortalité de 63–75 %. Des mesures des isotopes stables de carbone ont montré que qu'il y avait une stratification basée sur la disponibilité de l'eau qui se traduisait par un enrichissement significatif de δ^{13}C dans les traitements où il y avait de la compétition. L'humidité du sol est cruciale pour que les semis s'établissent rapidement après la plantation. Nos données indiquent que le choix du type de stock approprié dans les stations sujettes à la sécheresse ou la végétation peut avoir un effet bénéfique sur la survie et la croissance.

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Introduction

The challenges of dry-site reforestation are not new, but they continue to 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 establish root systems at depths where water is available.

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Natural ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *ponderosa* C. Lawson) regeneration can be low (Keyes et al. 2007) 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 (van Haverbeke 1963). The resulting ability of a seedling to take up water allows the initiation of a positive-feedback loop: water uptake increases stomatal 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 begin with roots placed deeper in the soil profile (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. The resultant changes in biomass partitioning, and the balance in shoot transpirational area versus root absorption area, can influence subsequent seedling growth and survival. Cregg (1994) suggests that an optimum shoot-to-root ratio (*S*:*R*) may exist 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 any case, seedlings that survive into the next growing season must somehow balance transpirational demand with root water uptake.

The reduction of stomatal conductance due to dry soils can also be correlated with plant discrimination against $^{13}CO_2$ relative to the more abundant $^{12}CO_2$. This discrimination reflects the balance of CO_2 entering through the stomata and demand for CO_2 by photosynthetic mesophyll and is positively related to intercellular CO_2. Plants discriminate more as intercellular CO_2 increases relative to ambient conditions (Farquhar et al. 1982). Thus, most discrimination generally occurs in plants with high stomatal conductance because intercellular CO_2 is more readily exchanged with atmospheric CO_2, leading to less enrichment of the intercellular pool. The stable carbon isotope composition of leaves is considered a time-integrated index of the ratio of intercellular to ambient CO_2 concentration that can be used to infer photosynthetic water-use efficiency and water availability (Ehleringer and Osmond 1989; ; Warren et al. 2001; Dawson et al. 2002). Although this technique is commonly used in ecological studies and for refining ecosystem modeling, it may also have utility for understanding plant water relations under various nursery and outplanting scenarios.

Dry soils result not only from low seasonal precipitation inputs, but also from water use by competing vegetation (Elliott and White 1987; Newton and Preest 1988). Research by Pearson (1942) suggests that grasses growing on burned forest sites extract 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) 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 can be more effective than natural regeneration (McDonald et al. 2009). Current reforestation options offer a plethora of seedling stock type choices, but studies offering sound data on the advantages of particular 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 among container types. 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). Consequently, our study aimed to objectively evaluate seedling stock type performance during a seasonal summer drought. We created seasonal drought using plant competition treatments and quantified competition effects on soil moisture and availability. We hypothesized that stock types with longer root systems, which have access to soil moisture deeper in the soil profile, would outperform smaller stock types in dry soil conditions. Our stock type evaluations included survival, gas exchange, carbon isotope composition, 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 stock types were grown in Superblock (Beaver Plastics Ltd., Acheson, Alberta) containers differing only in cavity depth and, therefore, volume (Table 1). 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, as recommended by Pinto et al. (2011*a*). This physiological uniformity ensured that treatment differences reflected only container type and were not confounded by nursery practices that can lead to differing seedling quality among stock types (Pinto et al. 2011*a*).

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

Table 1. Container specifications and morphological attributes of 2006 greenhouse-grown *Pinus ponderosa* seedlings (n = 36).

Container*	Container depth (cm)	Container volume (cm^3)	Height (cm)	RCD (mm)	Shoot dry mass (g)	Root dry mass (g)	Shoot–root ratio
C60	10.4	60	12.5 (0.44) a	3.2 (0.09) a	1.6 (0.08) a	1.1 (0.06) a	1.5 (0.06) ab
C90	15.1	90	16.3 (0.64) b	3.8 (0.11) b	2.4 (0.14) b	1.6 (0.09) b	1.4 (0.04) a
C120	22.7	120	19.4 (0.84) c	3.8 (0.12) b	2.8 (0.16) b	1.7 (0.10) b	1.7 (0.08) b

Note: Seedlings were harvested in December of 2006. Root-collar diameter (RCD) is the diameter of the main seedling stem approximately 1 cm above the ground line. Standard errors are in parentheses. Columns with the same letter are not significantly different, Tukey adjusted (P < 0.05).

*Superblock (Beaver Plastics Ltd., Acheson, Alberta) model nos. 160/60, 160/90, and 160/120, respectively.

sandy loam with an average 1 m depth to gravel. Seedling establishment was 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). When the seedlings were planted, ocular assessment estimated the winter wheat to be approximately 7.5 cm tall, well below the majority of photosynthetic leaf area of the seedlings. This allowed competition for water in the root zone with little competition for solar radiation in the canopy. Wheat was sown on 6 April at three densities (0, 150, and 300 plants·m^{-2}; D_0, D_{150}, and D_{300}, respectively) to induce drought effects; it was irrigated periodically to aid germination and establishment. After four weeks, D_0, D_{150}, and D_{300} were fertilized at 0, 22.4, and 44.8 kg N·ha^{-1}, respectively. The low N rate was recommended solely for wheat establishment but was expected to be depleted by the time the seedlings were planted (A.D. Eramian, personal communication, 2007). Winter wheat leaf area index was measured using a sunfleck ceptometer (model SF-80, Decagon Devices, Inc., Pullman, Washington) in early fall to quantify the aboveground biomass relative to the sowing rate. D_{300} measured 0.64 (±0.09, standard error (SE)) m^2·m^{-2}, whereas D_{150} measured 0.32 (±0.06) m^2·m^{-2}.

Seedling installation

On 29 May, seedlings were moved from freezer storage to cooler storage (5 °C) for thawing. Seedlings were planted on 5 June when wheat was approximately 7.5 cm tall. Competition treatments were replicated four times as wholeplots. Each wholeplot 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; container type was the split-plot) were planted into each level of competition within each replication (a randomized complete block (RCB) split-plot design).

Weather and soil measurements

Instrumentation and data loggers were used to collect weather and soil moisture data, and laboratory analysis was used to calibrate equipment and construct moisture characteristic curves. A weather station (model 2900ET, Spectrum Technologies, Inc., Plainfield, Illinois) collected air temperature (°C), 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 (%):

$$\mathrm{VPD} = \left(a\,\mathrm{e}^{\left(\frac{bT}{T+c}\right)}\right)(1-h_\mathrm{r})$$

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 soil temperature measurements were collected hourly in situ 17 cm from the soil surface using ECH$_2$O-TE soil moisture probes and Em50 data loggers (Decagon Devices, Inc., Pullman, Washington). Three replications of soil sensors were distributed randomly among the wholeplot wheat treatments. Three soil samples were taken at each depth where soil moisture probes were installed. Each of these soil samples was then split into two undisturbed subsamples. One subsample was used to generate a moisture characteristic curve at five 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. A three-parameter, single exponential rise-to-maximum model was fit to the data ($\Psi_\mathrm{soil} = -9.39 + 9.37(1 - \mathrm{e}^{-24.3\theta})$; R^2 = 0.90; SE = 0.17). The second subsample was used to generate soil-specific calibration equations for the soil moisture probes (Starr and Paltineanu 2002). 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 ±0.02 m^3·m^{-3} (Decagon Devices, Inc., Pullman, Washington).

Survival, growth, and biomass allocation

Morphology, biomass, and survival assessments were performed periodically. At the end of greenhouse production in December 2006, height, root-collar diameter (RCD), and postharvest 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 that ≥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 D_0 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, predawn needle water potential, and stable carbon isotopes

Using a portable photosynthesis system (model LI-6400, LI-COR, Lincoln, Nebraska) equipped with a blue–red LED light source and CO_2 injector, seedling gas exchange was measured eight times during the course of the first growing season (June to October). Measurements were conducted between 0800 and 1200 h and began two weeks after planting. The first four occurred at one-week intervals; measurements five and six occurred at two-week intervals, with the last two measured at three- to four-week intervals (19, 26 June; 3, 11, 23 July; 6, 28 August; 9 October 2007). One seedling from each container type and treatment was randomly chosen from each replication for gas exchange measurements (n = 36). 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 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR, 25 °C, 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2, and a flow rate of 400 $\mu\text{mol}\cdot\text{s}^{-1}$.

Leaf area calculation was adapted from the methods of Svenson and Davies (1992). Fascicle diameters were measured at the center of each fascicle segment placed inside the LI-6400 light chamber. Using the assumption that three needles of a fascicle form a cylinder, 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 the 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:

$$\text{LA} = \pi dl + 6\frac{d}{2}l$$

where d is the diameter of the three-needle fascicle, and l is the length of the 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 29 June, Ψ_{nwp} was measured on one seedling from each competition treatment × container × replication using a pressure chamber (PMS Instrument Company, Corvallis, Oregon). Between 0200 and 0400 h, one needle from a fascicle was randomly chosen for measurement.

At the end of the 2007 growing season, needle samples were collected for carbon isotope analysis. Three samples were tested: needles from the 2006 greenhouse production period; needles from the 2007 growing season (D_0 treatment); and needles from the surviving C120 seedlings (D_0, D_{150}, and D_{300} treatments). The ratio of ^{13}C to ^{12}C ($\delta^{13}C$), expressed in parts per thousand (‰) for shoots, was determined at the University of Idaho Stable Isotope Laboratory (Moscow, Idaho). Needle tissue was re-dried for 48 h at 70 °C and ground to a fine powder. Samples were flash-combusted in CE Instrument's NC 2500 elemental analyzer, interfaced with a Conflo II, and analyzed using the Finnigan-MAT Delta Plus isotope mass spectrometer. Carbon isotope ratio was expressed relative to the Pee Dee River belemnite standard (PDB; Craig 1957) as follows:

$$\delta^{13}\text{C} = [(^{13}\text{C sample}/^{12}\text{C sample})/(^{13}\text{C}_{\text{PDB}}/^{12}\text{C}_{\text{PDB}}) - 1] \times 1000$$

Experimental design and statistical analysis

Analysis of variance PROC MIXED (α = 0.05) (version 9.1.3, SAS Inc., Cary, North Carolina) 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 three competition levels × three container types × four replications in a RCB split-plot design, with competition as the wholeplot 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 four seedlings per competition × container × replication combination (144 total seedlings). Periodic observations of survival showed that all seedlings of the C60 and C90 container types had died in D_{150} and D_{300} before the experiment ended. This precluded a priori RCB split-plot analysis of net photosynthesis and growth for all competition × container combinations on 2007 measurements. Post hoc analyses included net photosynthesis on all competition × container combinations for the first measurement period only, growth analysis on C120 × competition combinations at the end of the 2007 growing season, and repeated measures net photosynthesis analysis on C120 × competition combinations (a RCB design analysis) for all eight measurement periods. Seedling survival was analyzed with 9 October data. Because of high mortality, survival data violated assumptions for normality and was consequently analyzed using the nonparametric Kruskal–Wallis and Friedman tests. Stable carbon isotope analyses were done on three sets of data: (*i*) the 2006 data collected from previously harvested seedlings grown in the greenhouse; (*ii*) the 2007 data collected from surviving trees in the D_0 treatment; and (*iii*) surviving C120 seedlings across all competition treatments. Analysis of variance was used to examine the effects of container and year on carbon isotope composition; container types were compared using the Tukey adjustment. Also, because of high mortality, post hoc analysis was performed to examine the effects of competition treatment on C120 seedlings.

Results

Site conditions

During the 2007 growing season, competition treatments D_{150} and D_{300} created drier conditions in the upper 17 cm of the soil profile than did D_0. Between the time of planting (5 June) and the last photosynthesis measurement (9 October), the average θ was 0.22 $m^3{\cdot}m^{-3}$ for D_0 compared with 0.14 and 0.12 $m^3{\cdot}m^{-3}$ for D_{150} and D_{300}, respectively. At planting, predicted Ψ_{soil} was –0.04 MPa for D_0, –0.06 MPa for D_{150}, and –0.12 MPa for D_{300} (Fig. 1A). Ψ_{soil} remained high due to precipitation inputs shortly after planting and then proceeded to decline (rapidly for D_{150} and D_{300}) 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. Five small rainfall events (four events > 5 mm and one event = 8 mm) occurred between July and early September (the typical dry period). These rainfall events may have lessened severe drought effects by changing the trajectories of soil moisture availability, but these changes were not quantified.

At the time of planting, the air temperature and VPD were 13.4 °C and 0.03 kPa, respectively. On July 5, maximum air temperature and VPD were reached (37.8 °C and 6.1 kPa, respectively). From June 5 to October 9, overall air temperature averaged 18.5 °C, and VPD averaged 1.4 kPa (Figs. 1B and 1C). 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 D_0 treatments, averaging 23.2 °C with a seasonal maximum of 34.2 °C. Wheat cover seemed to reduce surface soil heat flux for D_{150} and D_{300} such that 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. Significant differences were observed in height, RCD, shoot dry mass, root dry mass, and *S*:*R* (Table 1). Pairwise analyses indicated that C90 and C120 containers were the most similar.

All seedlings in the D_0 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 D_{150} and D_{300} treatments. Seven weeks after planting, all C60 seedlings in D_{300} were dead, and mortality was nearly 50% for the C90 seedlings in both D_{150} and D_{300} (Fig. 2). Twelve weeks after planting, all C90 seedlings in D_{150} and D_{300} 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). D_{150} and D_{300} seedlings had reduced RCDs ($P < 0.001$) and needle length (>74% reduction; $P < 0.0001$) relative to seedlings in D_0. C120 seedlings growing in D_0 remained the tallest of the container types ($P < 0.0001$) and had more total shoot biomass and 2007 needle biomass than C60 ($P > 0.002$) but not C90 ($P > 0.67$; Table 3). RCD and root biomass were not significantly different ($P \geq 0.06$) among container types in D_0 (Table 3). 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 ($P = 0.02$). Although 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 $\mu mol\ CO_2{\cdot}m^{-2}{\cdot}s^{-1}$; $P = 0.006$). For the subsequent five measurements, net photosynthesis (*A*) of seedlings in D_{150} and D_{300} treatments dropped precipitously (Fig. 3), corresponding to decreasing soil moisture and seasonal increases in temperatures and VPDs. Seven weeks after planting, *A* reached minimum values (0.14 $\mu mol\ CO_2{\cdot}m^{-2}{\cdot}\ s^{-1}$), 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 that D_0 C120 seedlings had higher photosynthetic rates than C120 seedlings in either D_{150} or D_{300} ($P < 0.01$; Fig. 3). *A* was not significantly different ($P > 0.6$) between D_{150} and D_{300}, and no date × treatment interactions were detected ($P = 0.4$).

Seventeen days after planting, both competition and container type treatment effects showed significantly different Ψ_{nwp} ($P \leq 0.0002$; Fig. 4). Seedlings in D_{150} and D_{300} already exhibited low Ψ_{nwp} (–2.18 and –2.23 MPa, respectively), whereas 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, respectively, whereas C120 seedlings measured slightly less negative at –1.44 MPa.

Variation in carbon isotope ratio ($\delta^{13}C$) was not significantly different ($P \geq 0.60$) among containers for either greenhouse-produced needles or needles produced after outplanting in the D_0 competition treatment (Fig. 5A). However, needles produced after outplanting were significantly more enriched in ^{13}C ($P < 0.0001$) than needles produced in the greenhouse. Among C120 seedlings, competition treatments contributed to significantly less discrimination against ^{13}C ($P = 0.022$) when compared with seedlings grown in the absence of competition (Fig. 5B).

Discussion

Forest practices advocate proper soil moisture management for enhancing survival and growth of seedlings on droughty sites (Nambiar 1990; Nambiar and Sands 1993; Powers and Reynolds 1999). However, both anecdotal and limited empirical evidence suggest that planting deep containers will also work (Stroempl 1990; Chirino et al. 2008). 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; Mohammed et al. 1998). These studies have been inconclusive, however, 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 confound-

Fig. 1. (A) Vegetation competition treatment soil water potential (Ψ_{soil}; 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. Vertical broken line indicates seedling planting (5 June); vertical dotted 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. Growing season (B) vapor pressure deficit and (C) air temperature conditions measured at the USDA Forest Service Nursery in Coeur d'Alene, Idaho, USA.

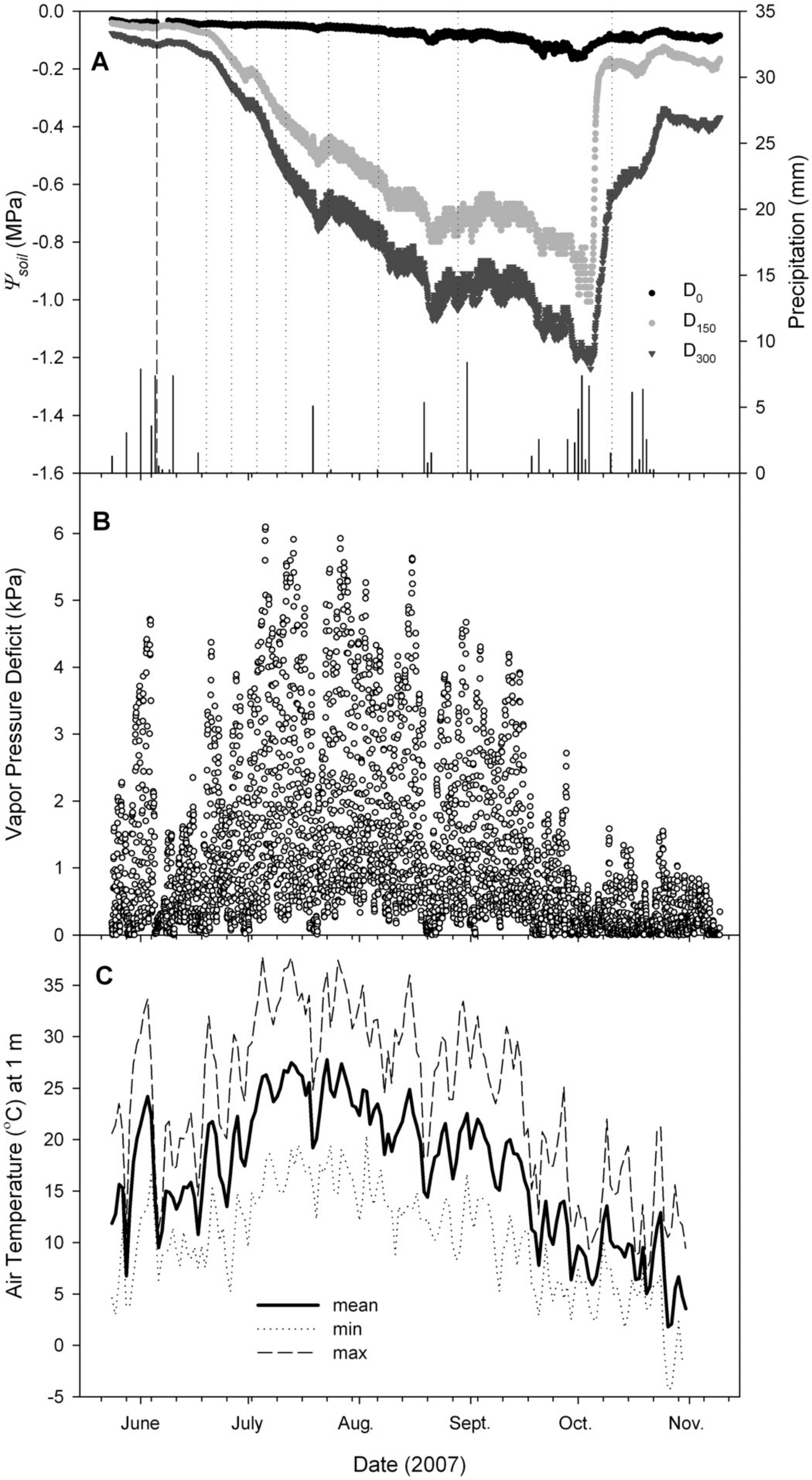


Fig. 2. Mortality levels for C60 (circles), C90 (triangles), and C120 (squares) seedlings in D_{150} (solid symbols, **A**) and D_{300} (open symbols, **B**) competition treatments over the course of the 2007 growing season. Vertical broken line indicates time of seedling planting (June 5). No mortality was observed in D_0. 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.

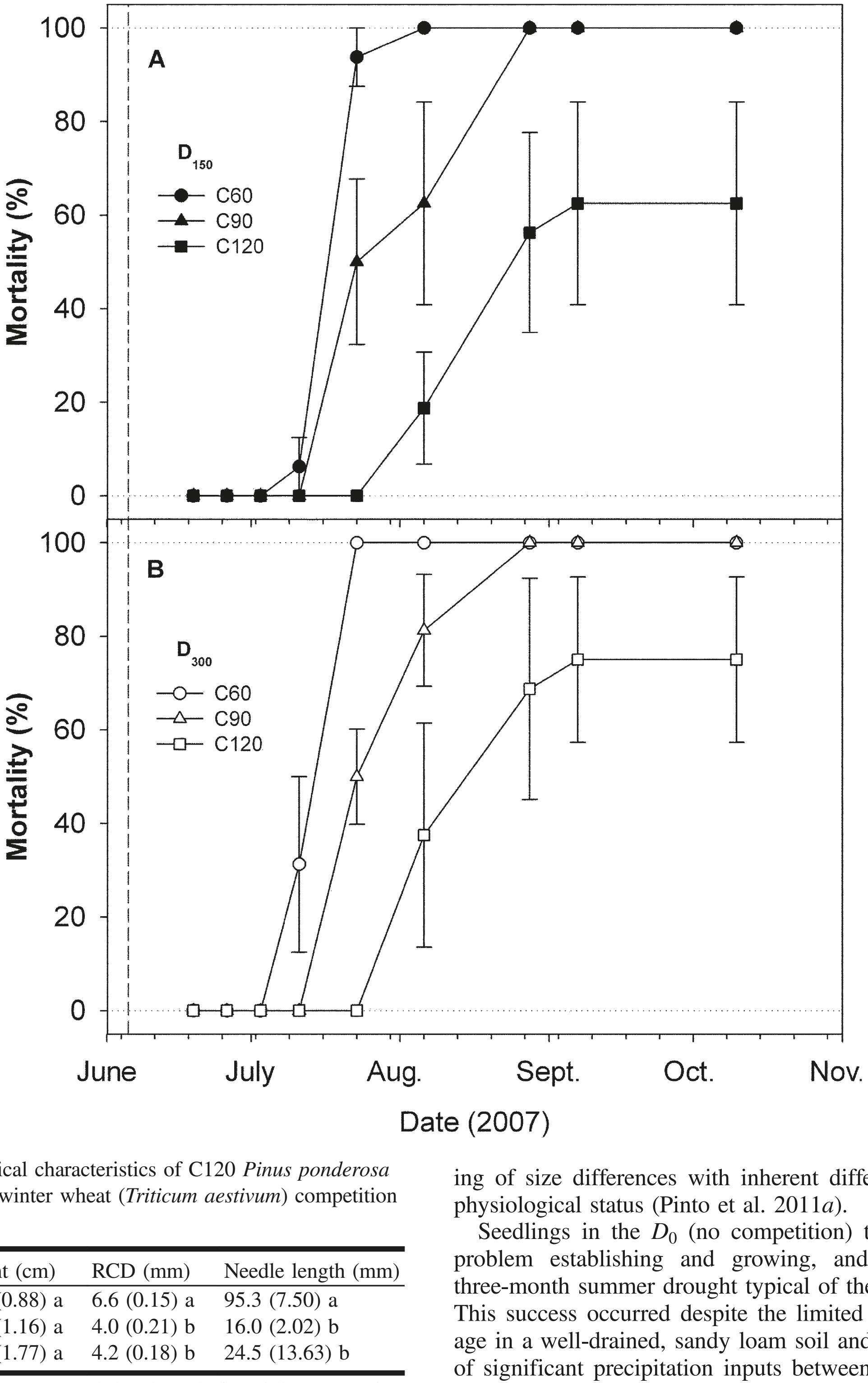


Table 2. Morphological characteristics of C120 *Pinus ponderosa* seedlings planted in winter wheat (*Triticum aestivum*) competition treatments (n = 18).

Treatment*	Height (cm)	RCD (mm)	Needle length (mm)
D_0	28.3 (0.88) a	6.6 (0.15) a	95.3 (7.50) a
D_{150}	24.4 (1.16) a	4.0 (0.21) b	16.0 (2.02) b
D_{300}	23.9 (1.77) a	4.2 (0.18) b	24.5 (13.63) b

Note: Measurements were recorded at the end of the fall 2007 growing season. Root-collar diameter (RCD) is the diameter of the main seedling stem approximately 1 cm above the ground line. Standard errors are in parentheses. Columns with the same letter are not significantly different, Tukey adjusted ($P < 0.05$).

*D_0, D_{150}, and D_{300} = 0, 150, and 300 wheat plants·m^{-2}.

ing of size differences with inherent differences in seedling physiological status (Pinto et al. 2011*a*).

Seedlings in the D_0 (no competition) treatment had little problem establishing and growing, and all survived the three-month summer drought typical of the northwestern US. 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 with D_{150} and D_{300} treatments. Pinto et al. (2011*b*) showed similar results in a field study in which seedlings were grown on a site with extensive site preparation (burning and herbicide

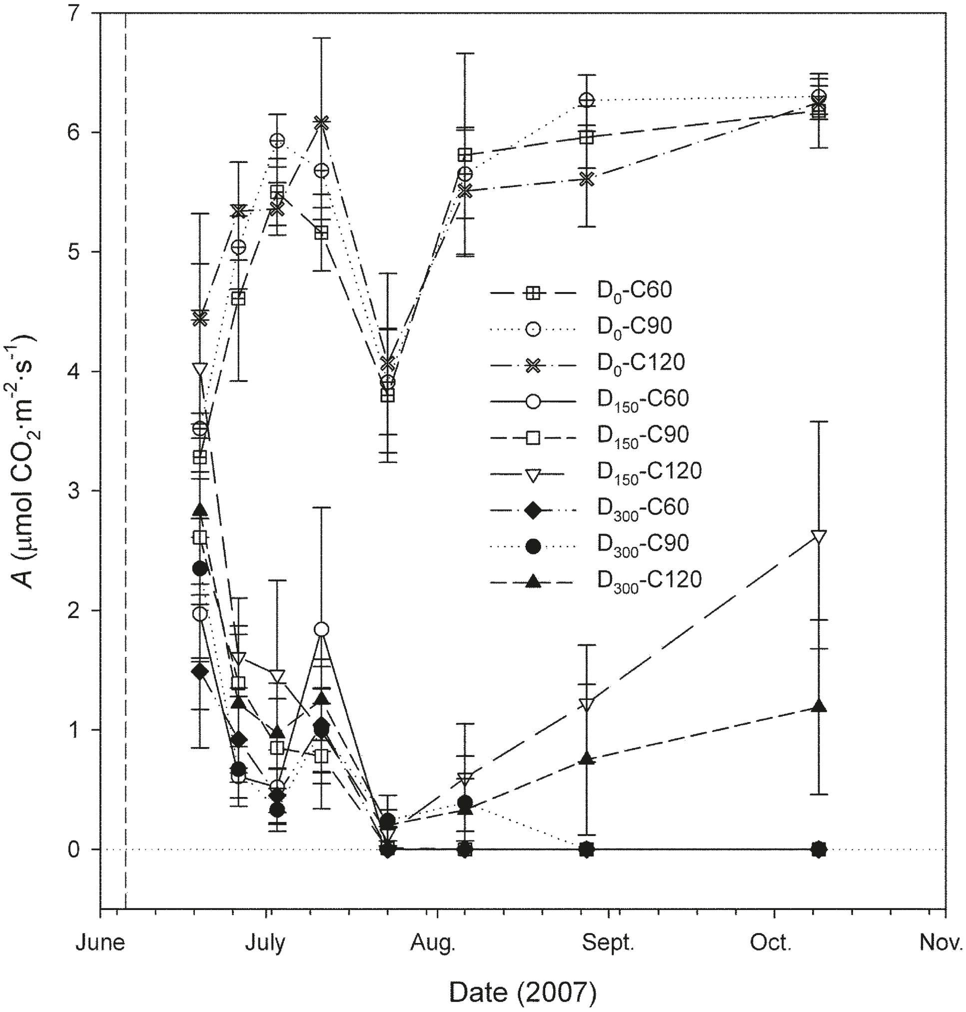
Table 3. Morphological characteristics of *Pinus ponderosa* seedlings planted in the D_0 winter wheat (*Triticum aestivum*) competition treatment (0 plants·m^{-2}; $n = 48$).

Container*	Height (cm)	RCD (mm)	Shoot dry mass (g)	Root dry mass (g)	Current-year needle dry mass (g)	Root egress (cm)
C60	20.0 (0.62) a	6.2 (0.13) a	7.5 (0.43) a	6.4 (0.42) a	3.7 (0.25) a	31.4 (1.53) a
C90	25.0 (0.71) b	6.5 (0.17) a	9.8 (0.0.48) b	7.2 (0.54) a	4.9 (0.35) b	30.8 (1.14) a
C120	28.3 (0.88) c	6.6 (0.15) a	10.5 (0.57) b	7.2 (0.77) a	5.0 (0.34) b	30.3 (1.09) a

Note: Measurements were recorded at the end of the fall 2007 growing season. Root-collar diameter (RCD) is the diameter of the main seedling stem approximately 1 cm above the ground line. Standard errors are in parentheses. Columns with the same letter are not significantly different, Tukey adjusted ($P < 0.05$).

*See Table 1 for container descriptions and codes.

Fig. 3. Net photosynthesis for all containers in all competition treatments. Each point represents a mean ± SE. 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.



vegetation control). These seedlings had high soil moisture, low rates of mortality, and rapid growth rates compared with another location with little site preparation. Similar results have been seen with the control of woody and herbaceous vegetation in ponderosa pine and Douglas-fir (*Pseudotsuga menziesii*; Ross et al. 1986; Powers and Reynolds 1999; Rose and Ketchum. 2002). Despite planting later than normal (June vs. April), the conservation of soil moisture in D_0 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

Fig. 4. Predawn water potential measurements (Ψ_{nwp}) taken on 29 June 2007. Solid points (circle, triangle, and square) on the left indicate split-plot container treatment effects; open squares on the right indicate wholeplot competition treatment effects. Points show least squares mean ± SE. For each treatment effect, points with the same letter are not significantly different, Tukey adjusted ($P < 0.05$). 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.

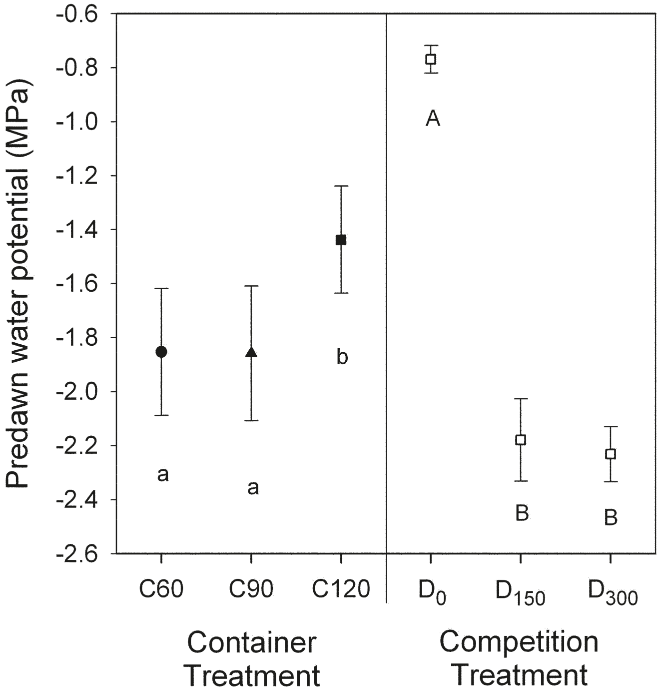
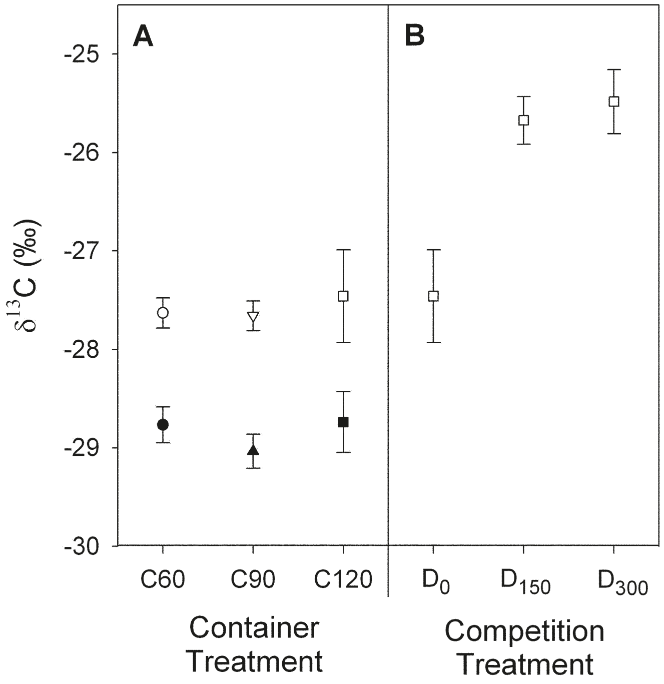


Fig. 5. Carbon isotopic ratio ($\delta^{13}C$) for containerized ponderosa pine seedlings: (A) $\delta^{13}C$ values for seedlings produced in the greenhouse (2006; solid points) and one year after outplanting (2007; open points) with no competition; (B) $\delta^{13}C$ values for C120 seedlings planted in competition plots one year after outplanting. Points show least squares mean ± SE. 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.



place roots deeper in the soil profile, where soil moisture may be more available during the summer drought, limiting water stress (Warren et al. 2005). van Haverbeke (1963) reports that naturally regenerated ponderosa pine seedlings produce an undisturbed tap root with the capacity to grow deep into the soil profile, thereby avoiding moisture stress. Kolb and Robberecht (1996) also show that 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 D_0 indicate that seedling quality was more than adequate. Although root egress of D_0 seedlings averaged 0.31 m, these measurements are underestimates because roots were severed during the excavation process.

Consequently, with sufficient root egress and control of soil moisture, seedlings of differing container sizes in D_0 were all able to maintain similar and sufficient rates of net photosynthesis throughout the growing season (Fig. 3). Net photosynthesis increased from 3.8 μmol $\mathrm{CO_2 \cdot m^{-2} \cdot s^{-1}}$ at planting to 5.6 μmol $\mathrm{CO_2 \cdot m^{-2} \cdot s^{-1}}$ 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 D_0, 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.

Vegetative competition enhanced the summer decline in soil moisture. Precipitous drops in predicted Ψ_{soil} (from –0.06 to –1.01 and –0.12 to –1.22 MPa for D_{150} and D_{300}, respectively) were observed after spring precipitation diminished. However, immediately after planting and before the rapid drop in soil moisture, net photosynthetic rates were similar among treatments (Fig. 3). 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 D_{150} and D_{300}.

Our study confirms previously reported relationships between stomatal limitation of photosynthesis and water availability based on stable carbon isotope values in leaf tissue (Zhang et al. 1997; Warren et al. 2001; Adams and Kolb 2004); however, we believe that this is the first study to use isotopes to explain seedling production and outplanting relationships. Because containers are finite in size, they contain finite water, which influences rates of transpiration, soil moisture depletion, and plant moisture stress (Landis et al. 1989). Seedlings harvested from the greenhouse had similar, and low, $\delta^{13}C$ values. This similarity indicates that each of the container types was managed to provide similar water availability. We feel that our greenhouse-obtained values are a reflection of similar seedling quality among container types (Pinto et al. 2011*a*). First-year outplanting data indicate a shift in the enrichment of ^{13}C on two levels. First, despite growing in a competition-free environment, seedlings were still faced with the harshness of growth in the field and were forced to regulate water loss via stomatal closure. The result was an average increase in $\delta^{13}C$ of 1.3‰. Secondly, surviving C120 seedlings in the D_{150} and D_{300} treatments were subjected to the most drought stress and consequently exhibited the highest $\delta^{13}C$ ratios, a further increase of 1.8‰. Despite not having a full set of data describing the competition treatment effects among container types (due to high rates of mortality), we believe that there could be some utility in using $\delta^{13}C$ values to describe relative differences in seedling establishment in drought vs. nondrought conditions or differences in the establishment of different stock types.

Recall the positive-feedback model of seedling establishment (Burdett 1990). It seems likely that lack of soil moisture and water uptake in the competition treatments 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. Drought effects might have also been mitigated by reductions in leaf area via leaf abscission or via limiting new needle expansion (Kozlowski and Pallardy 1997; Taiz and Zeiger 2006), but this too would limit photosynthesis. The abrupt change in soil moisture may also have limited opportunities for drought preconditioning. Cregg (1994) showed that ponderosa pine seedlings subjected to several dry-down periods after planting are preconditioned to photosynthesize at higher rates despite decreasing soil moisture — these dry-down cycles are something seedlings in D_{150} or D_{300} failed to receive. 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.

Among the most common and most limiting factors to seedling establishment are root growth and soil moisture (Stone 1955; Burdett 1990; Grossnickle 2005); however, a lesser known factor is the length of time that it takes seedlings to establish. This factor can become increasingly important if outplanting conditions are subject to the quick onset of drought. In this study, seedlings in D_0, where soil moisture remained high, were able to increase net photosynthetic rates within three weeks of planting. In contrast, seedlings in D_{150} began to see the abrupt decline in soil moisture between the second and third week, and seedlings in D_{300} were already 28% lower in soil moisture than D_0; concomitantly, net photosynthetic rates in D_{150} and D_{300} 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 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? Although 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.

It is possible that changing climate will alter outplanting conditions for establishing seedlings. Although some areas will see increases in precipitation and soil moisture, many areas will see increases in heat and drought stress. With this in mind, it will remain increasingly important to identify the limitations to seedling establishment and take action to overcome them. Stock type selection is one tool to overcome limitations and engage in the establishment process. Our results offer insight on the advantages of planting stock types with longer root systems to afford some buffer capacity for reduced upper-profile soil moisture. Placing root systems below root competition, or deeper in the soil profile where there is a known reservoir of soil moisture longer, can aid in survival and growth. However, many other limitations can exist, and stock type studies will need creative approaches to objectively discern the advantages of one stock type characteristic versus the other.

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