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Seedling establishment and physiological responses to temporal and spatial soil moisture changes

Jeremiah R. Pinto[1] · John D. Marshall[2] · R. Kasten Dumroese[1] · Anthony S. Davis[2] · Douglas R. Cobos[3]

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Abstract In many forests of the world, the summer season (temporal element) brings drought conditions causing low soil moisture in the upper soil profile (spatial element)—a potentially large barrier to seedling establishment. We evaluated the relationship between initial seedling root depth, temporal and spatial changes in soil moisture during drought after outplanting, and subsequent seedling performance using seedlings of *Pinus ponderosa* Laws. var. *ponderosa* grown in three containers similar in dimension except for depth (i.e. three stocktypes). Soil moisture patterns were quantified and growth, gas exchange, and carbon isotope analysis were used as metrics for stocktype evaluation. Soil moisture reached minimum volumetric soil moisture contents (θ) of 0.078 m^3 m^{-3} at a 15 cm depth and 0.15 m^3 m^{-3} at 90 cm by late summer, which also translated to estimated soil water potential (Ψ_{soil}) values of -2.29 and -0.02 MPa, respectively. Seedling photosynthesis (A) and transpiration (E) rates followed soil moisture trends, also reaching seasonal lows in late summer. In early fall, gas exchange rates nearly doubled following a replenishment of upper-profile soil moisture by precipitation. Over the course of the growing season, stocktypes did not differ in gas exchange rates ($P \geq 0.15$), biomass ($P \geq 0.45$), root penetration depth ($P = 0.60$), or carbon isotope signature ($P \geq 0.60$). For all seedlings, current-year needles showed greater capacity for A than previous-year needles ($P \leq 0.01$), and A was only significantly correlated with soil moisture in the upper soil profile (15 cm; $P \leq 0.03$). In this study, stocktype was not a significant factor, suggesting that seedling access to soil moisture was not different among them. The temporal and spatial variation observed in soil moisture availability, however, provides critical biophysical information on outplanting timing as it relates to subsequent seedling establishment and potential root growth. As well, needle formation, carbon gain, and the relationship to soil water depth

✉ Jeremiah R. Pinto
jpinto@fs.fed.us

[1] US Department of Agriculture Forest Service, Rocky Mountain Research Station, 1221 S Main St, Moscow, ID 83843, USA

[2] Department of Forest, Range, and Fire Sciences, University of Idaho, Moscow, ID 83843, USA

[3] Decagon Devices, 2365 NE Hopkins Ct., Pullman, WA 99163, USA

further indicate the importance for managing soil water or seedling stocktype for successful seedling survival and growth.

Keywords Ponderosa pine · Soil water potential · Container seedlings · Stocktype · Volumetric water content

Introduction

Two main limitations to plantation establishment are soil moisture availability and a planted seedling's ability to access it (Burdett 1990; Grossnickle 2005; Rietveld 1989). Reforestation practices often dictate that the optimum time for tree planting is followed by the onset of the dry season (Waring and Franklin 1979), resulting in progressively drying soil during seedling establishment. Reduced precipitation combined with plant transpiration commonly decrease soil moisture in the upper soil profile to below −1.5 MPa, 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 in regions around the world that exhibit similar moisture patterns (e.g. Padilla and Pugnaire 2007).

Seedlings have some capacity to mitigate this post-planting drought by changing their root system architecture. Naturally regenerated seedlings often grow a deep taproot and long laterals (Burdett et al. 1984; Kolb and Robberecht 1996a; Zwieniecki and Newton 1994), thereby exploiting any soil moisture not used by competing vegetation or depleted by evaporation (Pearson 1930; Van Haverbeke 1963). Conversely, when nursery-produced seedlings are first outplanted, their root systems are confined to a planting hole and are limited in soil moisture uptake by their initial root size. To survive this initial drought, outplanted seedlings must either close their stomata to conserve water or grow new roots into areas where soil moisture is more available (Burdett 1990). Alternatively, a seedling stocktype can be selected to pre-place root systems where they are able to access soil moisture more readily.

In situations when roots are not placed in moist soils or new root growth is insufficient to mitigate moisture stress, negative growth and seedling establishment effects are observed. In this negative feedback loop, stomatal closure can persist, leading to reduced photosynthesis, further reducing root growth (Burdett 1990; van den Driessche 1987). For outplanted *Pinus ponderosa* seedlings, seasonal effects of drought may be seen for years after planting (Irvine et al. 2002) and may be detectable through stable carbon isotopes from developing plant tissue. The stable carbon isotope composition of leaves can be considered a time-integrated index of the ratio of intercellular to ambient CO_2 concentration that can be used to infer relative photosynthetic water-use efficiency and water availability among seedlings (Dawson et al. 2002; Ehleringer and Osmond 1989; Warren et al. 2001).

Understanding and estimating soil moisture patterns during the first year of seedling establishment is valuable. 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 newly outplanted seedlings' roots are placed and where new root elongation is needed to overcome

transplant shock, but also 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 (i.e. deeper) 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 et al. 2012). The theory behind planting longer-rooted seedlings is that earlier placement of roots deep in the soil profile allows seedlings to grow roots deeper quicker, thus avoiding dry upper soil profile conditions during the onset of drought. This theory may be further extended to include growing roots deeper to avoid the pressures of competing vegetation (Anderson et al. 2001; Elliott and White 1987). Once 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 may be reduced (Kolb and Robberecht 1996b; McMillin and Wagner 1995; Olivas-Garcia et al. 2000; Panek and Goldstein 2001; Pinto et al. 2012; Zhang et al. 1997).

Placing a longer-rooted container seedling on a site may not always be the best choice to mitigate drought because of the associated planting problems (Robert and Lindgren 2006) and because longer-rooted containers are often larger in volume and incur extra costs with production (Scagel et al. 1998). It is therefore crucial to understand all site limiting factors, especially soil moisture, and their effects on seedling establishment before committing to a unique stock type (Pinto et al. 2011b). To date, few if any studies have integrated spatial and temporal soil moisture availability 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 *Pinus ponderosa* Laws. var. *ponderosa*, our study objectives were to (1) test the hypothesis that longer-rooted stock-types would perform better during seasonal drought conditions, (2) characterize the temporal and spatial attributes of soil moisture and model water availability, (3) measure the relative performance of the stocktypes by tracking seasonal changes in seedling gas exchange and measuring stable carbon isotopes, and (4) relate changes in soil moisture to resultant changes in growth.

Materials and methods

Seedling preparation

In 2006, *P. ponderosa* seedlings from a Confederated Tribes of the Colville Indian Reservation seed source appropriate for the site described below (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 length (i.e. depth; not in diameter or density); consequently, depth differences converted directly into volume differences (Table 1). Seedlings were grown with container-specific regimes to achieve

Table 1 Specifications of Styroblock® (Beaver Plastics Ltd., Acheson, Alberta) containers 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

uniform physiological characteristics (Pinto et al. 2011a) 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 outplanting site is an operational planting unit located approximately 13 km northwest of Nespelem, Washington, USA (48.2376°N, 119.1272°W; 890 m) on the Confederated Tribes of the Colville Indian Reservation. The open-range, partial-cut site received minimal site preparation (concentrated slash piling) prior to planting. This site is typical of those in the Interior West of the USA, where optimum spring outplanting conditions (April to early May) are followed by a typical, pronounced summer drought (July through September). On 1 May 2007, 20 seedlings from each container type were hand-planted 1.5 m apart within rows established 3 m apart within each of five blocks (100 total seedlings per container type) as a randomized complete block design to account for a slight elevational difference between plots. 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 (0.3–1.5 m). These soils are classified as moderate in available water holding capacity, averaging about 0.12 m^3 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, USA) collected hourly measurements of temperature (°C), relative humidity (%), and rainfall (mm) 1 m above the ground via a 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 ECH$_2$O 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 2. Seasonal trends in soil moisture are graphed in Fig. 1 while temperature and VPD trends are shown in Fig. 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

Table 2 Edaphic and atmospheric monitoring for Coyote Creek planting unit 1 May through 14 October 2007

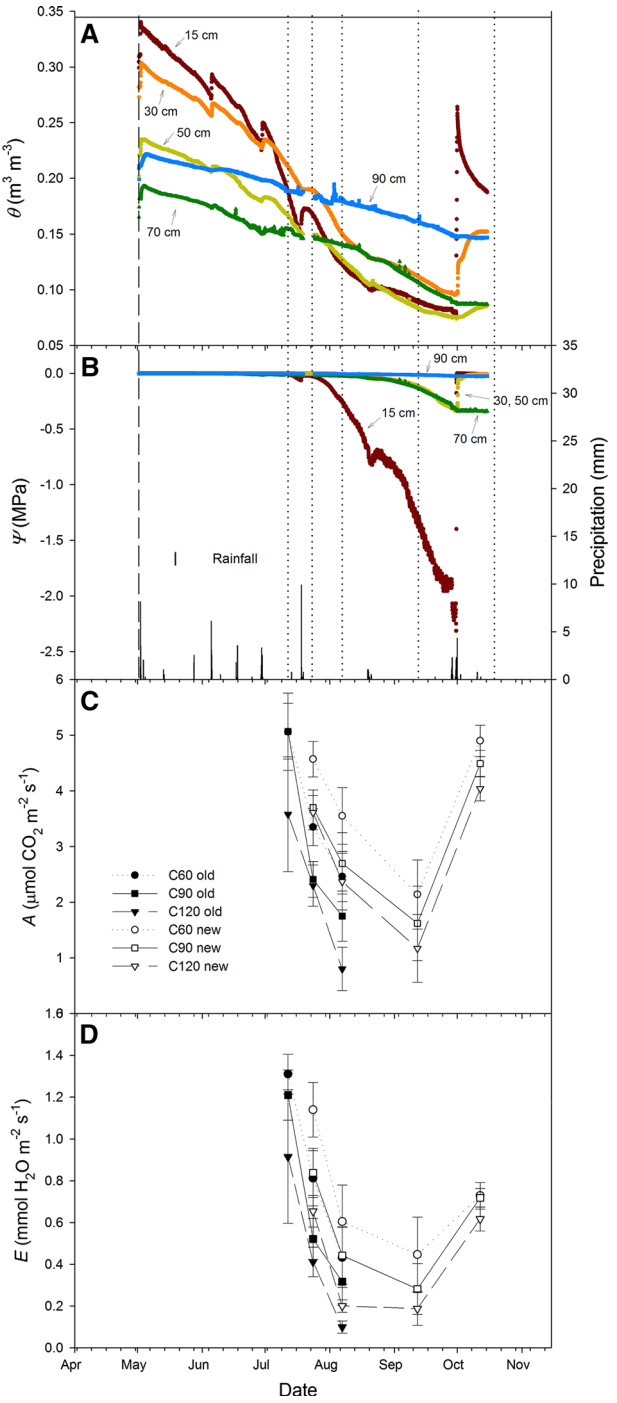
	Mean	Maximum	Minimum
Precipitation (mm)	135[a]	10	0
Air temperature (°C)	16.2	35.8	0.1
Vapor pressure deficit (kPa)	1.4	5.3	0
Soil temperature (°C) (cm)			
15	16.1	24.4	6.4
30	15.1	19.9	7.6
50	14.4	18.2	7.2
70	13.7	17.1	7.1
90	13.7	16.6	7.6
Volumetric soil moisture (m^3 m^{-3}) (cm)			
15	0.19	0.34	0.08
30	0.19	0.30	0.10
50	0.15	0.24	0.07
70	0.14	0.19	0.09
90	0.18	0.22	0.15

[a] Total precipitation for measurement period

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 ± 0.02 m^3 m^{-3} (Decagon Devices, Inc., Pullman, WA, USA). 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, USA). 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 measured water potentials were plotted against θ, and regression equations were fitted to model and predict soil water potential (Ψ_{soil}) in the field.

Seedling gas exchange, biomass allocation, and stable carbon isotope composition

A portable photosynthesis system (model LI-6400, Li-Cor, Lincoln, NE, USA) equipped with a blue/red LED light source and CO_2 injector was used to measure seedling gas exchange 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 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 chamber was set at 1400 μmol m^{-2} s^{-1} (photosynthetic active radiation), 25 °C, 400 μmol mol^{-1} CO_2, and an air flow rate of 400 μmol. 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 chamber. Using the assumption that three needles of a fascicle form a cylinder (Johnson 1984), the abaxial leaf area was calculated by multiplying the cylinder circumference by the length of the needle inside the chamber. Total adaxial leaf area is calculated by multiplying the


A
15 cm
30 cm
50 cm
90 cm
70 cm
θ (m³ m⁻³)
B
90 cm
30, 50 cm
15 cm
70 cm
Rainfall
Ψ (MPa)
Precipitation (mm)
C
C60 old
C90 old
C120 old
C60 new
C90 new
C120 new
A (µmol CO₂ m⁻² s⁻¹)
D
E (mmol H₂O m⁻² s⁻¹)
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Date

◀**Fig. 1** Volumetric soil moisture θ (**a**) and water potential Ψ_{soil} at five depths (15, 30, 50, 70 and 90 cm) with precipitation measurements (*bars*) (**b**) during the 2007 growing season. *Vertical dashed line* indicates seedling planting (1 May 2007); *Vertical*, *light-dotted lines* indicate dates of gas exchange measurements. Measured net photosynthesis A (**c**) and transpiration E (**d**) 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)

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). The following equation was used 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 chamber during gas exchange measurements, and the subsequent effects on stomatal conductance (Running 1976; Meinzer 1982), transpiration fluxes were calculated using ambient VPD. Seedling leaves (outside the chamber) were assumed to 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 initially performed on needles formed in the greenhouse, the main source of photosynthate for establishing trees. Because needle age classes can exhibit differences in photosynthetic capacity (Wang et al. 1995), a comparison was made between old and new needles once the new needles were long enough to measure inside the LI-6400 chamber on two dates. The formation of new needles and sufficient biomass represent the capacity for a seedling to photosynthesize and allocate resources for overall growth and establishment.

At the end of the 2007 growing season, needle samples were collected for carbon isotope analysis. Two samples were tested: needles from the 2006 greenhouse production period and needles from the 2007 growing season. 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, ID, USA). Needle tissue was dried 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 + isotope mass spectrometer. Carbon isotope ratio was expressed relative to the Pee Dee River belemnite standard (PDB; Craig 1957) as follows:

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

On 16 and 26 October 2007, three seedlings from each block × 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

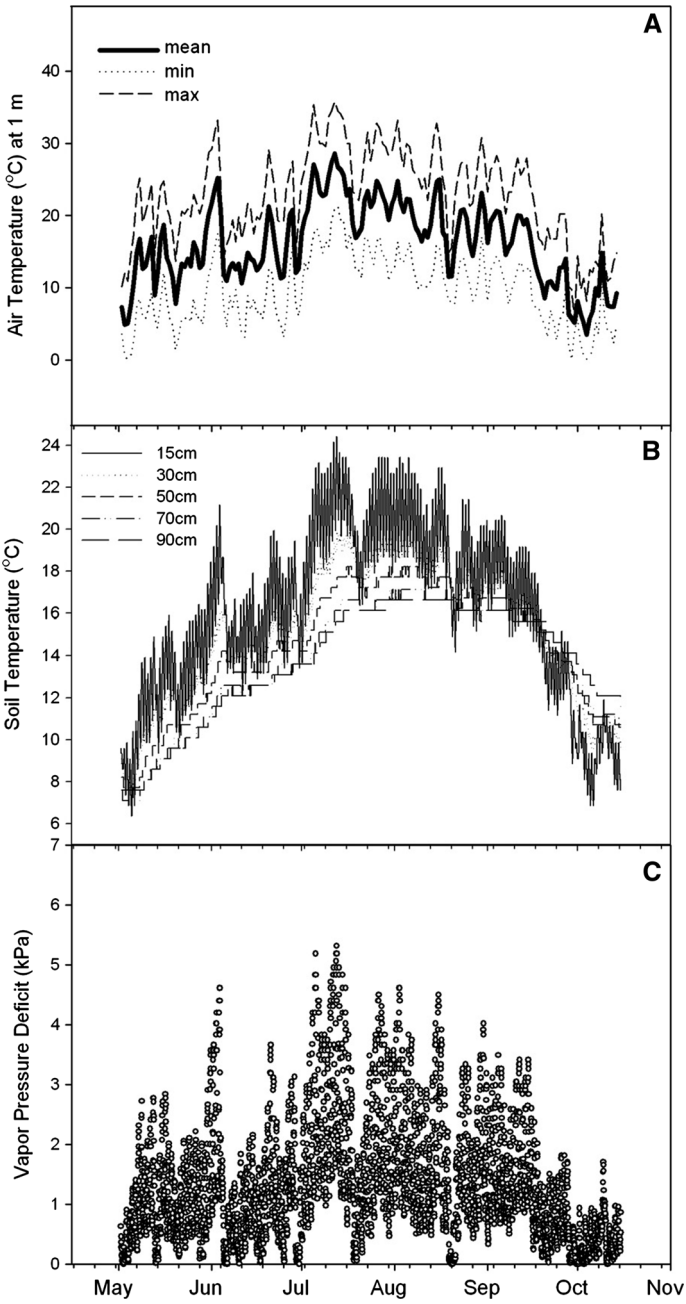


Fig. 2 2007 growing season air temperature (**a**), soil temperature (**b**), and vapor pressure deficit (**c**) conditions at the Coyote Creek planting unit

51.6 cm^2 g^{-1} from a nearby population of *P. ponderosa* (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, 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, USA), was performed for a randomized complete block design to identify differences among containers for seedling biomass (roots, shoots, needle dry weight, and root length), carbon isotope composition, 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, USA). 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 $\alpha = 0.05$. Using θ and modeled Ψ_{soil}, A was plotted to examine the degree in which physiological functioning was correlated with rooting depth. Linear and non-linear regression analyses were performed using SigmaPlot 10.

Results

Edaphic sampling

Soil water retention curves (SWRCs) varied with soil depth (Fig. 3). Best-fit regression models were either quadratic or linear depending on depth, with models explaining 72–98 % of the variation in Ψ_{soil} (Table 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 −2.29 MPa, while Ψ_{soil} at 30, 50, and 70 cm were −0.25, −0.50, and −0.27 MPa, respectively (Table 3; Fig. 1b). 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 ($\geq$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, biomass allocation, and stable carbon isotope composition

Gas exchange values changed over time in the old needles, making the date effect significant for 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 (Fig. 1b, c). Seedling transpiration rates (E) followed a similar pattern from July through October, with a 55 % reduction

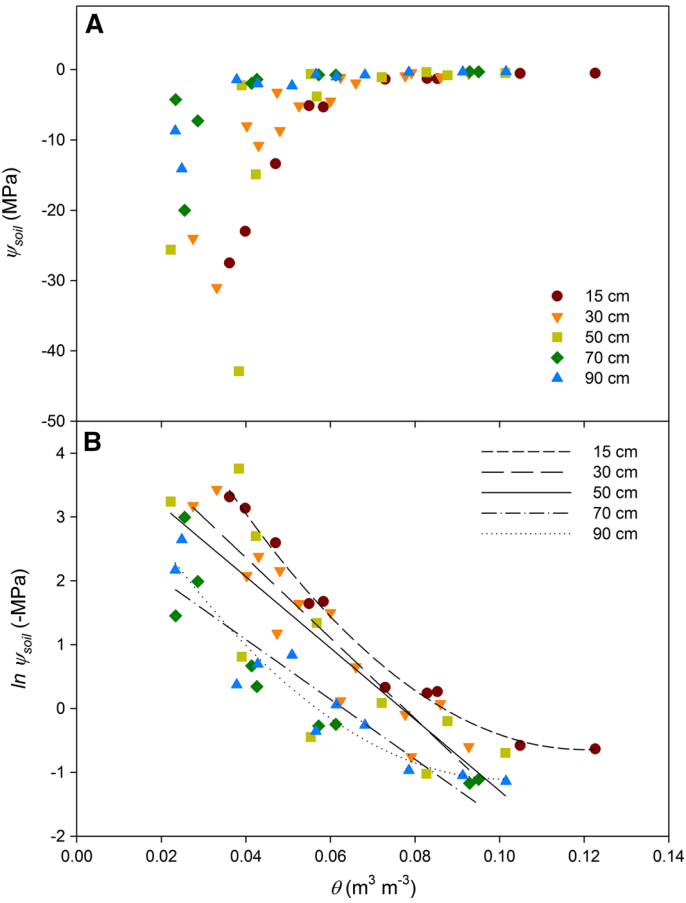


Fig. 3 Laboratory generated soil water retention curves for the Coyote Creek planting unit (**a**). Values of θ and log transformed values of Ψ_{soil} (**b**) are for model prediction purposes. Model results are presented in Table 3

observed over the first three measurements (Fig. 1d). As *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 new needle *A* and *E* ($P \leq 0.0001$). On the second and third date, gas exchange was measured on old and new needles. On July 24, new needle *A* was 47 % greater than old needles (3.96 vs. 2.70 μmol CO_2 m^{-2} s^{-1}, respectively; $P < 0.0001$) and by August 7 the difference reached 63 % (2.98 vs. 1.83 μmol m^{-2} s^{-1}, respectively; $P = 0.008$). Transpiration measures were 48 % greater for new versus old needles on the first date (0.97 vs. 0.66 mmol H_2O m^{-2} s^{-1}, respectively; $P = 0.001$) but not significant on the second ($P = 0.17$).

Repeated measures analysis of *A* and *E* on old needles showed no container effect and no container × date interaction ($P \geq 0.17$). No new needle differences were observed among containers from early August to mid-October for either *A* ($P = 0.11$) or *E* ($P = 0.06$) and the container × date interaction was absent ($P \geq 0.43$). In October, whole-seedling gas exchange calculations (data not presented) showed no differences in

Table 3 Soil water retention curve model parameters for 5 depths on the Coyote Creek planting unit

Soil depth (cm)	n	Minimum measured site θ	Predicted Ψ_{soil}	*P*	R^2	SE	Model type	Model parameters		
								y_o	a	b
15	10	0.08	−2.29	≤0.0001	0.90	0.48	Linear[a]	4.7	−49.3	–
			−1.33	≤0.0001	0.98	0.18	Quadratic[b]	7.7	−138.1	574.7
30	14	0.10	−0.25	≤0.0001	0.87	0.49	Linear	4.9	−62.9	–
50	9	0.07	−0.50	0.0024	0.72	0.84	Linear	2.7	−48.1	–
70	9	0.09	−0.27	0.0009	0.79	0.65	Linear	3.0	−46.9	–
90	11	0.15	−0.02	≤0.0001	0.82	0.54	Linear	3.2	−39.1	–
			−1.2	≤0.0001	0.89	0.42	Quadratic	4.6	−112.6	555.5

Models are derived from log transformed (ln) soil water potential data (Ψ_{soil}). Linear models are presented for all depths; quadratic models are presented only when they yielded better fits

[a] $\Psi_{soil} = y_o + a*\theta$

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

A ($P = 0.92$) or *E* ($P = 0.19$). Seedling shoot and root biomass increased 114 and 140 %, respectively, after one season of field growth (Table 4). Container types were not significantly different in biomass or root egress ($P \geq 0.45$).

Regression analysis on θ versus *A* and Ψ_{soil} versus *A* yielded a positive, and highly correlated ($R^2 > 0.82$) relationship with soil moisture in the upper 15 cm (Fig. 4a, f). For θ, the relationship was linear and it decreased below 30 cm ($R^2 > 0.42$; Fig. 4b–e). Similarly, Ψ_{soil} was better correlated above 30 cm in the soil profile ($R^2 > 0.70$). The relationship improved when the data were fitted with an exponential function ($R^2 > 0.75$). Below 30 cm, however, the relationship deteriorated significantly (Fig. 4g–j).

Variation in carbon isotope ratio ($\delta^{13}C$) was not significantly different among containers for greenhouse produced needles ($P = 0.6031$) or needles produced after outplanting ($P = 0.7053$; Fig. 5). Needles produced after outplanting were, however, significantly more enriched in ^{13}C ($P < 0.0001$) than needles produced in the greenhouse.

Discussion

A myriad of factors, including edaphic and atmospheric conditions, may limit seedling establishment. Immediately after outplanting, stems and foliage may be exposed to unfavorable temperatures (Larson 1967) including those well below 0 °C (Burr et al. 1989) or lethally high values near the soil surface (Kolb and Robberecht 1996a). Similarly, roots may be faced with temperatures not conducive for growth (Larson 1967; Lopushinsky and Max 1990), potentially reducing root-soil contact (Grossnickle 2005). In this study, above- or below-ground temperatures were not extreme (Fig. 2a, b) and although they were low in the upper soil profile at planting time, they rose to non-limiting values (Lopushinsky and Max 1990) by the time seedlings should have been ready to grow new 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 sufficient to cause stress (Grossnickle 2005), which may be expressed as reduction in stomatal conductance (Kavanagh and Zaerr 1997; Marshall and Waring 1984; Running 1976) or a

Table 4 *Pinus ponderosa* morphological and biomass characteristics after 2006 and 2007 growing season ($n = 45$)

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

Seedlings were outplanted 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

[a] Data from Pinto et al. 2012

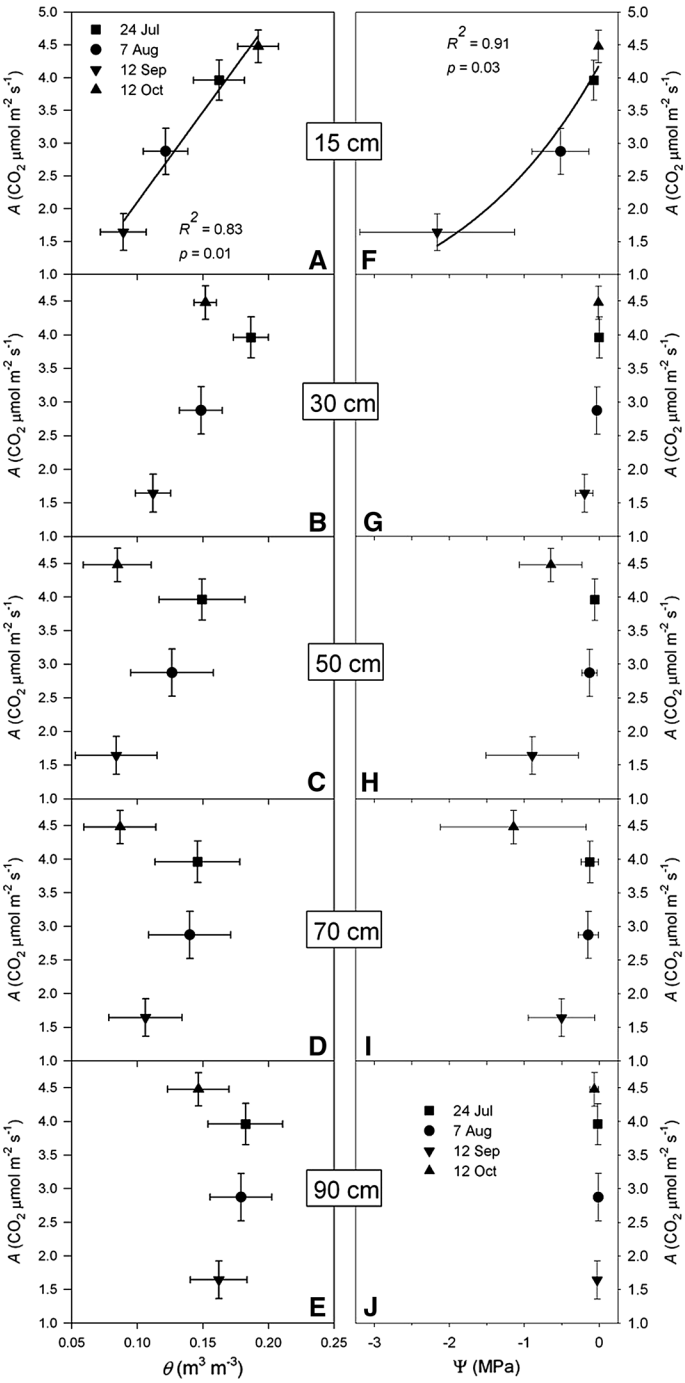
[b] Columns with the same letter are not significantly different, Tukey adjusted ($P < 0.05$)

reduction of A (Hubbard et al. 1999). In addition, elevated nighttime VPD may also increase resistance to seedling growth and establishment because it can drive nocturnal transpiration and maintain low leaf water potential throughout the night (Kavanagh et al. 2007). Such low water potential might exacerbate the stress already experienced by an establishing seedling in already dry conditions and appear as xylem cavitation or heat damage. Current data shows evidence of elevated nighttime VPDs (Fig. 2c) but its impact on the seedlings is not known. The more critical drivers to establishment are likely related to root growth and soil moisture conditions.

When seedlings were outplanted in May, θ and Ψ_{soil} at all measured depths were at their highest seasonal values. This is typical for spring conditions in the Pacific Northwest USA (Warren et al. 2005), where snowmelt typically recharges the soil profile. Shortly thereafter, pronounced and continual decreases in θ were seen at the 15 and 30 cm soil depths (Fig. 1) likely due to the well-drained, loamy-skeletal soils, low precipitation inputs, and the lack of site preparation to control competing vegetation. Conversely, Ψ_{soil} remained high because of the classic characteristic exponential relationship of Ψ_{soil} to θ. Only until soil moisture levels reached about 0.16 m^3 m^{-3} did Ψ_{soil} drop precipitously, and then only in the upper profile. 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, E reached seasonal minima coincident with surface-soil moisture reaching its minimum. In a Mediterranean climate, Cuesta et al. (2010) observed analogous results in A and stomatal conductance (g_s) for *Pinus halapensis.* 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 reduction in gas exchange, coupled with the reduction in soil moisture, indicates that seedling root systems may have not yet expanded deep enough to sustain the transpiration levels seen in older, established trees (Irvine et al. 2002; Weltzin and McPherson 1997). To our knowledge, our regression (Fig. 4) and Ψ_{soil} (Fig. 1b) data are the first of its kind to support this, demonstrating seedling roots in the upper soil profile contribute largely to current physiological functioning and carbon gain. Although Padilla and Pugnaire (2007) did not collect gas exchange data, their *Pinus halapensis* seedling survival results corroborate the importance of rooting depth on seedling establishment. *Pinus ponderosa* 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 in our October data. Again, the use of $\delta^{13}C$ provides data that suggests outplanted seedlings must exercise a greater amount of stomatal control (and consequently greater ^{13}C uptake) to maintain physiological functioning, which we observed the year after outplanting (Fig. 5).

The increases in root biomass (Table 4) and initial gas exchange measurements (Fig. 1c, d) support the assumption of adequate root-soil contact and root egress into the surrounding soil (Grossnickle 2012). Because rocky soils made root harvesting difficult, it is likely that the minimum rooting depth we measured (26 cm) beyond the shortest root plug (Table 4) underestimated actual rooting depth. The resultant root growth likely


24 Jul
7 Aug
12 Sep
12 Oct
R^2 = 0.83
p = 0.01
R^2 = 0.91
p = 0.03
15 cm
30 cm
50 cm
70 cm
90 cm
A
B
C
D
E
F
G
H
I
J
A (CO_2 µmol m^{-2} s^{-1})
θ (m^3 m^{-3})
Ψ (MPa)

◀**Fig. 4** Measured net photosynthesis (new needles) on four dates plotted against measured volumetric soil moisture (θ) at 5 depths below the soil surface (**a**–**e**). A linear model ($A = -0.65 + 27.5*\theta$) was fit to the data for the 15 cm depth; below 15 cm, models were not significant. The same net photosynthesis measurements plotted against modeled water potential (Ψ_{soil}) at 5 depths below the soil surface (**f**–**j**). A modified 2 parameter exponential model [$A = e^{(0.5*(\theta + 2.9)}$] was fit to the data for the 15 cm depth; below 15 cm, models were not significant

contributed to the sufficient levels of *A* observed early in July; these values were similar to the levels seen by Pinto et al. (2012) in established *P. ponderosa* seedlings before the onset of moisture stress. Further evidence of good initial root-soil contact is the elongation of new needles (Burdett et al. 1984; Pinto et al. 2012). 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 trade off potential for more photosynthesis and increased rates of carbon gain might have allowed seedlings to successfully engage and sustain the Burdett model of seedling establishment through root growth. Despite observing post-planting root growth in this study, we suspect that deep root penetration was insufficient to offset moisture stress and reduced gas exchange capacity (see Grossnickle 2005). Nevertheless, once soil moisture increased in October, the extra needles were beneficial by photosynthesizing (Fig. 1) and likely contributing to enhanced root growth during late fall and early winter before temperatures became limiting (Burr 1990; Marshall et al. 2001). This additional root growth could, in turn, provide seedlings more opportunity for growth the following season as *P. ponderosa* seedlings have potential for early season photosynthesis (Marshall et al. 2001).

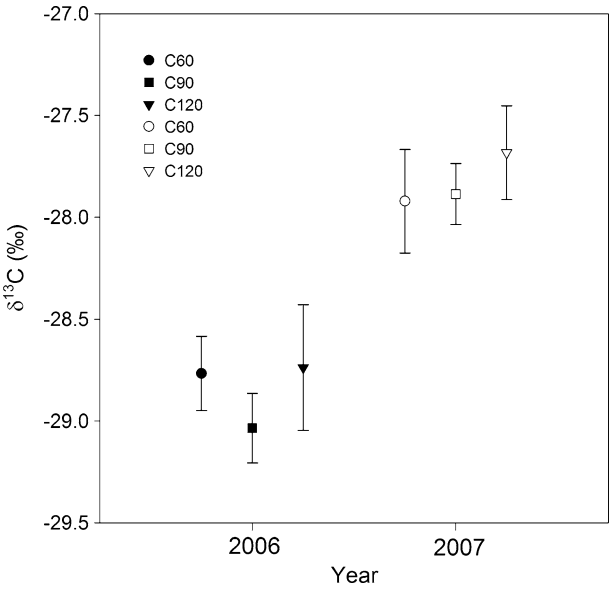


Fig. 5 Carbon isotopic ratio ($\delta^{13}C$) for ponderosa pine seedlings produced in the greenhouse (2006; *filled points*) and 1 year after outplanting (2007; *unfilled points*) at the Coyote Creek planting unit. *Circles* are 60, *triangles* are 90, and *squares* are 120 cm^3 sized containers. Points show least square mean ± SE

At outplanting, our estimates of Ψ_{soil}, particularly at the soil surface, suggest little free water was available to seedlings. In general, Ψ_{soil} increased with soil depth, which parallel earlier observations, for example by Warren et al. (2005) for an old-growth *P. ponderosa* site. Predicted minimum Ψ_{soil} values of −2.29 MPa were observed at 15 cm. It is possible that θ above this depth was lower due to water uptake by vegetation as well as evaporation from the soil surface (Pearson 1930). The shape of the SWRCs generated for our soils show that small decreases in soil moisture in dry conditions cause large changes in Ψ_{soil} (Fig. 3). Using the linear fitted model parameters (Table 3), a 2 % reduction in θ yields a Ψ_{soil} of −5.55 MPa, similar to values seen by Kolb and Robberecht (1996b) at depths above 25 cm. Considering that a large portion of a newly outplanted seedling's root system lies in this zone, it is reasonable to assume roots have little access to freely available water and the seedling is therefore under moisture stress (Grossnickle 2005). Seedlings can mitigate this water limitation by growing roots deeper into the soil profile. Our seedlings showed root penetration to at least 36 cm (Table 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 et al. (2012) measured similar upper profile Ψ_{soil} minima and analogous trends in gas exchange in an experiment that tested the survival and growth responses of *P. ponderosa* seedlings grown in containers of various sizes. That experiment found that seedlings grown in deeper containers, with longer root systems that could access deeper soil moisture quicker, showed better survival than seedlings grown in small containers with shorter root systems. Likewise, longer root systems have improved survival and growth of several oak species seedlings planted in dry areas of Spain (Chirino et al. 2008, 2009). For some pine species, deep planting of bareroot seedlings confers survival advantages (South and Mitchell 1999; VanderSchaaf and South 2003). In the current experiment, however, the seedlings showed no survival or gas exchange benefits due to the longer roots from deeper containers.

Conclusion

Seedlings planted in forest environments are faced with a myriad of barriers before they become coupled with their new surroundings. In this study, spatial and temporal changes in soil moisture had an impact on carbon gain by seedlings. The synchronicity of these changes can be the difference between plantation success and failure. In this study, the longer-rooted, larger stocktype provided no advantages in survival, growth, or carbon allocation compared with smaller stocktypes. Anywhere seasonal droughts occur soon after outplanting, and especially on harsh sites, the deleterious effects inhibiting seedling establishment can be ameliorated by access to moisture stored in the soil profile. The timing and degree of drought will dictate whether stocktype choice, deep planting, or adequate root growth will compensate for the low water potential conditions in the upper soil profile. We observed the importance of new needles to seedling establishment; these needles not only offer higher efficiency and carbon gain before the onset of drought conditions, but they also contribute to increased carbon gain before the onset of winter quiescence. In the latter case, these carbon gains are likely contributing to new root growth that will further aid seedling establishment during the second growing season.

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References

Anderson CP, Hogsett WE, Plocher M, Rodecap K, Lee EH (2001) Blue wild-rye grass competition increases the effect of ozone on ponderosa pine seedlings. Tree Physiol 21:319–327

Blake GR, Hartge KH (1986) Bulk density. In: Klute A (ed) Methods of soil analysis, part I. Physical and mineralogical methods: agronomy monograph no. 9 (2nd ed.). American Society of Agronomy, Madison, pp 363–375

Burdett AN (1990) Physiological processes in plantation establishment and the development of specifications for forest planting stock. Can J For Res 20:415–427

Burdett AN, Herring LJ, Thompson CF (1984) Early growth of planted spruce. Can J For Res 14:644–651

Burr KE (1990) The target seedling concepts: bud dormancy and cold hardiness. In: Rose R, Campbell SJ, Landis TD (eds) Target seedling symposium: proceedings, combined meeting of the western forest nursery associations. Gen. Tech. Rep. RM-200, USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, pp 1–8

Burr KE, Tinus RW, Wallner SJ, King RM (1989) Relationship among cold hardiness, root growth potential and bud dormancy in three conifers. Tree Physiol 5:291–306

Campbell GS, Norman JM (1998) An introduction to environmental biophysics, 2nd edn. Springer, New York

Chirino E, Vilagrosa A, Hernández EI, Matos A, Vallejo VR (2008) Effects of a deep container on morpho-functional characteristics and root colonization in *Quercus suber* L. seedlings for reforestation in Mediterranean climate. For Ecol Manage 256:779–785

Chirino E, Vilagrosa A, Cortina J, Valdecantos A, Fuentes D, Trubat R, Luis VC, Puértolas J, Bautista S, Baeza MJ, Peñuelas JL, Vallejo VR (2009) Ecological restoration in degraded drylands: the need to improve the seedling quality and site conditions in the field. In: Grossberg SP (ed) Forest management. Nova Publisher, New York, pp 85–158

Cobos DR (2007) Calibrating ECH2O soil moisture sensors. Application Note. Decagon Devices. http://www.decagon.com/appnotes/soil_moisture/. Accessed October 2008

Craig H (1957) Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. Geochim Cosmochim Acta 12:133–149

Cuesta B, Villar-Salvador P, Puértolas J, Jacobs DF, Benayas JMR (2010) Why do large, nitrogen rich seedlings better resist stressful transplanting conditions? A physiological analysis in two functionally contrasting Mediterranean forest species. For Ecol Manage 260:71–78

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Annu Rev Ecol Syst 33:507–559

Decagon Devices (2006) Generating a soil moisture characteristic using the WP4. Application Note. Decagon Devices. http://www.decagon.com/appnotes/soil_moisture/. Accessed Sept 2008

Dingman SL (2002) Physical hydrology, 2nd edn. Prentice Hall, New Jersey

Ehleringer JR, Osmond CB (1989) Stable isotopes. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW (eds) Plant physiological ecology. Chapman and Hall, New York, pp 281–300

Elliot KJ, White AS (1987) Competitive effects of various grasses and forbs on ponderosa pine seedlings. For Sci 33:356–366

Escudero A, Mediavilla S (2003) Decline in photosynthetic nitrogen use efficiency with leaf age and nitrogen resorption as determinants of leaf life span. J Ecol 91:880–889

Gardiner DT, Miller RW (2004) Soils in our environment, 10th edn. Prentice Hall, New Jersey

Grossnickle SC (2005) Importance of root growth in overcoming planting stress. New For 30:273–294

Grossnickle SC (2012) Why seedlings survive: importance of plant attributes. New For 43:711–738

Hubbard RM, Bond BJ, Ryan MG (1999) Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. Tree Physiol 19:165–172

Hultine KR, Marshall JD (2001) A comparison of three methods for determining the stomatal density of pine needles. J Exp Bot 52:369–373

Irvine J, Law BE, Anthoni PM, Meinzer FC (2002) Water limitations to carbon exchange in old-growth and young ponderosa pine stands. Tree Physiol 22:189–196

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED (1996) A global analysis of root distributions for terrestrial biomes. Oecologia 108:389–411

Johnson JD (1984) A rapid technique for estimating total surface area of pine needles. For Sci 30:913–921

Kavanagh KL, Zaerr JB (1997) Xylem cavitation and loss of hydraulic conductance in western hemlock following planting. Tree Physiol 17:59–63
Kavanagh KL, Pangle R, Schotzko AD (2007) Nocturnal transpiration causing disequilibrium between soil and stem predawn water potential in mixed conifer forests of Idaho. Tree Physiol 27:621–629
Kolb PF, Robberecht R (1996a) High temperature and drought stress effects on survival of *Pinus ponderosa* seedlings. Tree Physiol 16:665–672
Kolb PF, Robberecht R (1996b) *Pinus ponderosa* seedling establishment and the influence of competition with the bunchgrass *Agropyron spicatum*. Int J Plant Sci 157:509–515
Larson MM (1967) Effect of temperature on initial development of ponderosa pine seedlings from three sources. For Sci 13:286–294
Lopushinsky W, Max TA (1990) Effect of soil temperature on root and shoot growth and on budburst timing in conifer seedling transplants. New For 4:107–124
Maherali H, DeLucia EH (2000) Xylem conductivity and vulnerability to cavitation of ponderosa pine in contrasting climates. Tree Physiol 20:859–867
Marshall JD, Waring RH (1984) Conifers and broadleaf species: stomatal sensitivity differs in western Oregon. Can J For Res 14:905–908
Marshall JD, Rehfeldt GE, Monserud RA (2001) Family differences in height growth and photosynthetic traits in three conifers. Tree Physiol 21:727–734
McMillin JD, Wagner MR (1995) Effects of water stress on biomass partitioning of ponderosa pine seedlings during primary root growth and shoot growth periods. For Sci 41:594–610
Meinzer FC (1982) The effect of vapor pressure on stomatal control of gas exchange in Douglas-fir (*Pseudotsuga menziesii*) saplings. Oecologia 54:236–242
Meinzer FC, Brooks JR, Bucci S, Goldstein G, Scholz FG, Warren JM (2004) Converging patterns of uptake and hydraulic redistribution of soil water in contrasting woody vegetation types. Tree Physiol 24:919–928
Natural Resources Conservation Service (NRCS) (2009) Soil Survey Staff. United States Department of Agriculture. Web Soil Survey. http://websoilsurvey.nrcs.usda.gov/. Accessed Feb 2009
Olivas-Garcia JM, Cregg BM, Hennessey TC (2000) Genotypic variation in carbon isotope discrimination and gas exchange of ponderosa pine seedlings under two levels of water stress. Can J For Res 30:1581–1590
Padilla FM, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. Funct Ecol 21:489–495
Panek JA, Goldstein AH (2001) Response of stomatal conductance to drought in ponderosa pine: implications for carbon and ozone uptake. Tree Physiol 21:337–344
Pearson GA (1930) Light and moisture in forestry. Ecology 11:145–160
Pinol J, Sala A (2000) Ecological implications of xylem cavitation for several Pinaceae in the Pacific Northern USA. Funct Ecol 14:538–545
Pinto JR, Dumroese RK, Davis AS, Landis TD (2011a) Conducting seedling stocktype trials: a new approach to an old question. J For 109:293–299
Pinto JR, Marshall JD, Dumroese RK, Davis AS, Cobos DR (2011b) Establishment and growth of container seedlings for reforestation: a function of stock-type and edaphic conditions. For Ecol Manage 261:1876–1884
Pinto JR, Marshall JD, Dumroese RK, Davis AS, Cobos DR (2012) Photosynthetic response, carbon isotopic composition, survival, and growth of three stocktypes under water stress enhanced by vegetative competition. Can J For Res 42:333–344
Rietveld WJ (1989) Transplanting stress in bareroot conifer seedlings: its development and progression to establishment. North J Appl For 6:99–107
Robert JA, Lindgren BS (2006) Relationships between root form and growth, stability, and mortality in planted versus naturally regenerated lodgepole pine in north-central British Columbia. Can J For Res 36:2642–2653
Running SW (1976) Environmental control of leaf water conductance in conifers. Can J For Res 6:104–112
Scagel R, Bowden R, Madill M, Kooistra C (1998) Provincial seedling stock type selection and ordering guidelines. B.C. Ministry of Forests, Victoria, pp 16–19
South DB, Mitchell RJ (1999) Determining the "optimum" slash pine seedling size for use with four levels of vegetation management on a flatwoods site in Georgia, USA. Can J For Res 29:1030–1046
Starr JL, Paltineanu IC (2002) Methods for measurement of soil water content: capacitance devices. In: Dane JH, Topp GC (eds) Methods of soil analysis: part 4 physical methods. Soil Science Society of America, Inc., Madison, pp 463–474
Svenson SE, Davies FT Jr (1992) Comparison of methods for estimating surface area of water-stressed and fully hydrated pine needle segments for gas exchange analysis. Tree Physiol 10:417–421

van den Driessche R (1987) Importance of current photosynthesis to new root growth in planted conifer seedlings. Can J For Res 17:776–782
Van Haverbeke DF (1963) Root development of ponderosa pine seedlings in the Black Hills. Ecology 44:161–165
VanderSchaaf CL, South DB (2003) Effect of planting depth on growth of open-rooted *Pinus elliottii* and *Pinus taeda* seedlings in the United States. S Afr For J 198:63–73
Wang K, Kellomäki S, Laitinen K (1995) Effects of needle age, long-term temperature and CO_2 treatments on the photosynthesis of Scots pine. Tree Physiol 15:211–218
Waring RH, Franklin JF (1979) Evergreen coniferous forests of the Pacific Northwest. Science 204:1380–1386
Warren CR, McGrath JF, Adams MA (2001) Water availability and carbon isotope discrimination in conifers. Oecologia 127:476–486
Warren JM, Meinzer FC, Brooks JR, Domec JC (2005) Vertical stratification of soil water storage and release dynamics in Pacific Northwest coniferous forests. Agric For Meteorol 130:39–58
Weltzin JF, McPherson GR (1997) Spatial and temporal moisture resource partitioning by trees and grasses in a temperate savanna, Arizona, USA. Oecologia 112:156–164
Wykoff WR (2002) Measuring and modeling surface area of ponderosa pine needles. Can J For Res 32:1–8
Zhang JW, Marshall JD (1995) Variation in carbon isotope discrimination and photosynthetic gas exchange among populations of *Pseudotsuga menziesii* and *Pinus ponderosa* in different environments. Funct Ecol 9:402–412
Zhang JW, Feng Z, Cregg BM, Schumann CM (1997) Carbon isotopic composition, gas exchange, and growth of three populations of ponderosa pine differing in drought tolerance. Tree Physiol 17:461–466
Zwieniecki MA, Newton M (1994) Root distribution of 12-year-old forests at rocky sites in southwestern Oregon: effects of rock physical properties. Can J For Res 24:1791–1796