

Allometry, nitrogen status, and carbon stable isotope composition of *Pinus ponderosa* seedlings in two growing media with contrasting nursery irrigation regimes

R. Kasten Dumroese, Deborah S. Page-Dumroese, and Robert E. Brown

Abstract: Nursery irrigation regimes that recharged container capacity when target volumetric water content reached 72%, 58%, and 44% (by volume) influenced *Pinus ponderosa* Douglas ex Lawson & C. Lawson growth more than either a 1:1 (by volume) *Sphagnum* peat – vermiculite (PV) or a 7:3 (by volume) *Sphagnum* peat – sawdust (PS) medium. Exponential fertilization avoided confounding irrigation and fertilization. Temporary nitrogen (N) immobilization in PS caused transient allometric differences prior to hardening. Subsequent release of immobilized N during the onset of hardening, when daily N flux decreased from 4.2% to 1.6%, allowed PS seedlings to avoid foliar N dilution experienced by PV seedlings. Media yielded seedlings with similar final morphological characteristics, although PS seedlings had improved N status. At onset, particle density and volumetric water content were similar for both media, but PS held about 10% more water than PV at all water potentials at experiment conclusion. Exposure to the driest water content (44%) decreased seedling growth and root N status compared with cohorts that were provided ample moisture (72%). Despite maximum tissue heterogeneity within samples and regardless of irrigation regime, seedlings became 1.3‰ more depleted of ¹³C as the growing season progressed. Refinement of sampling procedures, with focus on ontogenetics, may improve subsequent use of stable carbon isotopes in nursery research.

Résumé : Les régimes d'irrigation en pépinière qui rechargeaient la capacité des récipients lorsque la teneur volumétrique cible en eau atteignait 72, 58 et 44 % (par volume) ont eu plus d'effet sur la croissance de *Pinus ponderosa* Douglas ex Lawson & C. Lawson que des substrats de tourbe de sphaigne et vermiculite (TV) 1:1 (par volume) ou de tourbe de sphaigne et de sciure de bois (TS) 7:3 (par volume). Une fertilisation exponentielle a évité de confondre l'irrigation et la fertilisation. L'immobilisation temporaire de l'azote (N) dans le substrat TS a entraîné des différences allométriques passagères avant la période d'endurcissement. La libération subséquente de N immobilisé au début de la période d'endurcissement, alors que le flux quotidien de N passait de 4,2 à 1,6 %, a permis aux semis dans le substrat TS d'éviter la dilution foliaire de N subie par les semis dans le substrat TV. Les substrats ont produit des semis dont les caractéristiques morphologiques finales étaient semblables même si la teneur en N des semis s'était améliorée dans le substrat TS. Au début, la densité des particules et la teneur volumétrique en eau étaient semblables dans les deux substrats mais le substrat TS a retenu 10 % plus d'eau que le substrat TV peu importe le potentiel hydrique à la fin de l'expérience. L'exposition à la plus faible teneur en eau (44 %) a réduit la croissance des semis et la teneur en N dans les racines comparativement aux cohortes irriguées avec abondamment d'eau (72 %). Malgré l'hétérogénéité maximale des tissus dans les échantillons et peu importe le régime d'irrigation, les semis ont perdu 1,3 ‰ de ¹³C à mesure que progressait la saison de croissance. Le raffinement des procédures d'échantillonnage, en mettant l'accent sur l'ontogénèse, pourrait améliorer l'utilisation subséquente d'isotopes stables du carbone dans les travaux de recherche en pépinière.

[Traduit par la Rédaction]

Introduction

Sphagnum peat is the most widely used growth substrate for container seedling production (Bunt 1988), especially for reforestation plants (Landis et al. 1990). Peat has inherent

characteristics ideal for container production, as well as plant growth, including low pH, high cation exchange capacity (CEC), low inherent fertility, a proper balance of aeration and water-holding porosity, and sufficient rigidity to support plants (Landis et al. 1990). Current manufacturing standards

Received 17 August 2010. Accepted 27 January 2011. Published at www.nrcresearchpress.com/cjfr on 18 April 2011.

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ensure other desired attributes as well: reasonable cost, low bulk density, uniformity, stability, and ease of use (Landis et al. 1990). Of these, porosity may be most important (Heiskanen 1995a, 1995b) because the proper balance of aeration and water-holding capacity is necessary for optimum seedling growth (Heiskanen 1993a).

Heiskanen (1993a, 1995b) concluded that high quality *Sphagnum* peat (light, medium texture and low humification (H1–H3) on the von Post scale) can be used alone for reforestation seedling production because of its excellent porosity characteristics, but also noted that poorer grades of pure peat may not always provide optimum balance depending on irrigation and fertilization practices. For this reason, as well as financial concerns when using high-quality *Sphagnum*, peat is often amended with other components such as perlite or vermiculite (Landis et al. 1990). In general, inorganic amendments are desired because they are usually inert, improve aeration porosity, and often have low bulk densities that reduce overall medium weight (Landis et al. 1990). Such multicomponent media may have a range of volume ratios; usually the amount of organic material ranges from 25% to 50% (Mastalerz 1977), with 40% to 50% thought to be best (Joiner and Conover 1965).

Vermiculite is one of the most popular inorganic amendments because peat serves as the principal water-holding matrix concomitantly providing high porosity, while the lightweight vermiculite adds water-holding capacity, as well as high CEC (Landis et al. 1990). The cost of vermiculite has, however, increased by more than 50% since 2004, and many growers retain fears about potential carcinogenic effects identified with some sources. This concern, combined with growers' interest in making use of locally available, environmentally friendly products, has led growers to look to other amendments (Landis and Morgan 2009).

One such amendment is sawdust. In the Pacific Northwest, USA, commercially available media having 7:3 (by volume) *Sphagnum* peat – Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) sawdust are available at less cost than peat-vermiculite media (Schaefer 2009). Because peat-sawdust media are 100% organic, nursery managers are concerned about root diseases favored by purported increases in water-holding porosity (James et al. 1994). In addition, the high carbon-to-nitrogen ratio (C:N) of the noncomposted sawdust might lead to N immobilization and reduced growth (Landis et al. 1990). Proper attention to irrigation and fertilization practices, which interact with media to affect seedling growth, may relieve these potential disadvantages. Several investigators report that seedling growth is dependent on medium-specific fertigation practices and that irrigation needs to be tailored for optimal seedling growth (Colombo and Smith 1988; Langerud and Sandvik 1988). Media that are robust, in terms of aeration and water-holding porosity, across a range of irrigation schedules would provide fertigation flexibility to nursery managers.

Proper amounts of aeration and water-holding porosity are needed for optimum conifer seedling growth (Timmer and Armstrong 1989). One measure of this function is water-use efficiency, the ratio of net photosynthesis to transpiration, which can be evaluated by plant discrimination against $^{13}\text{CO}_2$ relative to the more abundant $^{12}\text{CO}_2$. 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 transpiration because intercellular $^{13}\text{CO}_2$ is heavier and is more readily exchanged with the lighter atmospheric $^{12}\text{CO}_2$. Although this technique is commonly used in ecological studies (e.g., Miller et al. 2001) and for refining ecosystem modeling (e.g., Mortazavi et al. 2009), it may also have utility for understanding plant water relations under various nursery scenarios. In nurseries, however, seedlings rapidly progress through a series of changes in needle type (cotyledons to primary to secondary) accompanied by vascular differentiation in the hypocotyls and finally formation of terminal buds. Thus, seedling development (i.e., ontogeny) and associated allometry may impact the robustness of this measurement.

Our objective was to compare an all-organic medium (*Sphagnum* peat – sawdust (PS)) and an inorganic-amended medium (*Sphagnum* peat – vermiculite (PV)) across three contrasting irrigation regimes to determine subsequent effects on *Pinus ponderosa* Douglas ex Lawson & C. Lawson seedling growth, allometry, N status, and carbon stable isotope composition ($\delta^{13}\text{C}$). We believe that this is the first study to explore the use of $\delta^{13}\text{C}$ in an operational nursery setting to assess plant production techniques.

Materials and methods

To test hypotheses, we used two commercial media \times three irrigation regimes \times four replications in a completely randomized, factorial design. Media were a 1:1 (by volume) PV and a 7:3 (by volume) PS (SunGro Horticulture, Hubbard, Oregon). See Table 1 for particle size distribution of components.

The sawdust was fresh so the company amended the medium with $0.44 \text{ kg}\cdot\text{m}^{-3}$ Nitroform (38% N; Nu-Gro Technologies, Inc, Courtright, Ontario, Canada) to reduce problems with N immobilization caused by high C:N. Assuming a C:N of peat of 50:1 (Arenas et al. 2002) and Douglas-fir sawdust of 500:1 (Spano et al. 1982), PS has a C:N of 185, 370% greater than PV. Average values for PV and PS for pH (4.0 vs. 3.7) and soluble salts (0.07 vs. $0.125 \text{ dS}\cdot\text{m}^{-1}$) were similar and typical for media used to grow conifer seedlings for reforestation. Both media contained the same base N (excluding the Nitroform) at very low concentrations ($\leq 7.5 \text{ mg}\cdot\text{kg}^{-1}$).

We used Styrofoam containers, typical for reforestation, that were approximately 52 cm long and 35 cm wide, with 160 cavities (10 rows \times 16 columns) each having a volume of 90 mL (about 3 cm in diameter and 15 cm deep). Containers remained intact to evaluate seedling response to the media but were carefully dissected into individual cavities to evaluate media physical properties. At the University of Idaho Center for Forest Nursery and Seedling Research (Moscow, Idaho; 41.717°N , 117.00°W), media were mechanically added to intact and dissected containers using similar equipment settings. Media moisture content ranged from 40% to 46%. All cavities were sown with *P. ponderosa* seeds on 4 March (Julian day 63; hereafter, simply Julian 63) and placed into a greenhouse; containers were irrigated to con-

Table 1. Particle size distribution (%) of *Sphagnum* peat, vermiculite, and Douglas-fir sawdust.

	Particle size distribution (%)					
	6.35 (1/4)*	3.36 (6)	1.68 (12)	0.841 (20)	0.420 (40)	0.149 (100)
Peat [†]		0–20	65–95	40–70	20–45	0–20
Vermiculite [‡]	0–5	20–60	30–70	0–10	0–5	0–5
Sawdust [‡]		10–50	20–45	10–15	<5	

*Sieve size (mm (US)).

[†]SunGro Horticulture, Hubbard, Oregon.[‡]Estimated from Corder and Scott (1960).

tainer (field) capacity with an overhead, traveling boom system. Environmental conditions followed Wenny and Dumroese (1987) and are typical for production of conifer seedlings in greenhouses. Two weeks after sowing (18 March, Julian 77), we thinned germinants to one per cavity; two weeks later (30 March, Julian 89), we began irrigation treatments described below.

Seedling culture and assessment

Each of four containers described above served as a replicate (160 seedlings per replicate; 640 per media × irrigation combination). We modified the basic *P. ponderosa* regime (Wenny and Dumroese 1987) so that seedlings in each treatment combination received 40 mg N applied exponentially (Timmer and Aidelbaum 1996) as modified below.

The basic exponential fertilization equation is as follows:

$$N_T = N_S \cdot (e^{rt} - 1)$$

where r is the relative addition rate required to increase N_S (initial level of N in plant) to a final level ($N_T + N_S$), where N_T is the desired amount to be added over t , the number of fertilizer applications. Based on previous experience, $N_S = 1$ mg, $N_T = 40$ mg, and because we calculated the amount of fertilizer that could be added on a daily basis, $t = 150$ (the number of days between the first and last fertigation during the growing season). To ensure timely terminal bud formation (an important criteria in reforestation seedlings), we divided the 150 applications into pre- and post-bud initiation phases, because switching from a high fertilizer rate at the end of the first exponential curve to a relatively low rate at the beginning of the second exponential curve, along with changes in photoperiod, would stimulate seedlings to form terminal buds (Wenny and Dumroese 1987). For pre-bud applications, $N_S = 1$ mg, $N_T = 13$ mg, and $t = 63$ (30 March to 31 May, Julian 89 to Julian 151); therefore, $r = 0.042$. For post-bud applications, $N_S = 13$ mg, $N_T = 40$ mg, and $t = 87$ (1 June to 26 August, Julian 152 to Julian 238); therefore, $r = 0.016$. The amount to apply on a specific day was calculated as follows:

$$N_T = N_S \cdot (e^{rt} - 1) - N_{t-1}$$

where N_T is the amount of N to apply daily, N_{t-1} is the cumulative amount of N applied, and $t = 1, \dots, 63$ for pre-bud applications and $t = 1, \dots, 87$ for post-bud applications. Peters Professional Conifer Grower 20–7–19 (The Scotts Company, Marysville, Ohio) was the nutrient source, and subsequent nutrient ratios were 100N (58NO₃⁻:35NH₄⁺:7urea): 15P: 79K: 4S: 4Mg: 2Fe: 0.3Cu: 0.3Mn: 0.3Zn: 0.12B: 0.025Mo.

On Julian 77, each container was weighed approximately 60 min after watering to container capacity. Initial volumetric water content (VWC; (cm H₂O)³·(cm substrate)⁻³) was then determined using the following equation:

$$\text{VWC} = [M_{cc} - (M_{ec} + M_m)] / (V_c \cdot 160)$$

where M_{cc} is the mass of the container at capacity, M_{ec} is the mass of an empty container, M_m is the oven-dry mass of the medium, V_c is the filled volume of an individual cavity, and 160 is the number of cavities per container. Beginning on Julian 89, containers were weighed daily at 10:00 and irrigated when actual container mass reached a threshold of 90%, 75%, or 60% (± 5 percentage points) of container capacity mass. Therefore, at initiation of irrigation treatments, VWC thresholds for the media were 72%, 58%, and 44% (by volume). Container capacity mass was recalculated at Julian 112, Julian 167, and Julian 212 to adjust for media shrinkage. Seedlings were fertilized during each irrigation (fertigation). The necessary amount of fertilizer (cumulative daily amounts since the prior irrigation) was diluted in the calculated amount of water required to recharge the medium to container capacity. Containers were placed into a metal tray for fertigation so that leachate could be reapplied.

We randomly selected five seedlings, from interior portions of containers to avoid edge effects, from each medium × irrigation × replicate combination. Our a priori sampling design included four sample dates for these reasons: (i) 12 April (Julian 112) because seedlings had only brief exposure to the irrigation regimes; (ii) 16 June (Julian 167) because this was 14 days after transitioning from accelerated growth to the hardening phase and concomitant change in exponential fertilizer regime could be noted; (iii) 31 July (Julian 212) because seedlings had hardened 6 weeks; and (iv) 2 September (Julian 245) because this was 7 days after the conclusion of fertigation treatments. We sampled at the same time each day to reduce potential confounding of stable carbon isotope composition (Brendel et al. 2003). We measured height (top of medium to tip of growing point or terminal bud), stem diameter 5 mm above the medium, and shoot and root biomass after drying at 60 °C to constant mass. We decided to re-measure heights and stem diameters at Julian 154 because of slight chlorosis in PS seedlings and again at 196 as chlorosis faded. We measured root volume by displacement (Burdett 1979) on Julian 167 and Julian 245. Nitrogen status of shoots and roots was determined with a LECO-600 CHN analyzer (LECO Corp., St. Joseph, Michigan).

The ratio of ¹³C to ¹²C ($\delta^{13}\text{C}$; expressed in parts per thousand (‰)) for shoots was determined at the University of Idaho Stable Isotope Laboratory (Moscow, Idaho). Shoot tis-

Table 2. Initial volumetric water content (by volume) at –5, –10, –40, and –100 kPa for 1:1 (by volume) *Sphagnum* peat – coarse vermiculite (PV) and 7:3 (by volume) *Sphagnum* peat – Douglas-fir sawdust (PS) after irrigation to container capacity (Julian 77).

	Total porosity*	Initial volumetric water content			
		–5 kPa [†]	–10 kPa	–40 kPa	–100 kPa
PV	0.952	0.58	0.49	0.40	0.36
PS	0.954	0.56	0.46	0.38	0.34
<i>P</i> value		0.0329	0.0093	0.0684	0.0492

*Calculated from bulk and particle densities.

[†]From pressure plate analysis ($n = 4$).

sues (needles, stems, and buds when present) were 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+ isotope mass spectrometer. Carbon isotope ratio was expressed relative to the Pee Dee River belemnite standard (PDB; Craig 1957) as follows:

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

Media evaluation

For component particle densities, we assumed 1.6 g·cm⁻³ for peat (Heiskanen 1995a), 0.9 g·cm⁻³ for vermiculite (Raviv and Lieth 2008), and 0.44 g·cm⁻³ for Douglas-fir sawdust (Paul 1950). Using weighted averages, we determined that particle densities (D_p) for each media were 1.25 g·cm⁻³. Bulk density (D_b) was determined as the ratio of dry mass (60 °C) per 72 cm³, the average initial volume of media within each of 20 cavities. For PV and PS, $D_b = 0.060$ and 0.058 Mg·m⁻³, respectively. Total porosity (θ_s) was calculated from D_p and D_b as follows:

$$\theta_s = [(D_p - D_b) \cdot D_p^{-1}] \times 100$$

Treatments were identical to those described above, but individual cavities dissected from containers (described above) were replicated twice. Fertigation (timing and amount) was determined as described above and applied to an individual seedling in ± 5 mL aliquots using a syringe to reduce leachate. Leachate, captured in a metal pan, was re-applied. We randomly selected four cavities from each media \times irrigation \times replication on Julian 77 and Julian 245. Seedling shoots were clipped at the root collar. Samples were immediately frozen until analyzed.

We determined water retention curves (Klute 1965) for each medium \times irrigation combination. After cavities thawed at 20 °C, we saturated the medium during a 48 h period by slowly adding water until a thin film just covered the surface. Cavities were placed onto 1-bar ceramic plates, and fine sand was used to ensure substrate-to-plate contact. We determined gravimetric and volumetric water contents after equilibrating media in their original container cavities and water at tensions of –5, –10, –40, and –100 kPa.

Statistical analysis

Data were analyzed using SAS version 9.2 software (SAS,

Inc., Cary, North Carolina). After ensuring that model assumptions were met, differences in morphological variables (height, stem diameter, biomass of shoots and roots, and shoot–root ratio), N and C concentrations, C discrimination data, and retention data among treatments were identified using PROC GLIMMIX with an autoregressive adjustment and where raw data were averaged within replicate. Type III tests of fixed effects were used to examine interactions and main effects. Differences of least squares means were adjusted for multiple comparisons.

Results

Bulk density was similar for both media (0.060 Mg·m⁻³; $P = 0.6640$). Total porosity of PV and PS, calculated from particle and bulk densities, was also similar (95.2% and 95.4%, respectively). Gravimetric measurement of containers at initial container capacity indicated that both media had an average VWC of 79%, but VWC was significantly greater in PV than in PS at –5, –10, –40, and –100 kPa (Table 2). Retention data showed that the volume of water in containers at –5 (41.7 mL) and –10 (35.0 mL) kPa was approximately equal to our target irrigation thresholds of 55% and 42% (by volume), respectively. Therefore, the 72% (by volume) VWC threshold was between –5 kPa and –1 kPa, the probable maximum matric potential at container capacity.

Although the interval between irrigation events was unaffected by medium ($P = 0.1102$) and the medium \times VWC threshold interaction ($P = 0.7672$), target VWC threshold had a significant effect ($P < 0.0001$). The interval decreased as target VWC thresholds increased, with the 72%, 58%, and 44% (by volume) levels receiving irrigation every 2.1 ± 0.2 , 4.8 ± 0.3 , and 7.6 ± 0.4 days, respectively (mean \pm SE). The mean, actual percentages of container capacity that triggered irrigations were within 1.5 percentage points of target, and standard deviations were less than 5 percentage points (data not shown).

Because we lack shrinkage data of the media for the Julian 245 water retention measurements, we cannot determine transient VWC. Therefore, water content changes from Julian 77 to Julian 244 can only be compared on a per container volume basis. When comparing water contents at the end of the growing season with initial water contents, PV retained significantly less water (5% to 35%) at the higher matric potentials (–5 and –10 kPa), whereas PS values were within 10% for those same potentials (Fig. 1; Table 3). In general, PV

Fig. 1. The change in water content (mL) per container between Julian 77 and Julian 244 for containers filled with 1:1 (by volume) peat–vermiculite and 7:3 (by volume) peat–sawdust media and irrigated to container capacity when volumetric water content (by volume) reached 72%, 58%, or 44% based on initial (Julian 77) values ($n = 4$).

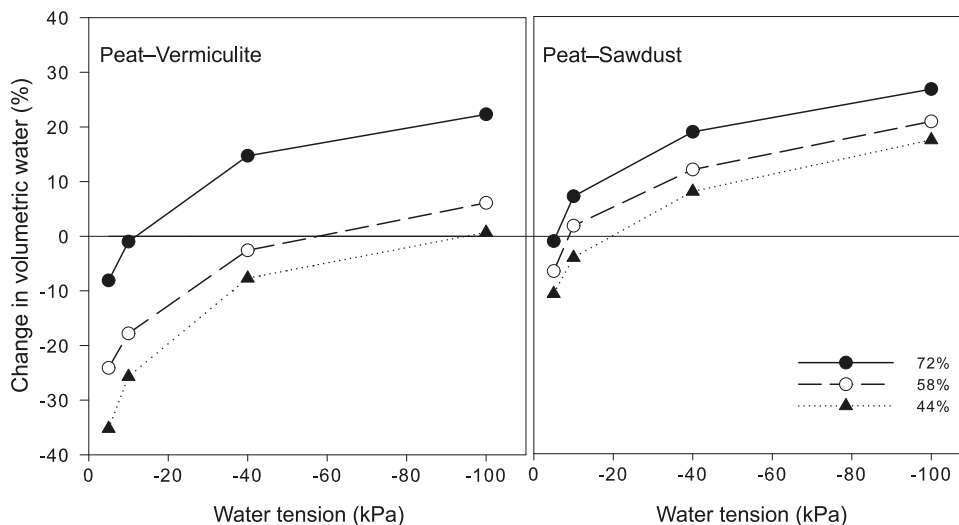


Table 3. (a) Changes in total water content of 1:1 (by volume) *Sphagnum* peat – coarse vermiculite (PV) and 7:3 (by volume) *Sphagnum* peat – Douglas-fir sawdust (PS) between Julian 77 and Julian 244 when irrigated at three initial (Julian 77) volumetric water contents (VWC) observed from pressure plate analysis. (b) Type III tests of fixed effects were used to examine interactions and main effects. Least square means have been adjusted for multiple comparisons (Tukey–Kramer).

(a) Changes in total water content.					
	<i>t</i> value (adjusted <i>P</i>)				
VWC (%) (container capacity (%))	–5 kPa	–10 kPa	–40 kPa	–100 kPa	
PV					
72 (90)	4.27 (0.0827)	0.03 (1.0000)	–6.92 (0.0085)	–12.25 (0.0004)	
58 (75)	10.94 (0.0007)	7.44 (0.0058)	1.71 (0.8169)	–2.03 (0.6760)	
44 (60)	14.07 (0.0002)	8.98 (0.0021)	2.82 (0.3450)	–0.46 (1.0000)	
PS					
72 (90)	0.07 (1.0000)	–3.33 (0.2090)	–9.15 (0.0019)	–14.78 (0.0001)	
58 (75)	2.78 (0.3573)	–0.68 (0.9996)	–5.02 (0.0408)	–10.61 (0.0008)	
44 (60)	5.08 (0.0386)	1.66 (0.8407)	–2.94 (0.3071)	–7.98 (0.0040)	
(b) Type III tests of fixed effects.					
	<i>F</i> statistic (<i>P</i> value)				
	df	–5 kPa	–10 kPa	–40 kPa	–100 kPa
Medium (M)	1	8.82 (0.0249)	1.24 (0.3086)	1.09 (0.3365)	1.48 (0.2701)
VWC	2	19.68 (0.0023)	18.82 (0.0026)	16.65 (0.0036)	12.38 (0.0074)
M × VWC	2	1.54 (0.2891)	2.05 (0.2095)	1.94 (0.2238)	2.46 (0.1661)
Julian date (D)	1	230.73 (<0.0001)	33.11 (0.0012)	63.42 (0.0002)	385.70 (<0.0001)
M × D	1	75.88 (0.0001)	58.89 (0.0003)	36.15 (0.0010)	57.85 (0.0003)
VWC × D	2	28.07(0.0009)	25.90 (0.0011)	35.64 (0.0005)	47.58 (0.0002)
M × VWC × D	2	3.27 (0.1098)	3.25 (0.1105)	2.81 (0.1379)	5.23 (0.0485)

Note: For the type III tests of fixed effects, $n = 24$; df, degrees of freedom.

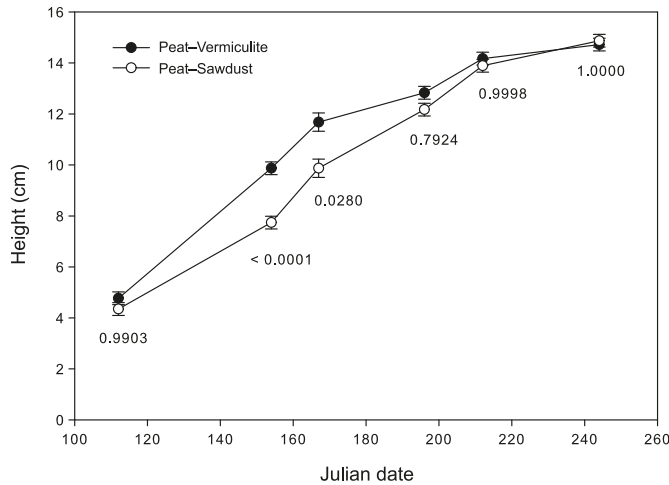
was more robust (less change in water content) than PS at the lower matric potentials (–40 and –100 kPa).

All seedlings had similar heights at Julian 112 (Fig. 2). At onset of hardening (Julian 154), PV seedlings were significantly taller (28.6%) than PS seedlings, and seedlings grown at 72% (by volume) initial VWC were 22.8% taller than those grown at 44% (by volume). From Julian 212 until the end of the growing season (Julian 244), medium had no effect on

height, but seedlings grown at 72% (by volume) remained significantly taller (14.0% to 19.4%) than those grown at 44% (by volume) (Fig. 2; Table 4).

Stem diameter was more robust in response to treatments, medium had no affect, and irrigation was significant only at the end of the growing season (Julian 244) when 72% (by volume) seedlings were 10% thicker than those grown at 44% (by volume). Shoot biomass (*S*) and root biomass (*R*)

Fig. 2. Heights (\pm SE) and *P* values of *Pinus ponderosa* seedlings grown in 1:1 (by volume) peat-vermiculite or 7:3 (by volume) peat-sawdust media ($n = 60$). Julian 167 is the transition point from the accelerated growth phase to the hardening phase.



were not significantly affected by medium or irrigation frequency. *S:R* was unaffected by medium, but irrigation caused seedlings grown at 72% (by volume) to have a 18% greater *S:R* than those grown at 44% (by volume) at Julian 112. Subsequently, *S:R* was unaffected by medium and irrigation, but significantly decreased from 2.8 to 2.1 to 1.6 as Julian date increased from 112 to 167 to 212. *S:R* was similar from Julian 212 through Julian 244.

Shoot N concentration was unaffected by irrigation frequency, but media caused significant differences. At Julian 112, PV had a 32.3% greater concentration than PS seedlings (Fig. 3). At Julian 167, all seedlings had similar N concentration ($15.2 \text{ mg}\cdot\text{g}^{-1}$). PS seedlings then had significantly higher (24.8% and 18.3%) N concentrations than PV seedlings at Julian 212 and Julian 244, respectively. For root N concentration at the end of the growing season (Julian 244), PS seedlings had significantly greater (12.2%) concentration than PV seedlings, and seedlings grown at 72% (by volume) initial VWC had significantly greater concentration (13.2%) than those grown at 44% (by volume).

Shoot C concentrations were unaffected by medium and irrigation frequency and were similar at Julian 112 and Julian 167, before increasing significantly (3.5%) at Julian 212. Similarly, stable carbon isotope compositions ($\delta^{13}\text{C}$) were unaffected by medium and irrigation. However, seedlings became significantly more (1.3‰) depleted of ^{13}C as they matured from Julian 112 to Julian 212 (Table 5).

Discussion

In general, container media with a high water-holding capacity have lower aeration porosity in relation to total porosity, which is a function of bulk density. For the media tested, bulk densities were similar ($0.06 \text{ Mg}\cdot\text{m}^{-3}$) and less than the range of values observed for peat ($0.08 \text{ Mg}\cdot\text{m}^{-3}$; Heiskanen 1993b) and peat amended with up to 50% inorganic materials (0.10 to $0.15 \text{ Mg}\cdot\text{m}^{-3}$; Heiskanen 1995a), although Heiskanen (1995a) did not evaluate either PV or PS. Thus, comparable bulk and particle densities for PV and PS resulted in similar average total porosity (95.3%), in agreement with

those reported by Heiskanen (1995a) for a variety of two-component media (96.3%). In addition, gravimetric measurement of initial container capacity indicated that both media had similar water-holding porosity.

Although peat shrinks as much as 12% to 15% during desorption (Heiskanen 1993b), peat amended with inorganic materials shrinks less, about 5% (Heiskanen 1995a). Heiskanen (1995a) found that most shrinkage occurs during the initial desorption from saturation to -1 kPa , but additional shrinkage due to compaction occurs during the growing season and can further decrease aeration porosity (Langerud 1986). Initial aeration porosity for both media was 37% to 39%, nearing the 40% aeration porosity threshold suggested by Heiskanen (1993a, 1995b) for pure peat substrate (Table 2).

Comparing water contents (volume per container) at the end of the growing season with initial water contents (volume per container), PV and PS retained less water at the higher matric potentials (-5 and -10 kPa) and, in general, more water at the lower potentials (-40 and -100 kPa) (Fig. 1). More frequent irrigations tended to retain water content levels at the highest matric potentials. In general, containers filled with PV held less water at the end of the growing season, whereas containers filled with PS held more. Less overall water content in PV is likely due to greater shrinkage of the peat, which is known to decrease total porosity (Heiskanen 1995a), and perhaps exacerbated by compression of vermiculite particles exposed to prolonged moist conditions (Ward et al. 1987). Data also suggest that PV exhibited much greater shrinkage during the growing season, not just during initial desorption, especially when desorption periods were longer and drier (75% and 60% target container capacity thresholds). PS appeared more robust across a variety of irrigation frequencies; water contents at the higher matric potentials (-5 and -10 kPa) were within 10 percentage points of initial volumes. The increased water contents of PS at matric potentials of -40 and -100 kPa were probably caused by loss of volume as the noncomposted organic components decomposed (Landis et al. 1990). Overall, at the end of the growing season, PS retained about 10% more water than PV, similar to the additional water (12.5%) required during the growing season to meet the threshold irrigation regimes (Table 3).

Despite observed differences in the media, only root volume and root and shoot N status were significantly affected by medium at the end of the growing cycle (Table 4). Because the amount of base nutrients in PV were minute and final N content of seedlings grown in PV was 40 mg and similar to the target amount of exponentially applied fertilizer, we conclude that the use of the exponential fertilization technique to avoid confounding application of liquid fertilizer and irrigation frequency was effective. Improved N status of PS seedlings, however, reflects the N delivered to each container cavity by the initial Nitroform application. Despite its addition to mitigate high C:N in PS, the N within the Nitroform and perhaps the fertigation solution was immobilized by microorganisms between Julian 63 and Julian 112. The significant 31% reduction in PS shoot N concentration compared with PV seedlings at Julian 112 was reflected in depressed shoot growth (Fig. 2). This temporal decline in shoot growth was relieved during the early stages of the hardening phase (Julian 167) as immobilized N was apparently

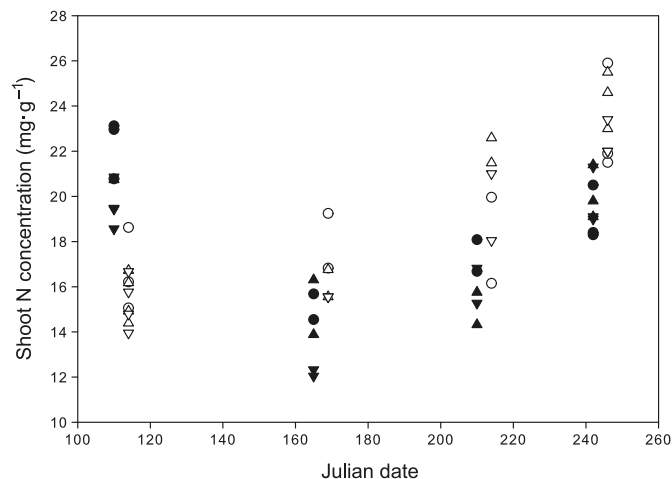
Table 4. (a) Final *Pinus ponderosa* seedling morphological characteristics (mean ± SE) when grown in either a 1:1 (by volume) peat-vermiculite (PV) or a 7:3 (by volume) peat-sawdust (PS) media and irrigated to container capacity when volumetric water content (VWC; by volume) reached 72%, 58%, or 44% based on initial (Julian 77) values. (b) Type III tests of fixed effects were used to examine interactions and main effects.

(a) Final seedling morphological characteristics.											
Medium (M)	Height (cm)	Stem diameter (mm)	Root volume (mL)	Root biomass (g)	Root N (mg g ⁻¹)	Shoot biomass (g)	Shoot N (mg g ⁻¹)	Total mass (g)			
PV	14.7±0.3a	3.30±0.04a	3.9±0.1b	0.82±0.02a	15.6±0.4b	1.38±0.03a	19.7±0.5b	2.20±0.03a			
PS	14.9±0.3a	3.46±0.04a	4.7±0.1a	0.83±0.02a	17.5±0.4a	1.40±0.03a	23.3±0.5a	2.22±0.03a			
VWC (%) (container capacity (%))											
72 (90)	16.0±0.3a	3.53±0.04a	4.7±0.1a	0.86±0.02a	17.1±0.5a	1.51±0.04a	21.1±0.6a	2.37±0.04a			
58 (75)	14.9±0.3ab	3.41±0.04ab	4.3±0.1ab	0.79±0.02a	17.4±0.5a	1.34±0.04a	22.2±0.6a	2.13±0.04b			
44 (60)	13.4±0.3b	3.21±0.04 b	3.9±0.1b	0.83±0.02a	15.1±0.5b	1.32±0.04a	21.1±0.6a	2.14±0.04b			

(b) Type III tests of fixed effects.											
<i>F</i> statistic (adjusted <i>P</i>)											
df	Height	Stem diameter	Root volume	Root biomass	Root N	Shoot biomass	Shoot N	Total mass			
M	1 27.99 (<0.0001)	0.03 (0.8583)	22.76 (0.0002)	0.01 (0.9434)	11.74 (0.0050)	1.75 (0.2022)	28.19 (0.0002)	1.09 (0.3109)			
VWC	2 36.82 (<0.0001)	10.49 (0.0010)	5.29 (0.0156)	2.30 (0.1290)	6.41 (0.0128)	3.74 (0.0437)	1.19 (0.3382)	2.98 (0.0764)			
M × VWC	2 3.70 (0.0450)	5.69 (0.0122)	10.48 (0.0010)	3.35 (0.0579)	2.65 (0.1113)	1.86 (0.1841)	0.53 (0.6044)	1.32 (0.2928)			
Julian date (D)	5 437.36 (<0.0001)	1181.76 (<0.0001)	931.85 (<0.0001)	976.80 (<0.0001)	—	537.69 (<0.0001)	—	1278.42 (<0.0001)			
M × D	5 5.57 (0.0002)	3.38 (0.0082)	9.84 (0.0202)	0.09 (0.9676)	—	1.18 (0.3305)	—	0.76 (0.5781)			
VWC × D	10 2.74 (0.0060)	1.87 (0.0628)	11.77 (0.0084)	1.21 (0.3194)	—	1.27 (0.2924)	—	1.70 (0.0956)			
M × VWC × D	10 1.05 (0.4076)	1.93 (0.0532)	19.72 (0.0023)	7.12 (<0.0001)	—	0.63 (0.7015)	—	1.17 (0.3212)			

Note: For each main effect and seedling characteristic combination, different letters indicate significant differences at $\alpha = 0.05$ using least square means, which were adjusted for multiple comparisons (Tukey-Kramer). For type III tests of fixed effects, $n = 144$. Degrees of freedom (df) for six sample dates (height and stem diameter data). For biomass and root volume, df for date = 3 and 1, respectively; df for interactions would change accordingly.

Fig. 3. Shoot N concentrations ($\text{mg}\cdot\text{g}^{-1}$) for each irrigation threshold and medium at four sampling dates ($n = 4$). Solid symbols represent 1:1 (by volume) peat-vermiculite (PV), open symbols represent 7:3 (by volume) peat-sawdust (PS); 72%, 58%, and 44% initial volumetric water contents (by volume) based on initial (Julian 77) values represented by circles, upward triangles, and downward triangles, respectively. PV and PS values are staggered around sample dates to improve clarity.



released. The reduction in shoot N status for PV seedlings during the early stages of the hardening phase reflects dilution caused by the abrupt reduction in applied N moving from the final stages of the pre-bud regime to that of the post-bud regime (Fig. 3). This dilution was probably circumvented for PS seedlings by the availability of previously immobilized N, allowing them to match the height of their PV cohorts by growing season conclusion.

Irrigation frequency and, hence, water availability had more impact on morphology than did media (Table 4). Seedling growth (height, stem diameter, root volume, and shoot biomass) decreased when seedlings were exposed to lower matric potentials. Heiskanen (1993b) indicates that water content retained at matric potentials higher than -10 kPa is the most available for plant use, and Handreck and Black (1994) conclude that container matric potential should remain higher than -10 kPa. At initiation of our irrigation treatments, the driest VWC threshold for irrigation, 44% (by volume), approximated a matric potential of -10 kPa. Even so, the reductions in growth observed at the -5 and -10 kPa levels (initially 58% and 44% (by volume)) concur with Langerud and Sandvik (1991), who found that seedlings grew largest when irrigated after containers lost 8% to 10% of the liquid held at container capacity, which was similar to our 72% (by volume) threshold.

We observed reduced root N status with decreasing irrigation frequency but similar shoot N concentrations, concurring with Timmer and Armstrong (1989) for conventionally fertilized *Pinus resinosa* and irrigation thresholds similar to our study. Timmer and Miller (1991) found the opposite, however, with exponentially fertilized *P. resinosa* irrigated at similar irrigation thresholds. This difference probably reflects the amount of N in the terminal application, which for Timmer and Miller (1991) accounted for nearly 20% of the total N applied, whereas our cumulative total for the last 7 days accounted for just 8%. The ratio of root N content to applied N

fertilizer, however, decreased as the interval between fertigation increased (Table 4). This may be a function of reduced solute movement from the media to roots caused by low hydraulic conductivity of the peat (Örlander and Due 1986), and (or) low solubilization and mineralization of applied N (Timmer and Miller 1991).

Although we observed significant differences among heights and stem diameters of seedlings grown at different irrigation thresholds (Table 4), seedlings irrigated at 44% (by volume) initial VWC were only 10% below target height and exceeded target stem diameter by 40% in this container type at this nursery, suggesting that reduced irrigation intervals need not sacrifice seedling morphological quality. Although Langerud and Sandvik (1991) concluded that frequent irrigations with small volumes would provide more consistent resource application and yield a more uniform crop, it may be possible that modifications to the exponential fertilization regime (i.e., a higher N_T) would provide sufficient resources to meet desired morphological targets. Reducing fertigation frequency and conditioning seedlings to grow at lower water availability may reduce root rot incidence (Dumroese and James 2005), and during hardening, it has been shown to improve drought resistance after outplanting (van den Driessche 1991). Moreover, recent work indicates species-specific reactions to reduced water availability (e.g., Bergeron et al. 2004; Lamhamedi et al. 2001), often with positive results, suggesting that growers may be using excessive irrigation amounts that potentially contribute to environmental degradation through increased leaching of nutrients and their subsequent discharge from nurseries (Dumroese et al. 2005; Tyler et al. 1996).

The ratio of ^{13}C to ^{12}C in tissues reflects conditions in which photosynthates fractionated into structural and soluble components. In this study, we analyzed entire shoots that included a range of plant tissue types with differing photosynthetic capacities, e.g., stems (Cernusak et al. 2001) and primary and secondary needles (Zobel 1969). Thus, compositing yielded heterogeneous samples. Our sampling also reflected a temporal average of these diverse tissues (e.g., different ages of primary needles), in which the observed $\delta^{13}\text{C}$ level may be dependent on the history (i.e., nursery environment, greenhouse air exchange) of that sample as well. Despite these shortcomings, seedlings, regardless of treatment, became more depleted of ^{13}C and had higher C concentrations as they progressed from the accelerated growth phase (Julian 112) into the hardening phase (Julian 212). The 1.3‰ shift in depletion probably reflects changes in ontogeny (changes in needle type and abundance and development of terminal buds) and (or) allometry rather than foliar N status. During the latter stages of rapid growth and progressing forward during hardening, nursery-grown *P. ponderosa* seedlings (i) begin to develop mature (secondary) needles in fascicles; (ii) grow more roots relative to shoots; (iii) increase their C concentration; and (iv) experience more shade on lower needles as canopies close. For several *Pinus* species in the western USA, primary needles have higher photosynthetic capacity than mature needles (Wright 1970), but for *Pinus roxburghii*, no correlation was found for stomatal conductance of water of primary and secondary needles (Zobel et al. 2001). We found no literature that compared $\delta^{13}\text{C}$ in primary and secondary needles of pines, although reduced

Table 5. (a) Stable carbon isotope composition ($\delta^{13}\text{C}$) and carbon and nitrogen concentrations (mean \pm SE) of *Pinus ponderosa* seedlings grown in either a 1:1 (by volume) peat–vermiculite (PV) or a 7:3 (by volume) peat–sawdust (PS) media and irrigated to container capacity when volumetric water content (VWC; by volume) reached 72%, 58%, or 44% based on initial (Julian 77) values. (b) Type III tests of fixed effects were used to examine interactions and main effects.

(a) Stable carbon isotope composition and carbon and nitrogen concentrations.			
	$\delta^{13}\text{C}$ (‰)	Carbon (mg·g ⁻¹)	Nitrogen (mg·g ⁻¹)
Medium (M)			
PV	-26.8 \pm 0.1a	481.0 \pm 2.0a	16.1 \pm 0.5b
PS	-26.6 \pm 0.1a	475.2 \pm 2.0a	20.1 \pm 0.5a
VWC (%) (container capacity (%))			
72 (90)	-27.0 \pm 0.2a	478.0 \pm 2.4a	17.8 \pm 0.7a
58 (75)	-26.7 \pm 0.2a	476.0 \pm 2.4a	18.7 \pm 0.7a
44 (60)	-26.4 \pm 0.2a	480.2 \pm 2.4a	17.9 \pm 0.7a
Julian date (D)			
112	-25.5 \pm 0.1a	462.4 \pm 1.3b	18.3 \pm 0.4a
167	-26.0 \pm 0.1b	466.6 \pm 1.4b	15.2 \pm 0.4b
212	-26.7 \pm 0.1c	478.1 \pm 1.4a	18.1 \pm 0.4a
(b) Type III tests of fixed effects.			
	df	F statistic (adjusted P)	
		$\delta^{13}\text{C}$	Carbon
M	1	<0.0001 (0.9465)	1.02 (0.3293)
VWC	2	2.26 (0.1382)	10.51 (0.0014)
M \times VWC	2	0.94 (0.4126)	3.73 (0.0485)
D	2	63.51 (<0.0001)	15.02 (0.0010)
M \times D	2	3.21 (0.0837)	51.85 (<0.0001)
VWC \times D	4	1.07 (0.4210)	1.51 (0.2709)
M \times VWC \times D	4	2.14 (0.1502)	2.07 (0.1601)

Note: For each main effect and variable combination, different letters indicate significant differences at $\alpha = 0.05$ using least square means, which were adjusted for multiple comparisons (Tukey–Kramer). For the type III fixed effects, $n = 54$; df, degrees of freedom.

photosynthesis in conjunction with increased transpiration by secondary needles could promote ^{13}C discrimination and yield the depletion values observed. As the *S*:*R* of our seedlings decreased from 2.8 to 2.0 to 1.6 (Julian 112, 167, and 212, respectively), discrimination increased from -25.5‰, to -26.0‰, to -26.7‰, respectively. Our *S*:*R* results concur with those of Pinto (2005) and suggest that the small root systems of rapidly growing seedlings limit hydraulic conductance. Improved hydraulic conductance provided by a larger root system in relation to the shoot (reduced *S*:*R*) would improve discrimination, as we observed. Total C concentration for this duration increased by about 3.5%, possibly reflecting an increase in the amount of lipids, which are known to be depleted of ^{13}C (DeNiro and Epstein 1977). In addition, as seedlings grown at high densities enter the hardening phase, appreciable intermingling of their canopies ensues. Reduced irradiance to lower needles may also have reduced photosynthetic capacity (Le Roux et al. 2001). Despite our inability to definitively describe the mechanism responsible for shifts in $\delta^{13}\text{C}$, we observed depletion of $\delta^{13}\text{C}$ that met currently accepted paradigms, as was the case with whole shoot (mixture of primary and secondary needles) samples of *Pinus contorta* seedlings (Guy and Holowachuk 2001). Thus, we believe that more discrete sampling of actively growing tissues for $\delta^{13}\text{C}$ relative to ontogenetic phase, which changes rapidly under controlled nursery conditions, could be a robust method for evaluating seedling response to a variety of operational nursery cultural practices.

In summary, we compared peat substrates amended with either an organic or inorganic amendment across a range of irrigation frequencies. Exponential fertilization appears to be an effective way to avoid confounding application of liquid fertilizers during irrigation experiments. When properly monitored, irrigation frequency has a stronger impact on seedling morphology than medium type, and a 100% inorganic substrate can yield suitable porosity characteristics for seedling growth. Although irrigation thresholds at lower substrate matric potentials significantly affected seedling morphology, on a practical nursery scale, these differences may be overcome by fine tuning of fertigation. Moreover, irrigation at lower matric thresholds may reduce disease incidence and has been proven to improve seedling drought tolerance. Measuring carbon stable isotope composition may be an effective tool for ascertaining irrigation and other effects of other nursery practices on crops. Better sampling methods, however, with focus on seedling development (ontogenetics) so homogeneous structures (e.g., primary or secondary needles) are sampled at discrete times (e.g., all primary needles of the same age), could improve subsequent interpretation.

Acknowledgements

We thank the members of the Intermountain Container Seedling Growers' Association for financial support; K.E. Quick, D.J. Swanson, and S.J. Morrison for assistance growing plants; J.D. Marshall for carbon isotope discussions and

comments on drafts; A. Ross-Davis and D.L. Turner for assistance with statistical analyses; and J. Heiskanen and J.R. Pinto, three anonymous reviewers, and an Associate Editor for insightful comments. This work was initiated when D.L. Wenny directed the University of Idaho nursery program; his generous support is noted.

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