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Determining the effect of age and drought stress on the hydraulic conductance and vulnerability to cavitation of Douglas-fir seedling root systems using the vacuum method

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Abstract

Reductions in a plant's ability to transport water, measured as hydraulic conductivity, can impact stomatal conductance and photosynthetic gas exchange. Roots are the plant organs least resistant to cavitation and, due to this, root hydraulic conductivity and vulnerability to cavitation have been proposed as an additional element for seedling quality evaluations. This trait, however, has been poorly characterized for important conifer species preventing the integration of this important physiological trait into criteria for genetic selection and nursery production. In the first part of this study, we modified the original design of the vacuum chamber method allowing for measurements of the whole root system of young conifer seedlings. This method was then compared with standard gravimetric measurements for stems of four tree species and our results confirmed that the vacuum chamber is a reliable technique to measure hydraulic conductance. We were able to perform up to 50 measurements per day of hydraulic conductance of whole root systems using a team of two people, opening the possibility for large scale assessments of root hydraulics, including genetic screening. In the second part of the study, the vacuum chamber method was used to assess the impact of nursery irrigation regime and seedling age on coastal Douglas-fir stem and whole root system hydraulic conductance. When coastal Douglas-fir seedlings grew under limited water availability, roots and stems both experienced a 65% reduction in xylem hydraulic conductance. In contrast to this, Douglas-fir seedling water transport efficiency was reduced more in roots than stems when seedlings were grown under well-watered conditions.

Keywords Pseudotsuga mensiezii · Plant hydraulics · Drought resistance · Roots

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Introduction

Soil moisture availability is the most common limiting factor for conifer seedlings planted on recently disturbed sites in the United States Pacific Northwest (PNW). This is often due to the combined effects of the prolonged summer drought period typical to the region and competition with aggressive early-seral vegetation species. Excessive water shortages can result in deleterious effects on seedling physiology, growth, and survival as moisture stress can lead to xylem embolism and stomatal closure (Kavanagh and Zaerr 1999; Pinto et al. 2012, 2016; Gonzalez-Benecke and Dinger 2018; Sloan et al. 2020). The reduction in hydraulic conductance associated with xylem cavitation can have cascading effects as stomatal conductance and photosynthetic gas exchange can be limited by the reduced efficiency of the water transport system (Domec et al. 2004), resulting in reduced growth and in many cases, mortality (Brodribb et al. 2019). Therefore, producing seedlings in the nursery with a higher resistance to xylem cavitation may help to increase seedling survival in areas with intense drought.

Roots are, in general, the plant organs least resistant to cavitation (Eissenstat 1997; Hacke et al. 2000). Even though root hydraulic conductance (k_r, water flow over a change in pressure) has been proposed as an additional element for seedling quality evaluations (Timmis 1980), this trait has been poorly characterized and integrated into criteria standards for tree nursery production and field performance for tree seedlings. This may be, in part, due to time and cost constraints related to the characterization of seedling hydraulics, including hydraulic conductance and vulnerability to cavitation.

Several methods and techniques exist for measuring k_r, such as evaporation flux, pressure chambers, and hydrostatic pressure-induced reverse flow chambers (Tyree 1998, 2003). The root hydraulics of trees are commonly measured using individual root segments (Ritchie and Shula 1984; Sperry and Ikeda 1997; Mencuccini 2003; Domec et al. 2004; Gonzalez-Benecke et al. 2010; Johnson et al. 2014) which provides important information about the hydraulic capacity of single root segments, but these results are hard to extrapolate to the whole root system, even for small trees or seedlings. The most common technique currently used for measuring k_r of whole root systems of small trees or seedlings is the high-pressure flow meter (HPFM; Tyree et al. 1993, 1995) and HPFM has been used to quantify k_r in several studies (Tsuda and Tyree 1997, 2000; Tyree et al. 1995; Alsina et al. 2011; Bogeat-Triboulot et al. 2002). Accurate measurements can be performed with this technique, but time and cost constraints may limit its use as only a few samples can be measured each day and the cost of the equipment is high.

An alternative method for measuring k_r of the whole root system was proposed by Kolb et al. (1996). In this technique, called the vacuum canister or vacuum chamber method, the whole root system is inserted into a custom made chamber capable of withstanding vacuum. The collar of the root system is connected to a tubing filled with water (generally a diluted solution with KCl) and connected to a water/solution reservoir located on an analytical balance. The chamber is connected to a vacuum pump with a regulator, and the flow of water through the root system is accounted for as the reduction in weight of the water reservoir on the balance. This alternative method for measure k_r offers advantages over the high-pressure flow meter as samples may be processed quicker and at a reduced cost. As the vacuum chambers used in this study were custom made in our lab, we compared the vacuum method against the standard gravimetric method, that is widely used for measuring the hydraulic conductance of stems (Sperry et al. 1988).

The incorporation of seedling vulnerability to cavitation in seedling genetic selection and nursery production practices offers potential opportunities to improve seedling performance at the outplanting site (Melcher et al. 2012; de Oliveira et al. 2018). For example, does altering irrigation regimes in the nursery effect seedling hydraulic conductance and vulnerability to cavitation? Does the hydraulic conductance and vulnerability to cavitation of seedlings change if they are grown in the greenhouse for an additional growing season? Do seedlings with higher vulnerability to cavitation perform better under droughty field conditions? Having more detailed information on how nursery practices impact the inherent drought resistance of Douglas-fir (*Pseudotsuga menziesii*) seedlings may help managers to produce seedlings better adapted to the intense drought conditions common to much of the PNW. This makes the vacuum chamber method an important tool in the future of reforestation research.

This study consisted of two parts: (1) the validation of the vacuum chamber method by comparison against the standard gravimetric method for measuring xylem hydraulic conductance across a range of species, and (2) the use of the vacuum chamber method to assess the effect of age and nursery irrigation regime on the hydraulic conductance and vulnerability to cavitation of Douglas-fir stems and whole root systems.

Materials and methods

Construction and use of the vacuum chamber to measure hydraulic conductance

In this study, the k_r of the whole root system of young seedlings was measured using a modified version of the vacuum chamber method proposed by Kolb et al. (1996). Two vacuum chambers of varying size were constructed from clear cast acrylic tube (standard: 3 inch OD, 2–1/2 inch ID, 30 cm length; large: 4.5 inch OD, 4 inch ID, 60 cm length). At one end of the acrylic tube, an aluminum coupling was attached. The coupling was custom made to fit with a pressure chamber compression gland lid (PMS Instruments Co., OR, USA) that allows opening and closing of the vacuum chamber. Inside the lid, tubing connectors were attached using vinyl tubing, polypropylene tubing adapters, and luer tubing connectors. At the other end of the acrylic tube, a rubber stopper was attached to seal the apparatus when vacuum is applied. A ½ inch quick connector was installed on the aluminum coupling to facilitate connecting to a vacuum pump (Fig. 1a).

To measure k_r, the whole root system was detached from the seedling two cm above the root collar. The cut was made underwater using an all-purpose cutter (CutZall, Trades Pro). The “neck” of the root system was attached to the tubing filled with degassed and filtered (0.2 μm) water containing 20 mM KCl (Pockman and Sperry 2000) installed at the lid (Fig. 1b). This tubing was connected to another tube which had a 1 ml micropipette suspended in a solution reservoir on a balance (APX-100, Denver Instruments, NY, USA) at the exit. The balance was connected to a computer and the tubing and micropipette where filled with solution. This allowed for solution to be pulled from the reservoir through the root system when vacuum was applied to the chamber. The root system was covered with a moist heavy duty paper towel to avoid desiccation and was left inside the vacuum chamber. After closing the lid and checking that no air bubbles were present in the tubing line, the vacuum pump was turned on and a pressure (vacuum) of 0.01 MPa was applied using

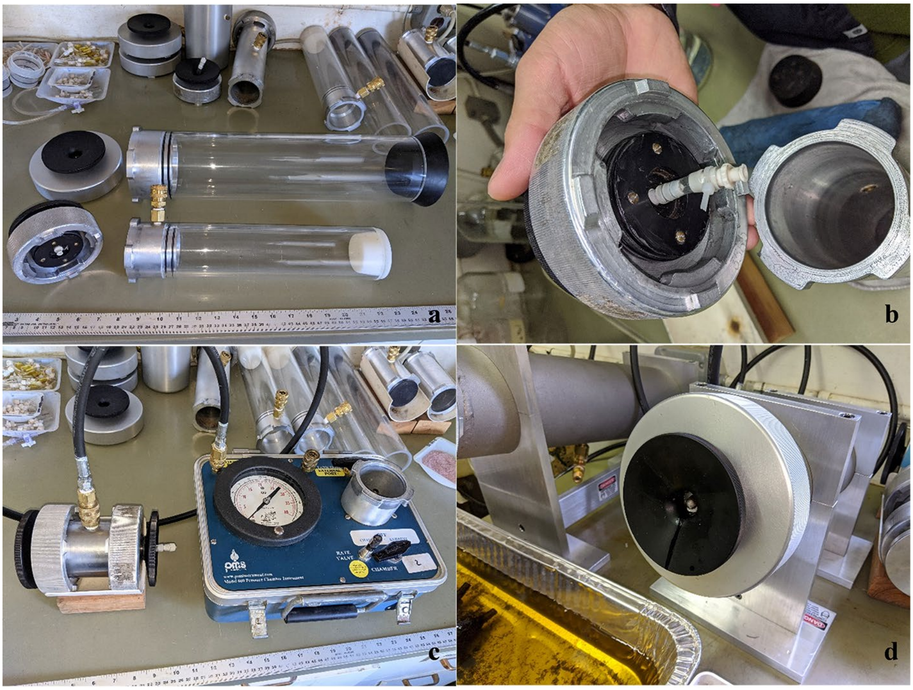

Fig. 1 Images of hydraulic apparatus used in this study: **a** vacuum chamber (two sizes are shown: 4.5 and 3 inch OD) with lid and coupling with quick connector, **b** tubing attachment inside the lid, **c** cavitation chamber for stems connected to a modified pressure chamber, and **d** pressure chamber inducing cavitation to roots (end of 2 cm root stem seen bubbling at the center)

a vacuum regulator (IRV20, Orange Coast Pneumatics, Inc., CA, USA). The vacuum was monitored with a vacuum meter (R3100, REED Instruments, NC, USA) and readings were recorded at the beginning and end of each measurement. Water volume changes in the reservoir were determined using the reduction in weight measured to the nearest 0.0001 g every 5 s over a 2 to 5 min interval (Gonzalez-Benecke et al. 2010). A correction was made to account for passive uptake (Kolb et al. 1996; Torres-Ruiz et al. 2012) by measuring "background flow" before and after each k_r measurement.

Hydraulic Conductance measurements using the gravimetric method

The standard gravimetric method was used to validate the vacuum chamber method by comparing sapwood-specific hydraulic conductivity (k_s, kg water s^{-1} m^{-2} sapwood MPa^{-1} m) measurements for stems. A Sperry tubing apparatus was used to conduct the gravimetric k_s measurements (Sperry et al. 1988). Stem segments of 10–15 cm in length and 2–6 mm in diameter were cut underwater. One end of the segment was attached to tubing filled with the same degassed and filtered 20 mM KCl solution. The other end of the segment was attached to the tube filled with the solution that conducted the efflux to a water reservoir on a scale connected to a computer. Similar to roots, water volume changes in the reservoir were determined using changes in weight measured to the nearest 0.0001 g every 5 s over a 2 to 5 min interval. A Mariotte tube (CL-060, SMS, CA, USA) was used as the solution reservoir and to control the hydrostatic pressure difference across the segment, maintaining a hydraulic pressure head of 0.008 MPa for all measurements. After the sample connection was made to the apparatus, flow into the segments without a pressure head (“background” flow) was measured after each gravimetric measurement (Davis et al. 1999). Background measurements were subtracted from regular gravimetric measurements to obtain a final k_s value.

Hydraulic conductance calculation

Hydraulic conductance (kg water s^{-1} MPa^{-1}) was calculated as the flow rate of water (kg water s^{-1}) for a given pressure gradient (MPa) through a sample (stem segment or whole root system) for both the vacuum chamber and gravimetric methods. The sapwood-specific hydraulic conductivity of stems (k_s, kg water s^{-1} m^{-2} sapwood MPa^{-1} m) was determined as hydraulic conductivity divided by the corresponding sapwood area (m^2) distal to the segment of known length (m). For roots, dry mass-specific hydraulic conductance (k_{rm}, mmol water s^{-1} g^{-1} root MPa^{-1}) and volume-specific hydraulic conductance (k_{rv}, mmol water s^{-1} cm^{-3} root MPa^{-1}) were determined as hydraulic conductance divided by the corresponding dry mass (g) or volume (cm^3) of the whole root system (unknown length).

For both stems and roots, initial “native” k ($k_{r\text{-}nat}$ for roots, $k_{s\text{-}nat}$ for stems) was determined as the first measurement immediately after sample collection. After “native” k determination, segments were soaked in a 20 mM KCl solution under vacuum for 72 h to refill the embolized tracheids/vessels (Domec and Gartner 2001; Gonzalez-Benecke et al. 2010). After removing tracheid/vessel embolisms through this process, the maximum k ($k_{r\text{-}max}$ for roots, $k_{s\text{-}max}$ for stems) was measured using the same corresponding hydraulics method.

Comparison gravimetric and vacuum methods

To test the adequacy of the vacuum chamber method for measuring k across a range of tree species, 49 stem segments of seedlings from four species were measured with both the gravimetric and vacuum methods. The species sampled were western larch (*Larix occidentalis* Nutt.; n = 20), black walnut *(Juglans nigra* L.; n = 9), interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca* (Beissn.) Franco; n = 10), and redwood (*Sequoia sempervirens* (Lamb. ex D. Don) Endl.; n = 10). All seedlings were younger than a year old and grown under greenhouse conditions. For black walnut, only $k_{s\text{-}nat}$ was measured. For western larch, redwood, and interior Douglas-fir; $k_{s\text{-}nat}$, $k_{s\text{-}max}$ and k_s at varying pressures were also measured, for a total of 199 observations (78 western larch, 9 black walnut, 53 interior Douglas-fir and 59 redwood). All k_s measurements were carried out following the methods outlined previously.

Douglas-fir seedling production

After validating the vacuum chamber method described previously, seedlings of coastal Douglas- fir (*Pseudotsuga menziesii* var. *menziesii*) were produced in the greenhouse to assess the effects of age and irrigation regime on root system and stem hydraulic conductance and vulnerability to cavitation. Seedlings were sown and grown at the greenhouse of the College of Forestry, at Oregon State University in Corvallis, OR. Seedlings were sown in March 2019 and grown in styroblocks of 170 cm^3 cavity volume (77/170, Beaver Plastic) using a commercial growing media (Wilco Professional Potting Mix). Seedling nutrition was carried out through fertigation with Peters Professional soluble fertilizers (ICL) using a Dosatron® injector. The application doses and frequency was established using the guidelines by Landis (1989) in accordance with seedling growth stages: Early growth, (Plant Starter 10–30–20), accelerated growth (General Purpose 20–20–20), and hardening (Flowering special 15–3–25). Additional micronutrients were added on each stage using Peters STEM. The irrigation regime for the first six months of seedling production was designed to maintain the relative weight of containers above 70% of the saturated weight ("manager technique", Dumroese et al. 2015). The weight of a subset of containers was measured multiple times a week to assess water status and guide irrigation treatments. At the end of the first growing season in the greenhouse (September 2019), 20 seedlings were sampled for morphology and hydraulic measurements, the details of which are described below. Seedlings were six months old at that time.

In February 2020, a cohort of seedlings not used for the previous measurements, that overwintered in the greenhouse, were transplanted into 710 cm^3 individual plastic containers (D43L Deepot cells) using the same growing media and grown for a second year. The slow-release fertilizer Osmocote (14–9–12) was included in the potting soil when these seedlings were transplanted and no additional fertilizer was applied. Between June and August 2020, the seedlings were divided in two groups, and each received one of two contrasting watering treatments: (i) well-watered (WW), relative weight of containers maintained above 70% of saturated weight, and (ii) water stress (WS), relative weight of containers maintained between 50 and 80% of saturated weight (Fig. 2b). These levels of threshold relative weight were determined using the relationship between relative weight and pre-dawn water potential determined from a previous study (Fig. 2a). This relationship demonstrates that seedling water stress, measured as pre-dawn water potential, begins to increase below 70% container relative saturated weight. Similar to the first six months, the weight of a subset of containers was measured multiple times per week to guide irrigation treatments. When the seedlings were

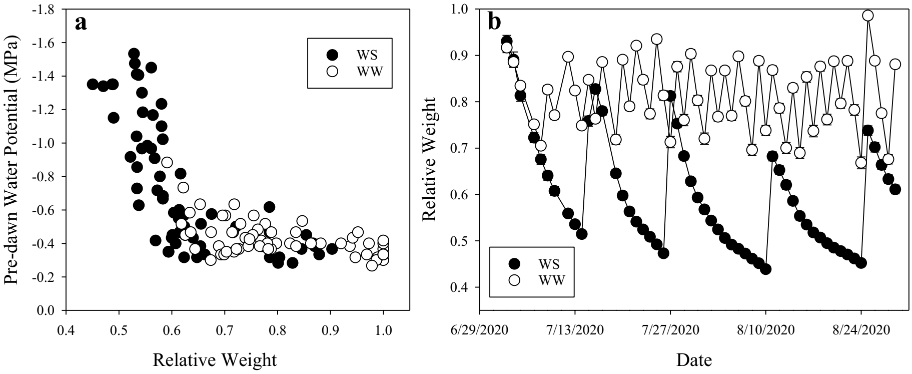

Fig. 2 Effect of nursery irrigation regime on Douglas-fir seedling pre-dawn water potential: **a** relationship between container relative weight and seedling pre-dawn water potential (from a previous study, using similar containers and growing media as current study), and **b** relative weight of well-watered (WW) and water stressed (WS) nursery irrigation regimes over time (from the current study)

18 months old (September 2020), 20 seedlings from each watering treatment were sampled for morphology and hydraulic measurements.

Vulnerability to cavitation

The determination of vulnerability to cavitation differed for the 6- and 18-month-old seedlings. For the 6-month-old seedlings, to determine vulnerability to cavitation curves (VC-curve), we exposed the samples (stems and roots) to increasing pressures using a cavitation chamber in the lab. Stem segments were placed in a double-ended custom-made cavitation chamber (Gonzalez-Benecke et al. 2010) with both ends protruding. The cavitation chamber was then connected to a modified pressure chamber (Model 600-EXP, PMS Instruments Co., OR, USA) in order to control the applied pressure (Fig. 1c). For roots, the whole root system was introduced into the pressure chamber (single cut end method; Sperry and Tyree 1998) with a portion of the 2 cm stem above the root collar protruding from the sealing lid of the pressure chamber (Fig. 1d). For both stems and roots, in order to obtain a VC-curve, the chamber was first pressurized to 0.5 MPa for 10 min. Samples were then removed from the cavitation chamber and *k* was measured until it stabilized (1–3 min), as described previously. After this initial measurement, flow through the segment was reduced by closing the valve, and the sample was introduced back into the cavitation chamber and air pressure was increased to a prescribed value (0.5 or 1.0 MPa steps) and held for 10 min. Then, air pressure was lowered back to atmospheric pressure for 1–3 min. Next, the sample was reconnected to the tubing with the solution, and flow was re-introduced by opening the valve (stems) or by turning on the vacuum pump (roots). Hydraulic conductance was then re-measured and the process continued. Exposure of the stem segments and whole root systems to progressively higher air pressures continued until reaching 4 MPa (the maximum pressure that the PMS600-EXP chamber can reach). A manifold was used to connect four cavitation chambers to the same source of pressurized gas, allowing for multiple simultaneous pressurizations.

The VC-curves of stems and roots for the 18-month-old seedlings were determined using *in-situ* measurements of pre-dawn (Ψ_{PD}, 4:00–6:00 am) and midday (Ψ_{MD}, 12:00–14:00 pm) xylem water potential. Water potential measurements of branch tips were conducted the day of destructive sampling using a portable pressure chamber (Model 600, PMS Instruments). The contrasting nursery irrigation regime treatments (WW and WS) created conditions of varying water availability and a range of Ψ_{PD} and Ψ_{MD} values was expected. After measuring Ψ_{MD}, seedlings were taken to the lab were *k-nat* and *k-max* were measured for stems and roots as described previously. An *in-situ* VC-curve was developed by correlating Ψ_{PD} (roots) or Ψ_{MD} (stems) with percent loss of *k* (PLC).

The PLC at a given pressure or xylem water potential was calculated using the equation given by Domec and Gartner (2001):

$$PLC(\Psi) = 100 \bullet \frac{k_{max} - k_{\Psi}}{k_{max}} \tag{1}$$

where $PLC(\Psi)$ is the percentage loss of *k* at pressure Ψ (MPa), k_{Ψ} is the *k* measured after applying pressure Ψ, and k_{max} is the maximum *k* measured previously after vacuum soaking.

For the 6-month-old seedlings, VC-curves were constructed for each sample showing the cumulative percentage loss in *k* (PLC) versus the negative of air-injection pressure applied. For 18-months old seedlings, one *in-situ* VC-curve was constructed for each set of roots and stem samples, using the PLC (determined from k_{nat} and k_{max} measurements). Instead of pressure applied, for in-situ VC-curves we used the corresponding Ψ_{PD} or Ψ_{MD}, for roots or stems, respectively (Sperry et al. 1998). For all cases, we used a sigmoidal equation for model fitting following Domec and Gartner (2001) and calculating biological parameters from the VC-curve:

$$PLC(\Psi) = \frac{100}{1 + e^{a\bullet(\Psi - b)}} \tag{2}$$

where a is an indicator of the slope, b represents the inflection point (the pressure at which 50% loss of k occurred) and Ψ is the pressure applied (6-month-old seedling) or the corresponding xylem water potential at pe-dawn (roots of 18-month-old seedlings) or at midday (stems of 18-month-old seedlings). Several parameters can be calculated in order to compare different curves:

$$\Psi 50 = b \tag{3}$$

where Ψ50 is the xylem tension (MPa) at which 50% loss of k occurs.

$$\Psi \mathrm{air} = 2/a + b \tag{4}$$

where Ψair is the air entry point, an estimate of xylem tension (MPa) when cavitation starts.

$$\Psi \max = b - 2/a \tag{5}$$

where Ψmax is the full embolism point, an estimate of the maximum tension (MPa) in the xylem before failing and becoming non-conductive.

$$s = 25 \cdot a \tag{6}$$

where s is the slope of the linear portion of the VC-curve (% loss of k per MPa), an estimate of loss of k per unit change in xylem tension. All the above parameters represent physiological traits widely used to compare drought resistance across treatments, sites and/or genotypes.

Seedling morphology, leaf area, wood density and dry mass

Seedling height (root collar to base of the terminal bud) and diameter of the root collar were measured for all seedlings before sample excision in September. After measuring VC-curves, volume (cm^3) of stem segments (without bark) and roots (with bark) of each seedling was determined using the water displacement method (Harrington et al. 1994). Dry mass (g) of the stem, and root system of each seedling was determined after oven-drying seedlings at 75 °C for 72 h. The three tissue types were then separated and weighed to determine biomass. Sapwood area of each stem segment was determined by directly measuring the area of scanned stem sections cut with a razor blade on both ends using image analysis software (ImageJ, National Institute of Health, USA). The sapwood area of the ends was then averaged. Foliage dry mass (g) of each 18 month-old coastal Douglas-fir seedling was determined after oven-drying all living needles at 75 °C for 72 h. Projected leaf area (LA, cm^2) was determined using foliage dry mass in conjunction with seedling-specific estimations of projected specific leaf area (SLA, cm^2 g^{-1}). For each seedling, a sample of 50 needles were scanned and the projected area of the 50 needles was estimated on the scanned images using ImageJ software version IJ 1.46r (https://imagej.nih.gov). The mass of these 50 needles was then measured. The SLA of each seedling was determined as the ratio between the projected leaf area and dry mass of each 50 needle sample. The LA of each seedling was then calculated as the product of SLA and dry weight of foliage (Gonzalez-Benecke et al. 2018). Wood density was calculated as the ratio between volume (cm^3) and dry mass (g) for both stems and whole root systems.

Statistical analysis

All statistical analyses were performed using SAS 9.4 (SAS Institute). Paired t-test was used to compare methods for measuring *k*. One-way analysis of variance (ANOVA) with Tukey Post-Hoc tests were used to determine species differences in seedling morphology. For VC-curve model fitting, a non-linear model fitting was carried out using the procedure PROC NLIN. Data from both nursery irrigation regimes for the 18-month-old costal Douglas-fir seedlings was combined in order to produce the range of values required for in situ determination of VC-curve. Two-way ANOVA with Tukey Post-Hoc tests were used to determine the effect of irrigation regimes on 18-month-old coastal Douglas-fir seedling water potential and hydraulics.

Results

Mean values of morphological traits for seedlings used to determine hydraulic conductivity/conductance and VC-curves of stems and whole root systems or to compare methods for measuring hydraulic conductivity of stems is provided in Table 1. For species used for comparing hydraulic methods, the smaller seedlings were western larch and the larger seedlings were black walnut, ranging between 36.6 and 76.3 cm height, 4.4 to 6.9 mm RCD, 6.8 to 61.6 cm^3 root volume and 1.2 to 23.5 g root mass. The density of whole root system wood (WDr) ranged between 0.176 and 0.381 g cm^{-3}, for western larch and black walnut, respectively.

There was no difference in WDr between 6 and 18-month-old Coastal Douglas-fir seedlings growing under WW conditions ($P=0.302$), even though older seedlings had bigger root system (expressed as mass and volume, $P<0.0001$). For 18-month old coastal Douglas-fir, seedlings growing under WS conditions showed smaller RCD, stem mass, root volume and root mass, but larger WDr and SLA than seedlings growing under WW conditions ($P<0.0001$). On the other hand, there was no effect of watering on height ($P=0.139$), LA ($P=0.794$) and foliage mass ($P=0.094$). The ratio between root and total dry mass averaged 0.433 and 0.366 for WW and WS seedlings, respectively ($P=0.0017$).

Comparing hydraulic methods (gravimetric and vacuum)

There was good agreement between gravimetric and vacuum methods for measuring k_s ($R^2=0.93$; Fig. 3). Within each species tested, there was no difference between observed and predicted values ($P>0.062$) and the difference between k_s estimated with gravimetric and vacuum methods ranged between a 0.9% overestimation (Interior Douglas-fir) to a 7.9% underestimation (redwood). Across species, the slope of the relationship between k_s determined with gravimetric and vacuum methods was 0.9841. Overall, estimates of

Table 1 Sample size (n) and average height, root collar diameter (RCD), root volume, root mass, root wood density (WDr), projected leaf area (LA, cm^2), foliage mass (g) and projected specific leaf area (SLA, $cm^2\ g^{-1}$) for seedlings used to measure hydraulic conductivity/conductance of stems and whole root systems (coastal Douglas-fir) or to compare methods for measuring hydraulic conductivity of stems (western larch, black walnut, redwood and interior Douglas-fir). Values in parenthesis represent standard deviation

Trait	Western larch	Black walnut	Interior Douglas-fir	Redwood	Coastal Douglas-fir		
					6 m WW	18 m WW	18 m WS
n	20*	9*	10*	10*	20**	20**	20**
Height (cm)	36.6 (3.5)	76.8 (13.8)	39.3 (2.9)	42.6 (11.6)	30.8 (3.5)	29.7 (3.9)	28.1 (3.4)
RCD (mm)	4.4 (0.38)	6.9 (0.67)	4.6 (0.38)	5.3 (0.09)	3.3 (0.54)	7.3 (0.89)	5.0 (0.68)
Root volume (cm^3)	6.8 (2.2)	61.6 (13.4)	10.9 (2.2)	20.3 (13.1)	2.3 (1.9)	22.4 (5.7)	8.4 (4.1)
Root mass (g)	1.2 (0.30)	23.5 (5.6)	2.0 (0.60)	4.3 (2.29)	0.48 (0.30)	3.8 (1.19)	1.9 (0.55)
WDr ($g\ cm^{-3}$)	0.176 (0.02)	0.381 (0.03)	0.183 (0.02)	0.212 (0.08)	0.174 (0.03)	0.170 (0.02)	0.240 (0.03)
Foliage mass (g)***	–	–	–	–	–	2.02 (0.18)	1.66 (0.12)
Stems mass (g)***	–	–	–	–	–	3.06 (0.27)	1.67 (0.09)
LA (cm^2)***	–	–	–	–	–	158.6 (15.0)	164.1 (14.3)
SLA ($cm^2\ g^{-1}$)***	–	–	–	–	–	78.8 (2.98)	94.7 (2.45)

6 m: 6 month-old; 18 m: 18 month-old; WW: well-watered; WS: water stress

[*] Used to measured *k* on stems using gravimetric and vacuum methods

[**] Used to measure *k* on stems and whole root system

[***] Only measured for 18-month-old coastal Douglas-fir

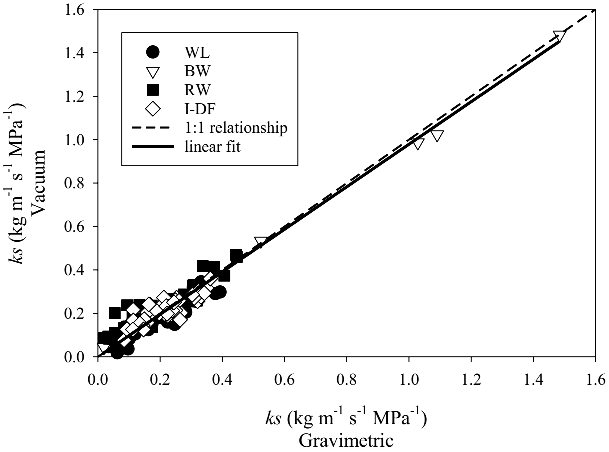

Fig. 3 Validation of vacuum chamber method. Relationship between sapwood-specific hydraulic conductivity (k_s) of stems of western larch (WL, filled circle), black walnut (BW, open triangle), redwood (RW, filled square), and interior Douglas-fir (I-DF, open diamond) seedlings measured with the gravimetric (Sperry apparatus) and vacuum (vacuum chamber) methods. The solid line represents linear fit between observed and predicted values. Dashed line represents a 1:1 relationship

stem k_s were not different across measurement methods (P = 0.069), having mean values of 0.2104 and 0.2155 kg s^{-1} m^{-1} MPa^{-1}, for gravimetric and vacuum methods, respectively. This represents an average difference of 2.5%.

Comparing hydraulic traits of whole root systems and stems of coastal Douglas-fir seedlings

For both roots and stems, the vacuum soaking method used for removing embolism was not able to refill all cavitated tracheids on 6 Douglas-fir seedlings grown under WS conditions ($\Psi_{PD} < -1.5$ MPa and PLC > 70%; Fig. 4a). In these cases, we assumed that k_{max} of those WS seedlings corresponded with mean k_{max} of WW seedlings. Though the methodologies

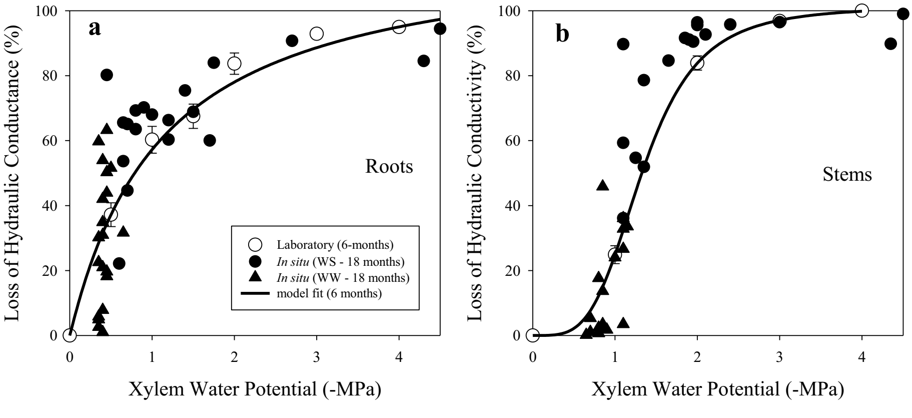

Fig. 4 Relationship between xylem water potential and loss of hydraulic conductance/conductivity (PLC) for whole-root system **a** and stems **b** of coastal Douglas-fir seedlings. Open circles represent lab observations of *k* under varying pressures applied (VC-curves) at an age of 6 months. Filled symbols represent in situ observations using *knat* at predawn (roots, a) or midday (stems, b) water potential under well-watered (triangle) and water-stress (circle) conditions at an age of 18 months. Error bars represent standard error (n = 20). Model fit shown for VC-curves at age 6 months

for measuring VC-curves were different, there was some similarity in the VC-curves of 6 and 18-month-old Douglas-fir seedling roots (Fig. 4a) and stems (Fig. 4b). For lab VC-curves, k was correlated with the negative of the pressure applied. For in situ VC-curves, k_{nat} was correlated with Ψ_{PD} (roots) and Ψ_{MD} (stems), respectively (Sperry et al. 1998). Data for the WS and WW 18-month-old seedlings (in situ measurements) was combined in order to have a sufficient range of values to determine the VC-curve. There were no differences in Ψ_{50} between lab measured VC-curves at age 6-months and in situ measured at age 18-months for both roots ($P=0.251$) and stems ($P=0.158$) (Table 2). Two reference water potentials (− 1 and − 2.5 MPa) were selected for comparison purposes at low and high water stress, respectively. At a xylem water potential of − 1 MPa, the PLC of roots was 45 and 37%, for 6 and 18-months-old seedlings, respectively. The PLC of stems was 24 and 26% for 6 and 18-months-old seedlings, respectively. When xylem water potential decreased to − 2.5 MPa, PLC of roots was 92 and 84%, for 6 and 18-months-old seedlings, respectively, while PLC of stems was 96 and 99%, for 6 and 18-months-old seedlings, respectively.

Average k_{max} attained for WS seedlings was 58% (roots) and 71% (stems) lower than for WW seedlings ($P<0.05$; data not shown). In Table 2, k_{max} for 18-month-old seedlings corresponds to values observed for WW seedlings. The average k_{max} of the whole root system of coastal Douglas-fir seedlings (both, $k_{rm\text{-}max}$ and $k_{rv\text{-}max}$) was much larger at age 6 months than at 18 months ($P<0.005$; Table 2). This was not the case for stems which has similar maximum sapwood-specific hydraulic conductivity ($k_{s\text{-}max}$) at 6 and 18 months ($P=0.23$; Table 2), averaging 0.325 and 0.36 kg s^{-1} m^{-1} MPa^{-1}, respectively. The Ψ50 of both roots

Table 2 Least square means of xylem hydraulic traits evaluated for stems and whole root system of 6 and 18-month-old coastal Douglas-fir seedlings growing under well-watered conditions

Organ	Trait	Units	DF	
			6 m	18 m
Root	$k_{rm\text{-}max}$	mmol s^{-1} MPa^{-1} g^{-1}	1.204 (0.125)	0.324 (0.034)
	$k_{rv\text{-}max}$	mmol s^{-1} MPa^{-1} cm^{-3}	0.211 (0.021)	0.055 (0.006)
	Ψ50	MPa	− 1.13 (0.064)	− 1.36 (0.096)
	Ψair	MPa	− 0.052 (0.065)	− 0.001
	Ψmax	MPa	− 2.21 (0.120)	− 2.73
	S	% loss of k MPa^{-1}	45.5 (3.37)	36.3 (7.39)
Stem	$k_{s\text{-}max}$	kg s^{-1} m^{-1} MPa^{-1}	0.325 (0.014)	0.360 (0.034)
	Ψ50	MPa	− 1.41 (0.049)	− 1.22 (0.042)
	Ψair	MPa	− 0.72 (0.062)	− 0.81
	Ψmax	MPa	− 2.10 (0.072)	− 1.62
	s	% loss of k MPa^{-1}	36.3 (3.68)	122.6 (24.79)

For roots, $k_{rm\text{-}max}$ is maximum mass-specific hydraulic conductance (mmol s^{-1} MPa^{-1} g^{-1}), $k_{rv\text{-}max}$ is maximum volume-specific hydraulic conductance (mmol s^{-1} MPa^{-1} cm^{-3}). For stems, $k_{s\text{-}max}$ is maximum sapwood-specific hydraulic conductivity (kg s^{-1} m^{-1} MPa^{-1}). Ψ50 is the xylem tension at which 50% of loss of hydraulic conductance occurs (MPa), Ψair is the air entry point (MPa), Ψmax is the full embolism point and s is the slope of the linear portion of the VC-curve (% loss of k per MPa). Values in parenthesis represent standard error ($n=20$)

Standard error for Ψair and Ψmax for 18-month-old seedlings were not computed as those traits were calculated from parameter estimates of VC-curves using Eqs. 4 and 5

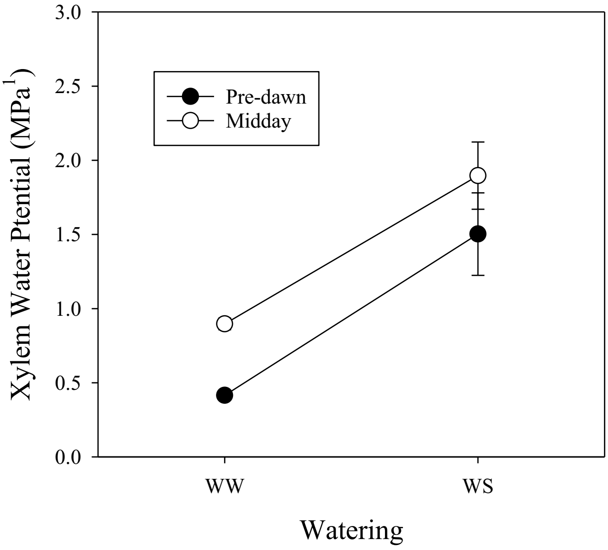

Fig. 5 Xylem water potential during pre-dawn (filled circle) and midday (open circle) coastal Douglas-fir seedlings grown under well-watered (WW) and water stress (WS) conditions at 18 months age. Error bars represent standard error (n = 20)

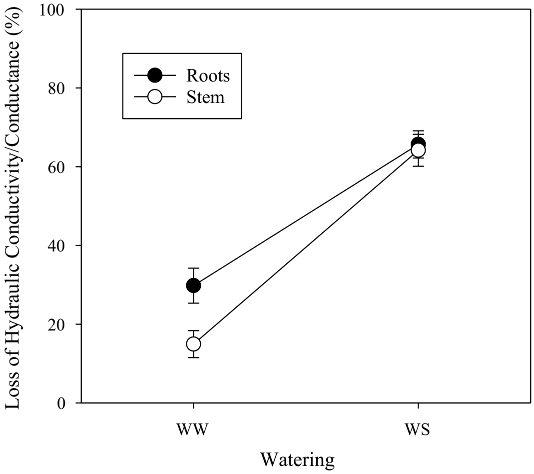

Fig. 6 Percent loss of hydraulic conductance/conductivity for whole-root systems (filled circle) and stems (open circle) of coastal Douglas-fir seedlings grown under well-watered (WW) and water stress (WS) conditions at 18 months age. Error bars represent standard error (n = 20)

and stems was also not different for 6 or 18-month-old seedlings ($P > 0.18$; Table 2), averaging 1.26 and 1.31 MPa, respectively.

The watering treatments applied were reflected in contrasting xylem water potentials. Coastal Douglas-fir seedlings growing under WW conditions averaged Ψ_{PD} and Ψ_{MD} of about -0.42 and − 0.90 MPa, respectively, while seedlings growing under WS conditions averaged Ψ_{PD} and Ψ_{MD} of about − 1.50 and − 1.96 MPa, respectively (Fig. 5a). The maximum gradient in Ψ (difference between Ψ_{PD} and Ψ_{MD}) averaged 0.48 MPa and was similar across watering treatments ($P = 0.119$).

For 18-month-old Coastal Douglas-fir seedlings growing under WS and WW conditions, PLC was computed using values of k_{nat} and k_{max}, as shown in Eq. 1. The PLC of roots was larger than stems for seedlings grown under WW conditions ($P = 0.011$), averaging

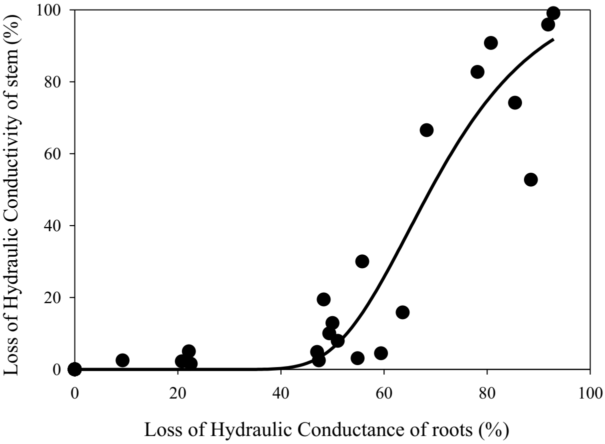

Fig. 7 Relationship between loss of hydraulic conductance/conductivity (PLC) for whole-root systems and stems of for 6-month-old coastal Douglas-fir seedlings. Figure include only paired observations of PLC of roots and stems of the same sampled seedling at the same pressure applied

30% and 15%, respectively. For seedlings grown under WS conditions, PLC was similar for roots and stems, averaging 65% (P = 0.782; Fig. 6).

Using paired observations of PLC from VC-curves of roots and stems of 6-month-old seedlings measured in the lab (same seedlings where *k* of roots and stems were measured at the same pressure applied), a sigmoidal relationship was observed (Fig. 7). When roots had up to 40% PLC (about − 0.5 MPa in Fig. 4), stems showed negligible loss of *k*. A rapid increase in the PLC of stems from 30 to 80% was observed when roots had PLC of 60–80%, respectively.

Discussion

In this study, we conducted a validation of the vacuum chamber method for measuring xylem hydraulic conductance and then applied the method to assess the vulnerability to cavitation of whole root systems. Several techniques have been developed for measuring root hydraulics. Our results confirmed that the vacuum chamber method (Kolb et al. 1996) is a reliable technique to measure *k* of whole root systems of tree seedlings, as similar results were found when compared to the standard gravimetric method (Sperry et al. 1988). This validation was performed for several tree species and included an angiosperm and several gymnosperms indicating it may be applied broadly. Our vacuum chamber was developed as a modified version of the original design of Kolb et al. (1996) and used plexiglass tubing and standard pressure chamber parts. This modified design facilitates the measurement of root systems of varying sizes as custom chambers may be produced to meet study requirements.

The vacuum chamber method can also be used for assessments of *k* on branches (Kolb and Sperry 1999; Venturas et al. 2018) or the whole above ground portion of tree seedlings (Venturas et al. 2019). These types of measurements have a varied range of applicability, from genotype characterization (Wang et al. 2020) to planetary flux systems analysis (Anderegg and Venturas 2020). We were able to perform up to 50 measurements per day of *k* of whole root systems using a team of two people, opening the possibility for large scale assessments of root hydraulics, including genotype screening. As Brodribb et al. (2015) pointed out, root hydraulic architecture, a poorly characterized trait for nursery production, needs to be considered along with phenotype variations in response to the environment when selecting genotypes for tree breeding programs.

After validating the vacuum chamber method, we assess the effect of seedling age and nursery irrigation regime on the k of whole root systems of young coastal Douglas-fir seedlings. The maximum k of the whole root system (expressed per unit root volume or root mass) was much larger for 6-month-old seedlings. This aging effect has been reported elsewhere, as roots of new plants have wider conducting cells, less suberization, less branching and reduced path length which reduces radial resistance to water transport (Eissenstat 1997; Eissenstat et al. 2000; Robinson et al. 2003; Gretchen and Paterson 2005). Nevertheless, Ψ50 of roots was not different for 6- or 18-month-old seedlings indicating a similar vulnerability to xylem embolism.

The type of unit for scaling k is an important biological/ecological question for each experiment (Tyree et al. 1998). In our study, the unit of scaling k_r (per unit mass or volume) showed no effect on the magnitude of the relative differences between k_r of coastal Douglas-fir seedlings of different ages (6 vs. 18 months). This response, which indicates no differences in wood density between seedlings growing under WW conditions, was not true for 18-month-old seedling growing under different water availability conditions. When scaled from k_{rv} and root volume, WW seedlings showed 2.7 times larger k_r than WS seedlings. That difference was reduced to two times when k_r was scaled from k_{rs} and root mass, as seedlings growing under WW conditions showed larger biomass allocation to roots than WS seedlings.

VC-curves determined using lab measurements at age 6 months and in situ measurements at age 18 months showed good agreement for both roots and stems. These results indicate no effect of seedling age on the loss of stem or root hydraulic conductance under varying levels of xylem water potential. There was also no effect of irrigation regime on the VC-curves of 18-month-old seedlings indicating that water availability did not affect inherent seedling drought resistance. These results have important implications on the applicability of nursery stage evaluations of drought resistance (VC-curves) and the expected responses of seedlings during the first growing season under field conditions.

Although there was no effect of seedling age or irrigation regime on seedling VC, large differences in VC were observed for costal Douglas-fir roots and stems (Fig. 4). The relationship shown in Fig. 4 demonstrate the higher sensitivity of roots in terms of regulating k in response to drying soil (Hacke et al. 2000; Brodribb et al. 2015). Roots showed a high degree of loss of k at water potentials considered adequate or at least not deleterious for stem k (i.e. $\Psi > -1$ MPa). Others have also noted that roots start reducing k (i.e. cavitation) at water potentials that have negligible effects on stems k (Eissenstat 1997; Sperry et al. 2002a, b). In our dataset, negligible reductions in stem k were observed when roots showed up to 40% loss of k. Above this threshold, however, a sharp decrease in stem k was observed with root and stem k becoming equal when loss of root k equaled about 80%.

The differences between root and stem vulnerability to xylem embolism was reflected in the results for 18-month-old coastal Douglas-fir seedlings growing under conditions of contrasting water availability. When grown under WW conditions the reduction in the water transport efficiency of roots was much higher than for stems, but still averaged less than the 40% threshold described above. Under conditions of water limitation (WS), however, the loss of conductivity of roots passes this threshold and averaged a 65% reduction of k. This resulted in a sharp increase in the loss of stem k which also averaged 65%. These results support the importance of understanding the VC of whole root systems due to their higher sensitivity to xylem cavitation.

Conclusion

Our results confirmed that the vacuum chamber method is a reliable technique to measure *k* of whole root systems of tree seedlings. The method was tested against the standard gravimetric method for stems of four tree species including several gymnosperms and one angiosperm and no differences between the methods were found. After validating the vacuum chamber method, the effect of nursery irrigation regime and seedling age on the hydraulic conductance and vulnerability to cavitation of coastal Douglas-fir seedlings was assessed. Under well-watered conditions, roots were found to have a higher loss of hydraulic conductivity than stems, reflecting the higher sensitivity of these organs to cavitation. When growing under conditions of water limitation, the percent loss of conductivity of roots and stems was found to be similar. These results suggest that although roots may buffer stems from cavitation under conditions of moderate drought stress, this effect is lost as drought conditions worsen. The drought resistance index (i.e. $\Psi50$) of coastal Douglas-fir seedlings measured at age 6 and 18 months were not different. The results from this study support the potential applicability of measuring whole root system hydraulics as an evaluation of seedlings drought resistance for both the nursery growth phase and first growing season after nursery production.

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Declaration

Conflict of interest The authors declare that they have no conflict of interest.

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