Original article

Fertilization at planting impairs root system development and drought avoidance of Douglas-fir (*Pseudotsuga menziesii*) seedlings

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Abstract – Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings (1 + 1 bareroot) were divided into 8–13 cm^3 and 23–35 cm^3 initial root-volume categories and either not fertilized or fertilized with 60 g Apex® in the planting hole at a drought-prone clearcut site in the Oregon Coast Range, USA. Regardless of initial root volume, fertilized seedlings consistently reached lower predawn xylem water potential (Ψ_{xylem}) values than non-fertilized seedlings during summer. Roots did not proliferate relative to fertilizer placement and mean values for lateral-root length, root tips, and root biomass were less for fertilized seedlings in each of four vertical root zones. Poor root system development of fertilized seedlings may have been associated with the rapid release of high fertilizer salt concentrations into the rhizosphere. Root-volume growth was positively correlated with Ψ_{xylem}, emphasizing the importance of new root growth following planting for drought avoidance. Criteria for successful field fertilization must consider the anticipated drought level of the site.

seedling / controlled-release fertilizer / root architecture / drought avoidance / *Pseudotsuga menziesii*

Résumé – La fertilisation à la plantation perturbe le développement du système radiculaire et réduit la résistance à la sécheresse des plants de Douglas (*Pseudotsuga menziesii*). On a utilisé deux catégories de plants à racines nues (1 + 1) de Douglas (*Pseudotsuga menziesii* (Mirb.) Franco) dont le volume initial des racines était respectivement de 8 à 13 cm^3 et de 23 à 35 cm^3. Ces plants furent installés dans une station de clairière plutôt sèche, située dans la Chaîne côtière de l'Oregon aux USA, et soumis à deux traitements : pas de fertilisation ou fertilisation avec 60 g d'Apex® introduits dans le trou de plantation. Quelque soit le volume racinaire initial, les plants fertilisés révèlent, au cours de l'été, des valeurs de potentiel hydrique du xylème en fin de nuit (Ψ_{xylem}) toujours inférieures à celles obtenues sur plants non fertilisés. Les racines ne se sont pas développées dans les zones en contact avec l'engrais, et les valeurs moyennes de longueur de racines latérales, de nombre de pointes racinaires, et de biomasse racinaire sont inférieures pour les plants fertilisés, dans les quatre plans verticaux. Ce mauvais développement du système racinaire des plants fertilisés peut être associé à la libération rapide d'engrais à forte concentration minérale dans la rhizosphère. La croissance racinaire en volume était corrélée positivement avec le Ψ_{xylem} ce qui permet de souligner l'importance de la croissance des racines après plantation pour limiter les effets de la sécheresse. Les critères à adopter pour réussir une opération de fertilisation de terrain doivent donc prendre en considération l'éventuel degré de sécheresse de la station.

plant / engrais à libération contrôlée / architecture racinaire / limitation des effets de sécheresse / *Pseudotsuga menziesii*

1. INTRODUCTION

The success of reforestation operations may be improved if seedlings remain on a growth trajectory following outplanting similar to that in the nursery. Transplant shock often limits the ability of bareroot seedlings to grow vigorously the first one to two years following planting [50]. Fertilization at planting offers a potential means to reduce transplant shock and accelerate initial plantation growth [2, 14]. Interest in using controlled-release fertilizer (CRF) at planting has increased over the past decade [26] due to advantages in fertilizer-use efficiency that this fertilizer type offers over conventional fertilizer forms [28]. With a single application, CRF gradually supplies planted seedlings with supplemental nutrition for several months. The ideal CRF product delivers nutrients at a rate matching plant demand, thus improving crop yield and minimizing nutrient loss due to leaching [24, 29].

Reports of seedling growth response to CRF at outplanting vary considerably [8] and one possible reason may be method of CRF application. Fertilizer may be applied to the planting hole, to an adjacent hole, or surface broadcast [8]. To facilitate nutrient uptake, many researchers have recommended applying CRF directly in the planting hole, such that nutrients are released in the immediate vicinity of the root system [2, 12, 14, 23, 52]. However, fertilization in the planting hole may limit root system expansion because roots tend to proliferate in areas

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of high nutrient supply, as observed in numerous controlled experiments [16, 19, 21]. The diffusion of fertilizer salts into the soil solution may also restrict root penetration into subsoil zones, as excessive fertilizer salts kill root apical meristems due to the buildup of toxic ion concentrations and osmotic effects [18, 33].

Restrictions to root growth may reduce the ability of seedlings to maintain adequate xylem water potential, termed drought avoidance [59], during the critical establishment period following outplanting. Shoot growth following planting is generally most limited by water availability [10]. When bareroot seedlings are lifted from the nursery, a significant portion of roots, particularly fine roots, are lost [43]. Although there is evidence that planting seedlings with large root volumes may reduce moisture stress and subsequent transplant shock [13, 25], survival of newly-planted seedlings is largely dependent on the rapid extension of roots, which reestablish root-soil contact and thereby absorb water to reduce stress from transpirational loss [51, 54]. Without efficient water extraction, seedlings continue to transpire, resulting in a condition of physiological drought which contributes to transplant shock and limits whole-plant growth [50].

Following planting, seedlings usually increase root growth relative to shoot growth [37]. A decreased shoot:root ratio allows a greater soil volume to be exploited with relatively less transpirational demand from the shoot [32, 40]. Fertilization at outplanting when soils are wet, however, may act to increase shoot:root ratios relative to non-fertilized seedlings, as high nutrient availability and sufficient water can limit the need for root expansion [31, 32, 57]. Reduced root expansion relative to shoot growth associated with field fertilization could further increase water stress after soils dry [22].

Few field experiments have studied seedling root proliferation in response to localized nutrient sources. Additionally, little information is available concerning the ability of field-fertilized seedlings to avoid drought immediately following planting. This experiment was designed to examine the influence of initial root volume and field fertilization at a relatively high application rate on seedling root architectural development and drought avoidance. A sub-sample of seedlings in four treatments from a larger experiment [1] was used. We hypothesized that relative to a non-fertilized control, field fertilization would result in (i) greater allocation of biomass to shoot relative to root tissues, (ii) localized root proliferation at the point of CRF placement and decreased subsoil penetration, and (iii) reductions in xylem water potential and stomatal conductance, which would be less pronounced for seedlings with a larger initial root volume.

2. MATERIALS AND METHODS

2.1. Plant material

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings (Seed Zone 262; Western Forest Tree Seed Council, State of Oregon Tree Seed Zones) were grown for 2 yr (1 + 1 bareroot) using standard nursery practices at Weyerhaeuser's Aurora, OR, USA nursery until lifting in December 1999. Following lifting, seedlings were graded to operational specifications and placed in cold storage at 3 °C. Several

Table I. Nutrient composition of the three Apex® controlled-release fertilizer (CRF) types blended to create the composite fertilizer used in the experiment. Estimated manufacturer timeframe for nutrient release at 21 °C media temperature is listed for each CRF type.

Nutrient	CRF 1 (3–4 month)	CRF 2 (5–6 month)	CRF 3 (8–9 month)	CRF composite
	Composition (%)			
N (NH$_4$)	8.2	8.2	7.7	8.1
N (NO$_3$)	10.8	10.8	10.3	10.6
P (P$_2$O$_5$)	5.0	6.0	6.0	5.7
K (K$_2$O)	12.0	12.0	12.0	12.0
Mg	1.0	0.9	0.9	0.9
S	1.8	1.7	1.7	1.7
Fe	0.5	0.5	0.5	0.5
Mn	0.2	0.2	0.2	0.2
Mo	0.01	0.01	0.01	0.01
Zn	0.06	0.06	0.05	0.05

days before outplanting, seedlings were washed free of soil and measured for root volume by water displacement [9]. Each seedling was then numbered, tagged, and returned to cold storage. Seedlings were divided into categories based on initial root volume. For this study, seedlings examined were from initial root-volume categories of 8–13 cm^3 (11.2 ± 0.2) (mean ± SE) (RVC1) and 23–35 cm^3 (27.0 ± 0.4) (RVC2).

2.2. Fertilizer treatments

Seedlings were either non-fertilized or fertilized with 60 g of a blended CRF (Apex®, J.R. Simplot Co., Boise, ID, USA) containing equal amounts of three fertilizers with differing release rates (manufacturer's estimates at 21 °C media temperature) (Tab. I). By using a CRF with a mixture of release rates, a more steady supply of nutrients through the desired release period may potentially be achieved. The 60-g rate was the highest rate used in the larger study [1] and we chose to closely examine seedling development at this rate relative to controls based on previous trials showing negative growth results from similar rates applied at planting on sites subject to dry summers [45]. This CRF consists of prills containing water-soluble nutrients encapsulated within a polyurethane coating, which acts to slow nutrient dissolution. Nutrient release from polymer-coated CRF is primarily controlled by soil temperature and a more rapid rate of release occurs with increasing temperature [34]. Fertilizer was applied to the bottom of the approximately 25-cm planting hole, covered by 1–2 cm of soil, and the seedling was planted with lateral roots generally extending to within 2 cm of the fertilizer layer.

Soon after planting, a companion study was installed adjacent to this study to quantify the release of nutrient ions from this CRF [27]. Fertilizer nutrients released relatively rapidly, with 9, 22, 34, and 48% of the composite fertilizer releasing by weight during the first 13, 20, 27, and 35 weeks, respectively. Specifically, 26, 33, 55, and 65% of total N, 6, 15, 15, and 24% of P$_2$O$_5$, and 9, 16, 34, and 51% of K$_2$O released from the fertilizer during the first 13, 20, 27, and 35 weeks, respectively. During the approximately 48-week period of the present study, 55% of the fertilizer released by weight and 73% of total N, 26% of P$_2$O$_5$, and of 63% of K$_2$O released. This was 87–92% of the total released for all variables during a 62-week sampling period.

2.3. Field site

The outplanting site was in Oregon State University's Dunn Experimental Research Forest (44° 43' N, 123° 20' W). This site is on the eastern edge of the Oregon Coast Range, an area characterized by mild winters with heavy precipitation and hot, dry summers. The 38-ha site was clearcut in 1998 and slash was piled and burned shortly thereafter. The aspect of the site was predominantly east, elevation averaged 317 m, slopes ranged from 5–10%, and the soil was a deep, well-drained silty-clay loam (Waldo series). Seedlings were planted on 19 January 2000 at a 3 m × 3 m spacing. Immediately following planting, Vexar® tubing was installed to protect seedlings from animal damage.

Pre-plant (30 August 1999) vegetation was controlled on the site using metsulfuron (Escort®, 0.05 kg/ha), sulfometuron (Oust®, 0.21 kg/ha), glyphosate (Accord®, 4.67 L/ha), and imazapyr (Arsenal®, 0.44 L/ha). Subsequent control included hexazinone (Velpar®, 28 March 2000, 7.00 L/ha) and clopyralid (Transline®, 9 June 2000, 0.73 L/ha). The objective was to attain maximum vegetation control to minimize variation in soil water potential across the site.

2.4. Measurements

Seedlings were sampled for predawn xylem water potential (Ψ_{xylem}) on four dates during 2000 (3 July, 31 July, 31 August, and 27 September). Four (3 July) or five (all other dates) seedlings from each treatment replication were sampled. A lateral branch from the lower half of the terminal shoot was cut from each seedling and within 5 min, a measurement of Ψ_{xylem} using a Scholander-type pressure chamber (Model 600, PMS Instruments, Inc., Corvallis, OR, USA) as per methodology in [15] was recorded.

Morning and afternoon stomatal conductance (g_s) was sampled using a porometer (Model LI-1600, Li-Cor, Inc., Lincoln, NE, USA) on five seedlings from each treatment replication on 28 and 29 September 2000. Two blocks were sampled on the first day and the remaining two blocks on the second day. The weather was clear each day. The same lateral branch on the lower half of the terminal shoot was sampled for both morning (0800–1000 h, solar time) and afternoon (1300–1500 h, solar time) g_s. The sampled foliage was then excised and placed into cold storage at 3 °C. Leaf area of each foliage sample was assessed using a Panasonic® video image recorder (Model WV-CD20, Matsushita Communication Industrial Co., Ltd., Yokohama, Japan) and AgVision software (Decagon Devices, Inc., Pullman, WA, USA) to adjust gas exchange measurements for variation in leaf area.

On 8 December 2000, each seedling used for sampling of Ψ_{xylem} and g_s was excavated from the soil with care to preserve the root system and placed into cold storage at 3 °C. Within 7 d, seedlings were measured for height from cotyledon scar to base of terminal bud, root-collar diameter, taproot length, shoot and root volume, and shoot dry weight.

Root architectural development was assessed based on the vertical distribution of roots within the soil profile. Because seedlings were sampled soon after excavation, were dormant when excavated, and had relatively well-lignified root structures, we assumed that roots held approximately the same vertical architectural orientation that was present in the soil. Seedlings were clipped at cotyledon scar and again 5 cm below this point, which was the minimum distance from cotyledon scar where lateral roots initiated. The remaining roots were then sliced into four 7.5-cm vertical sections (S1-S4), with roots infrequently extending into the deepest section (S4). The fertilizer was placed at a depth of approximately 25 cm in the soil and therefore generally associated with S3. Roots in each section were divided into tap and lateral roots, with the taproot never extending beyond S2. Within each root section, the number of root tips (white tips > 1 mm in length) and first-order lateral-root length were assessed. Root sections were then dried at 70 °C for 72 h and dry weights of tap and lateral roots were recorded for each vertical root section.
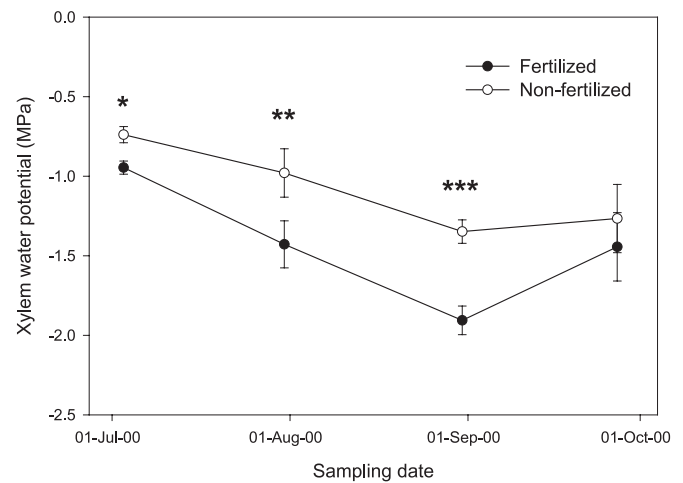

Figure 1. Predawn xylem water potential of fertilized and non-fertilized seedlings (averaged across root-volume categories) at four sampling points. Data points are means, and error bars are SEs. At each sampling point, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

2.5. Experimental design and statistical analysis

The experiment was arranged as a randomized complete block design with four blocks and factorial treatments (two initial root-volume categories × two fertilization rates). Five seedlings were sampled from each treatment within a block (with exception of 3 July Ψ_{xylem}), for a total of 80 seedlings in the experiment. The experimental unit was the group of seedlings within a treatment replication and the sampling unit was the individual seedling.

Data were subjected to analysis of variance (ANOVA) for a randomized complete block design with factorial treatments. Tests for normality and constant variance were performed to ensure the validity of the assumptions of ANOVA and no transformations were necessary. Only in the absence of a significant ($P < 0.05$ in F test) root volume × fertilization rate interaction were the main effects (root volume and fertilization) analyzed to determine if significant differences were present. Fisher's Protected Least Significant Difference procedure was used to identify significant differences ($\alpha = 0.05$) among treatments. Regression analyses were used to determine the relationship between root-volume growth and Ψ_{xylem} or g_s. Orthogonal contrasts were used to determine the statistical significance of higher-order regression models but only linear relationships were significant. The mean values of each of the four replicates for each treatment were used in the regression and an adjusted R^2 value was determined to indicate the fit of the model. SAS® software (SAS Institute Inc., Cary, NC, USA) was used for analysis of all data.

3. RESULTS

3.1. Xylem water potential and stomatal conductance

Water stress of seedlings in all treatments increased through time during summer (Fig. 1). However, the Ψ_{xylem} of fertilized seedlings was lower (i.e., more drought stressed) than non-fertilized seedlings on 3 July ($P = 0.0113$), 31 July ($P = 0.0020$), and 31 August ($P = 0.0006$) (Fig. 1). From 15 June through

Table II. Mean values ± SE for seedling morphology of fertilizer treatments (averaged across root-volume categories) and root-volume categories (averaged across fertilization treatments) following excavation in December 2000. For each parameter for either fertilizer treatment or root-volume category, means followed by the same letter in a row did not differ significantly ($P < 0.05$).

	Fertilizer treatment		Root-volume category	
	Non-fertilized	Fertilized	8–13 cm^3	23–35 cm^3
Height (cm)	36.0 ± 1.8 a	37.1 ± 2.3 a	31.5 ± 0.7 b	42.0 ± 1.2 a
Diameter (mm)	8.3 ± 0.5 a	7.8 ± 0.4 b	7.0 ± 0.3 b	9.1 ± 0.2 a
Taproot length (cm)	13.6 ± 0.6 a	12.6 ± 0.3 a	12.9 ± 0.5 a	13.3 ± 0.4 a
Shoot volume (cm^3)	35.3 ± 6.3 a	29.6 ± 4.2 a	19.6 ± 2.3 b	45.3 ± 3.3 a
Root volume (cm^3)[1]	27.1 ± 4.3	18.5 ± 2.4	15.8 ± 2.2	29.9 ± 3.5
Shoot:root volume	1.3 ± 0.1 b	1.6 ± 0.1 a	1.3 ± 0.1 b	1.6 ± 0.1 a
Shoot dry weight (g)	12.4 ± 2.0 a	11.0 ± 1.6 a	7.4 ± 0.8 b	16.0 ± 1.1 a
Root dry weight (g)[1]	9.0 ± 1.4	6.1 ± 0.8	5.0 ± 0.7	10.1 ± 1.1
Shoot:root dry weight	1.4 ± 0.1 b	1.9 ± 0.1 a	1.6 ± 0.1 a	1.7 ± 0.1 a

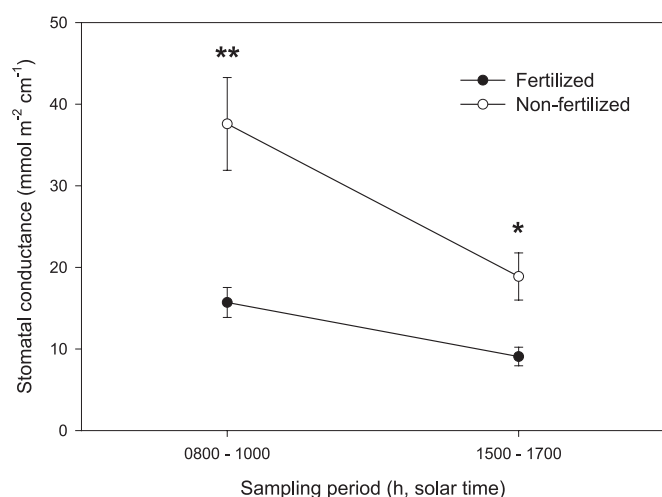[1] A significant root volume × fertilization rate interaction occurred.

Figure 2. Stomatal conductance for fertilized and non-fertilized seedlings (averaged across root-volume categories) sampled during morning and afternoon hours on 28 and 29 September 2000. Data points are means, and error bars are SEs. At each sampling point, * $P < 0.05$ and ** $P < 0.01$.
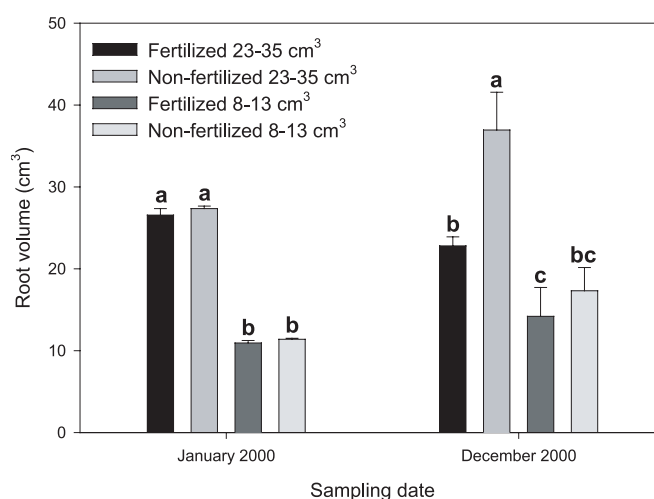

Figure 3. Initial seedling root volume at time of planting (January 2000) and following excavation (December 2000). Bars are means, and error bars are SEs. At each sampling point, treatments with the same letter did not differ significantly ($\alpha = 0.05$).

1 September, only 0.46 cm precipitation was recorded in Corvallis, OR, approximately 20 km south of the study site [47]. In early September, 1.63 cm of precipitation was recorded in Corvallis, OR. Mean Ψ_{xylem} on 27 September increased for all treatments, particularly for fertilized seedlings. There was no significant difference in Ψ_{xylem} at any sampling between root-volume categories (data not shown). There was also no significant root volume × fertilization rate interaction for Ψ_{xylem} at any sampling point, indicating that effects of fertilization on Ψ_{xylem} were not dependent on initial root volume.

Values of g_s were higher for non-fertilized seedlings than fertilized seedlings during both morning ($P = 0.0052$) and afternoon ($P = 0.0126$) samplings (Fig. 2). There was no significant difference in g_s between root-volume categories during the morning or afternoon (data not shown).

3.2. Whole plant morphology

Following seedling excavation in December 2000, root-collar diameter of non-fertilized seedlings was greater than that of fertilized seedlings ($P = 0.0270$) (Tab. II). Shoot:root volume ($P = 0.0243$) and shoot:root dry weight ($P = 0.0100$) were greater for fertilized than for non-fertilized seedlings. Mean height ($P = 0.4245$), shoot volume ($P = 0.0516$), and shoot dry weight ($P = 0.0991$) did not differ between fertilizer treatments. Height, diameter, and shoot volume were greater ($P < 0.0001$) for RVC2 than RVC1 (Tab. II). Shoot:root volume was also greater for seedlings in RVC2 than in RVC1 ($P = 0.0421$), though shoot:root dry weight was not ($P = 0.6610$).

Initial root volume was greater for RVC2 seedlings than RVC1 seedlings ($P < 0.0001$) (Fig. 3). At excavation, means

Table III. Mean values ± SE for root architecture parameters of fertilized and non-fertilized seedlings (averaged across root-volume categories) following excavation in December 2000. Root systems were divided into 7.5-cm vertical soil segments (S1–S4), beginning 5 cm below cotyledon scar, with roots infrequently extending into the deepest section (S4). For each parameter, means followed by the same letter in a row did not differ significantly ($P < 0.05$).

	Fertilizer treatment	
	Non-fertilized	Fertilized
S1 number of root tips	137 ± 16 a	101 ± 11 b
S2 number of root tips	126 ± 15 a	94 ± 17 a
S3 number of root tips	54 ± 10 a	30 ± 7 b
S4 number of root tips	11 ± 4 a	4 ± 2 a
Total number of root tips	328 ± 40 a	229 ± 34 b
S1 lateral-root length (cm)	94 ± 7 a	88 ± 8 a
S2 lateral-root length (cm)	95 ± 10 a	83 ± 10 a
S3 lateral-root length (cm)	36 ± 7 a	23 ± 4 a
S4 lateral-root length (cm)	11 ± 4 a	3 ± 2 a
Total root length (cm)	236 ± 24 a	197 ± 21 b
S1 taproot dry weight (g)	1.28 ± 0.16 a	0.88 ± 0.11 b
S2 taproot dry weight (g)	0.04 ± 0.012 a	0.01 ± 0.003 b
Total taproot dry weight (g)[1]	2.98 ± 0.16 a	2.36 ± 0.10 b
S1 lateral-root dry weight (g)[2]	3.15 ± 0.54	2.15 ± 0.30
S2 lateral-root dry weight (g)[2]	2.13 ± 0.40	1.28 ± 0.22
S3 lateral-root dry weight (g)	0.64 ± 0.17 a	0.31 ± 0.07 a
S4 lateral-root dry weight (g)	0.11 ± 0.04 a	0.03 ± 0.01 a
Total lateral-root dry weight (g)[2]	6.03 ± 1.10	3.76 ± 0.55

[1] Includes portion of taproot that was excluded in root zone analysis.
[2] A significant root volume × fertilization rate interaction occurred.

for both root volume and root dry weight were greater for non-fertilized than fertilized seedlings (Tab. II). However, there was a significant root volume × fertilization rate interaction for these parameters ($P = 0.0429$ and 0.0250, respectively). Mean root volume was greatest for the non-fertilized RVC2 treatment (Fig. 3). Interestingly, mean root volume of the fertilized RVC2 treatment did not differ from the non-fertilized RVC1 treatment, despite significant differences at the onset of the study. Treatment differences were similar for root dry weight at excavation, except that mean root dry weight of fertilized RVC2 seedlings was significantly greater than that of non-fertilized RVC1 seedlings (data not shown).

Pre-dawn Ψ_{xylem} increased linearly with root-volume growth on 3 July ($P = 0.0016$, $R^2 = 0.49$) (Fig. 4), 31 July ($P = 0.0034$, $R^2 = 0.43$), and 31 August ($P = 0.0195$, $R^2 = 0.28$). Differences in the grouping of data points for non-fertilized and fertilized seedlings reflected the lower root-volume growth and Ψ_{xylem} for fertilized seedlings (Fig. 4). Both morning ($P = 0.0258$, $R^2 = 0.26$) and afternoon ($P = 0.0149$, $R^2 = 0.31$) g_s also increased linearly with root-volume growth.

3.3. Root architecture

The mean number of root tips tended to be greater for non-fertilized than fertilized seedlings in all root zones (Tab. III). Differences were significant in the S1 ($P = 0.0313$) and S3 ($P = 0.0497$) root zones, though not in S2 ($P = 0.1113$) or S4 ($P = 0.1948$). The total number of root tips was also greater for non-fertilized than fertilized seedlings ($P = 0.0327$) (Tab. III).

Mean total first-order lateral-root length was greater ($P = 0.0185$) (Tab. III) for non-fertilized than fertilized seedlings. Taproot length did not differ between fertilizer treatments ($P = 0.1473$) (Tab. II). Total taproot dry weight, which included the 2.5-cm segment excluded from the root-zone analysis, was greater for non-fertilized than fertilized seedlings ($P = 0.0017$), as was taproot dry weight in S1 ($P = 0.0061$) and S2 ($P = 0.0296$) (Tab. III). Means for lateral-root dry weight tended to be greater for non-fertilized than fertilized seedlings in all root zones, though not statistically significant in S3 ($P = 0.0679$) or S4 ($P = 0.1207$) (Tab. III). A root volume × fertilization rate interaction was detected for lateral-root dry weight in S1 ($P = 0.0168$), S2 ($P = 0.0296$), and for total lateral-root dry weight
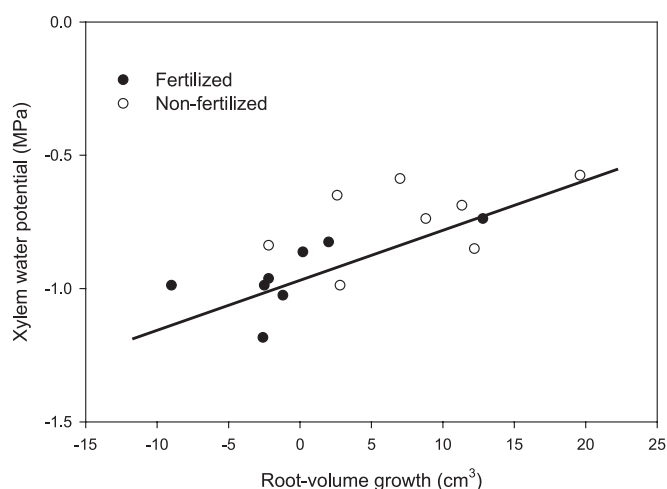
Figure 4. Regression of root-volume growth on xylem water potential (Ψ_{xylem}) sampled 3 July 2000 for fertilized and non-fertilized seedlings. Regression equation is: $\Psi_{xylem} = -0.904 + 0.017$ (root-volume growth); adjusted $R^2 = 0.49$, $P = 0.0016$.

($P = 0.0377$) (data not shown). Similar to treatment differences noted for root volume, non-fertilized RVC2 seedlings had the greatest total lateral-root dry weight, while that of the fertilized RVC2 and non-fertilized RVC1 did not differ. The mean lateral-root dry weight of non-fertilized RVC1 did not differ from the fertilized RVC1.

4. DISCUSSION

4.1. Influence of fertilization on root development

Fertilization clearly inhibited root growth in this study (Tab. III and Fig. 3). This may have been partly associated with the release of fertilizer nutrients acting to decrease the need for new root growth to extract soil nutrients. Under conditions of high nutrient availability and low water stress during spring, seedlings may allocate more biomass to shoots and less to roots since below-ground resources are sufficient for growth [39]. Reports of increased seedling shoot:root ratios in response to fertilization are common [14, 49, 57] and consistent with the greater shoot:root observed for fertilized seedlings (Tab. II). However, root elongation was not only reduced for fertilized seedlings relative to non-fertilized seedlings, but root volume actually decreased for fertilized RVC2 from time of planting to excavation (mean 26.6 vs. 22.8 cm³) (Fig. 3).

Thus, root system development of fertilized seedlings may have been inhibited by the buildup of excessive fertilizer salts in the soil solution, as observed in a previous study in which Douglas-fir seedling roots were unable to penetrate beyond a localized fertilizer layer [33]. Nutrient release of polymer-coated CRF is determined by the migration of water vapor through the fertilizer prill membrane, and this process is largely dependent on soil temperature [34]. Soil moisture between 50–100% of field capacity has essentially no influence on release

rate, release is slower at 25% field capacity, and no release occurs in dry soil [34]. Periodic excavation of fertilizer samples over time indicated that nutrients continued to release into the soil solution at a relatively consistent rate during the summer dry period, and 55% of total N had released within 27 weeks of application [27]. As fertilizer nutrients are released, the electrical conductivity (EC) of the soil solution increases and solute potential decreases. Without adequate water to leach nutrients from the soil, concentrations of fertilizer salts likely increased in the soil solution, leading to root dehydration and limiting water uptake. Though magnified under dry soil conditions, this type of physiological drought associated with high solute concentrations can occur even when soil moisture is at field capacity [35].

Conifers, and Douglas-fir in particular, are sensitive to high EC levels [36]. Tolerance to high salt concentrations varies with size of root stock and generally increases with age [61]. Thus, young Douglas-fir seedling roots may be highly vulnerable to injury associated with excessive fertilization, which may help to explain the poor root development of seedlings fertilized at a relatively high rate in this study.

We expected increased root proliferation at or above the position of CRF placement and greater root damage in the lower root sections compared to the upper root sections, as observed in a greenhouse experiment with Douglas-fir seedlings grown in containers [33]. However, there was no distinct proliferation of roots at the approximate position of CRF (vertical root zone S3) or any other root zone, and mean values for root parameters tended to be lower in all root zones for fertilized compared to non-fertilized seedlings (Tab. III). There are several possible explanations for this result. White root tips are anatomically suited for efficient ion uptake [48] and tend to increase in nutrient-rich solutions [17]. The lack of increased numbers of root tips, root length, or root biomass in any root zone for fertilized seedlings suggests that nutrient toxicities throughout the rhizosphere associated with rapid nutrient release from this high fertilizer application may have inhibited root proliferation in all zones. Additionally, the greater drought stress incurred by fertilized as compared to non-fertilized seedlings may have reduced whole-plant physiological vigor, further limiting root system development in all root zones. It is also possible that the inherent variation in studying root proliferation relative to fertilizer placement in the field (i.e., precision of planting hole size, fertilizer depth, seedling placement, etc.) and the division of root systems following whole-plant removal from the soil (as compared to separation with soil core intact) limited our ability to detect localized changes in root proliferation.

Seedling root development in the largest root-volume category (RVC2) was more negatively affected by fertilization than in the smallest root-volume category (RVC1) (Fig. 3). This may have been due to increased root-fertilizer contact for seedlings with a larger initial root volume (RVC2). Additionally, this effect was likely magnified by the greater root-volume growth of RVC2 non-fertilized seedlings compared to RVC1 non-fertilized seedlings (Fig. 3). Seedlings with a large initial root volume have higher root growth potential [13], tend to better tolerate transplant shock over time [25], and may have more rapid early growth rates compared to seedlings with a smaller initial root volume [52, 53].

4.2. Fertilization and moisture availability

The ability of seedlings to respond positively to fertilization seems to depend on soil moisture availability. Application of Osmocote® CRF improved Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedling growth compared to non-fertilized seedlings under well-watered conditions, but decreased growth slightly under droughty conditions [58]. N fertilization at outplanting on sites with low soil moisture reduced growth and survival of Douglas-fir [56] and Monterey pine (*Pinus radiata* D. Don) [38] seedlings. Survival and dry weight of container-grown Douglas-fir seedlings increased with greater N levels under well-watered conditions, but decreased under drier conditions [60]. Growth response of eastern white pine (*Pinus strobus* L.) seedlings at various levels of fertilizer nutrients in solution depended on irrigation schedule, as mean seedling weight decreased with reduced moisture to a greater extent in the full-strength than the half-strength solution [55].

Poor performance of fertilized seedlings under low soil moisture availability may be associated with the influence of fertilization on drought resistance. Although fertilization of established trees may reduce drought stress [7], contradictory results have often been reported for seedlings. Controlled experiments with lodgepole pine (*Pinus contorta* Dougl. ex Loud.) [20] and jack pine (*Pinus banksiana* Lamb.) [57] found that seedlings grown under the lowest N treatment were better suited to tolerate drought stress than seedlings in higher N treatments. Similarly, seedlings of loblolly pine (*Pinus taeda* L.) grown at high N levels were more negatively affected by drought stress than seedlings grown at moderate N levels [49]. Despite these reports, the role of N as a modifier of seedling morphology and physiology in relation to drought resistance remains unclear [57]. Data from the current experiment, however, demonstrated that there may be an interrelationship among fertilization, root system establishment, and drought avoidance.

4.3. Influence of root development on drought avoidance

Fertilized seedlings were significantly more drought stressed than non-fertilized seedlings during summer (Fig. 1). This may be at least partly explained by restricted root development (Tab. III and Fig. 3), resulting in access of roots to a smaller volume of soil water. Seedlings have little capacity to store water due to a low ratio of sapwood volume to water transfer rate and lack of other storage reservoirs [44]. Thus, to avoid drought stress following transplant, roots must rapidly extend through the soil profile to extract water. N fertilization at high rates reduced taproot penetration in jack pine and it was suggested that this would increase susceptibility to drought stress [57]. A significant positive correlation ($R^2 = 0.65$) was found between the number of new roots initiated by ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) seedlings and predawn leaf water potential [46]. Similarly, leaf water potential of shortleaf pine (*Pinus echinata* Mill.) increased exponentially with new root projected surface area [5]. In the present study, greater root growth of non-fertilized seedlings was also correlated with increasing ability to avoid drought (Fig. 4). Additionally, non-fertilized seedlings had greater numbers of white root tips

(Tab. III), which are important sites for water uptake as there is anatomically less resistance to water passage [48].

Fertilized seedlings also allocated proportionally more biomass to shoot relative to root tissues than non-fertilized seedlings (Tab. II). Higher shoot:root deter seedling establishment under dry conditions [4, 11], because less root area is available to supply a proportionally greater transpirational demand from the shoot.

There were no differences between root volume categories in Ψ_{xylem}. It was suggested that improved survival after eight growing seasons for ponderosa pine seedlings with a larger initial root volume on a harsh, dry site was associated with greater access of larger root systems to limited water and nutrients [53]. However, no difference at 95 or 115 d following transplant in predawn Ψ_{xylem} was reported among Douglas-fir seedlings graded into different root volume classes [25]. Thus, targeting for a larger initial root volume in Douglas-fir seedlings may not reduce drought avoidance at least during the first season following planting.

As Ψ_{xylem} decreases, g_s also declines [41] and photosynthesis is directly related to g_s [30]. Although healthy, established Douglas-fir trees can survive drought stress to –11 MPa, photosynthesis typically begins to decline at –1 MPa [6]. A seasonal decline in g_s of Douglas-fir seedlings of nearly 50% was reported when Ψ_{xylem} reached –1.5 MPa [42]. In the present study, mean predawn Ψ_{xylem} of fertilized seedlings reached –1.9 MPa for fertilized seedlings on 31 August, compared to –1.3 MPa for non-fertilized seedlings (Fig. 1). Values of g_s were also significantly lower for fertilized than non-fertilized seedlings at the end of September, even when Ψ_{xylem} no longer differed between treatments (Fig. 2). The low Ψ_{xylem} and g_s for seedlings in all treatments illustrates the severity of drought on this site. However, non-fertilized seedlings were better able to avoid drought stress, which likely enabled these seedlings to photosynthesize at higher rates than fertilized seedlings. Fertilized seedlings may have been less able to balance the energy costs associated with respiration with gains from photosynthesis, increasing dependence on stored carbohydrates. It is well documented that plant growth is restricted under conditions of prolonged drought [3, 4, 25]. Thus, limitations to drought avoidance during the summer following transplant associated with excessive fertilization may act to reduce seedling growth and survival in subsequent years.

5. CONCLUSIONS

The high rate of fertilization at outplanting substantially modified seedling root system development during establishment. No localized root proliferation associated with placement of fertilizer in the planting hole was observed. Mean values for root system parameters of fertilized seedlings were depressed in all root zones compared to non-fertilized seedlings, which may have been associated with the rapid release of high concentrations of fertilizer salts in the soil solution. Further quantitative research examining both specific changes in rhizosphere EC levels associated with CRF release and corresponding rates of plant nutrient uptake is needed to determine threshold EC values which maximize soil nutrient availability, while not deterring root proliferation or causing plant toxicities.

Fertilized seedlings were less able to avoid drought stress following transplant, as indicated by significantly lower predawn Ψ_{xylem} values during summer. This was likely a function of greater allocation of biomass to shoot relative to root tissues and the reductions in root biomass, first-order lateral-root length, and number of white root tips, which limited the ability of fertilized seedlings to exploit water from the soil profile. In an effort to conserve water, fertilized seedlings had lower rates of g_s and were, therefore, unable to photosynthesize to the degree of non-fertilized seedlings.

These results illustrate the importance of adjusting field fertilization protocols for the anticipated degree of moisture stress on the site. The 60 g fertilization rate applied to the vicinity of the root zone inhibited root system proliferation, thereby limiting the ability of seedlings to avoid drought stress. To improve seedling response to field fertilization on droughty sites, alternative methods of fertilizer placement may be preferable. Additional strategies for minimizing potential drought stress may include use of conservative CRF application rates, employing a CRF product with longer timeframe for nutrient release, or use of a CRF type with mechanisms of nutrient release that are more dependent on soil moisture availability (e.g., urea formaldehyde or isobutylidene diurea). Consideration should also be given to delaying fertilizer application to one or two years following planting when seedling root systems have established. On sites where drought is extreme, it may be advisable to avoid field fertilization entirely.

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