Development of Douglas-fir seedling root architecture in response to localized nutrient supply

Douglass F. Jacobs, Robin Rose, and Diane L. Haase

Abstract: Three months following sowing, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings were transplanted into pots with controlled-release fertilizer (CRF) applied at rates of 0, 8, 16, and 24 g/2200 cm^3 soil as a single uniform layer beneath the root system. Seedlings were destructively harvested periodically, and roots were divided into vertical segments above (S1), within (S2), and below (S3) the fertilizer layer. Two months following transplant, the number of active root tips was positively correlated with CRF rate in S1 and negatively correlated with rate in S2 and S3. At 6 months, root penetration into S3 was severely restricted at 16 and 24 g. This was attributed to detrimental changes in soil osmotic potential in S2. Fertilizer improved seedling growth at 8 g after 6 months compared with controls but was inhibitory at 24 g. Photochemical quantum yield was higher in all CRF treatments compared with controls 3 months following transplant, which corresponded with rapid initial CRF nutrient release. Despite improvements in nutrient release technology with CRF, high application rates may result in excessive concentrations of fertilizer nutrients in media, which can restrict root penetration and negatively affect seedling growth. Conservative application rates and improvements in CRF technology will help reduce the potential for adverse effects on seedling development.

Résumé : Trois mois après l'ensemencement, des semis de douglas (*Pseudotsuga menziesii* (Mirb.) Franco) ont été transplantés dans des pots avec un fertilisant à libération contrôlée (FLC) appliqué en une seule couche sous le système racinaire à des taux de 0, 8, 16 et 24 g/2200 cm^3 de sol. Les semis ont été récoltés périodiquement de façon destructive et les racines ont été séparées en segments verticaux au-dessus (S1), dans (S2) et sous (S3) la couche de fertilisant. Deux mois après la transplantation, le nombre d'apex racinaires actifs était positivement corrélé avec le taux de FLC dans le segment S1 et négativement corrélé avec le taux dans les segments S2 et S3. Après 6 mois, la pénétration des racines dans le segment S3 était fortement limitée avec 16 et 24 g de fertilisant. Ce fait a été attribué aux changements préjudiciables du potentiel osmotique dans le segment S2. Le fertilisant a amélioré la croissance des semis à 8 g après 6 mois en comparaison des témoins mais avait un effet inhibiteur à 24 g. Le rendement quantique photochimique était plus élevé dans tous les traitements FLC comparativement aux témoins 3 mois après la transplantation, ce qui correspond à une libération initiale rapide des nutriments du FLC. Malgré les améliorations dans la technologie de libération des éléments nutritifs avec les FLC, de forts taux d'application peuvent entraîner des concentrations excessives de nutriments dans le medium, restreignant la pénétration des racines et affectant négativement la croissance des semis. Des taux d'application conservateurs et des améliorations dans la technologique des FLC aideront à réduire les effets potentiellement néfastes pour le développement des semis.

[Traduit par la Rédaction]

Introduction

Interest in using controlled-release fertilizer (CRF) to enhance forest seedling productivity in the nursery and field has increased (Haase and Rose 1997). As compared with conventional water-soluble or immediately available forms of fertilizer, CRF can supply seedlings with nutrients for extended periods with a single application. Because of the gradual nutrient release, the potential for seedling damage associated with nutrient toxicities and nutrient loss through leaching may be reduced (Hauck 1985; Donald 1991). Benefits associated with using CRF in reforestation projects depend on a complex interaction of factors including CRF type, formulation, or rate; CRF placement; stock type; and environmental growing conditions (Brockley 1988). Variable results in the nursery and field indicate that a better understanding of the mechanisms by which CRF affects seedling morphology and physiology is needed.

Nutrient release of polymer-coated CRF is determined by the temperature-driven diffusion of water through the semipermeable prill membrane, with little influence associated with moisture when media water content is within 50–100% of field capacity (Kochba et al. 1990). Several types of coating materials are used in polymer-coated CRF. Patterns of nutrient release vary with coating type, with some CRF types releasing nutrients at a rapid initial rate (Huett and

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D.F. Jacobs,[1,2] R. Rose, and D.L. Haase. Department of Forest Science, Oregon State University, Corvallis, OR 97331-5752, U.S.A.

[1]Corresponding author (e-mail: djacobs@fnr.purdue.edu).
[2]Present address: Hardwood Tree Improvement and Regeneration Center (HTIRC), Purdue University, Department of Forestry and Natural Resources, West Lafayette, IN 47907-2033, U.S.A.

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Gogel 2000). This may affect plant growth because of the release of high concentrations of fertilizer salts into the soil solution.

The greatest potential for negative effects from salt damage may occur when CRF is positioned in the root zone. Researchers have advocated the application of CRF directly in the planting hole to facilitate efficient nutrient uptake (Carlson 1981; Carlson and Preisig 1981; Gleason et al. 1990). Roots then grow through the localized source of fertilizer while extending into the soil profile. While experiments have documented the proliferation of roots in areas of high nutrient concentrations in solution for Sitka spruce (*Picea sitchensis* (Bong.) Carrière) (Coutts and Philipson 1976), lodgepole pine (*Pinus contorta* Dougl. ex. Loud.) (Coutts and Philipson 1977), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Friend et al. 1990), no localized proliferation of roots due to CRF placement have been reported for outplanted Douglas-fir (Carlson and Preisig 1981) or western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) (Carlson 1981).

Toxicities associated with localized fertilization in the root zone could impair root architectural development, which may then have a negative influence on whole plant growth. As fertilizer is released, salts diffuse outward into the soil, which acts to lower soil osmotic potential and alter soil pH. High concentrations of fertilizer salts can then cause the death of root apical meristems (Drew 1975), which may restrict root expansion and limit access to water and nutrients (Kozlowski 1987). Thus, examining root architectural development in relation to localized CRF supply may help to identify mechanisms for negative responses associated with CRF application.

Chlorophyll fluorescence, an inexpensive and nondestructive method to evaluate plant physiological status (Vidaver et al. 1989), may also be useful for examining seedling response to localized CRF application. Chlorophyll fluorescence has been used to detect changes in the physiological status of Douglas-fir due to dormancy (Hawkins and Lister 1985; Roberts et al. 1991), freezing stress (Fisker et al. 1995), and shading (Khan et al. 2000). Few studies have reported the use of chlorophyll fluorescence to detect physiological changes associated with fertilization in forest tree seedlings.

The objectives of this study were to quantitatively assess the effects of a wide range of CRF rates applied as a single layer beneath the transplanted root system of Douglas-fir seedlings on (*i*) growth rates, (*ii*) root architectural development in relation to the proximity of the fertilizer layer, and (*iii*) chlorophyll fluorescence.

Materials and methods

Plant material

Douglas-fir seeds (seedlot No. 274; Western Forest Tree Seed Council, State of Oregon Tree Seed Zones) were sown into 39-cm^3 containers in March 2000 at the Timber Company's nursery near Cottage Grove, Oreg. Seedlings were grown under standard nursery cultural practices until being lifted and transplanted into pots in mid-June 2000. At the time of transplanting, seedlings had a height of 5.6 ± 0.1 cm (mean ± SE) and a stem diameter of 1.07 ± 0.02 mm.

Treatments

Seedlings were transplanted into cylindrical pots 30.5 cm in length and 10.2 cm in diameter, with a metal screen for drainage installed 1 cm from the bottom. Pots were filled to a depth of 13 cm with a 4:4:1:1 (v/v/v/v) peat – composted plant material – pumice – perlite (Organic mix, Pacific Soil). Osmocote Plus® (O.M. Scotts Company) 15:9:12 (N:P:K) plus micronutrients CRF (5- to 6-month nutrient release at 21°C media temperature) was applied at three rates (8, 16, and 24 g) as a single uniform layer. A control treatment was also included in which no CRF was added. An additional 2.5 cm of potting mix was applied above the fertilizer layer. Seedlings were then transplanted, and soil was filled to 3 cm below the top of the pots. The total volume of soil in each pot was approximately 2200 cm^3. The purpose of layering the CRF beneath the transplanted root system was to examine seedling vertical root architectural development over time in relation to the proximity of the CRF layer.

All pots were thoroughly watered following transplanting and placed in a controlled-environment greenhouse at Oregon State University's Oak Creek Plant Facility (44°38′N, 123°30′W). Fans and coolers were used to keep the greenhouse below 32°C. Pots were watered to field capacity when a representative sample (two pots/block) dried to a water content of 39%, providing a soil moisture range known to promote optimum Douglas-fir seedling morphological and physiological development (Khan et al. 1996). Watering occurred approximately every 10 days during the first 4 months of the experiment and every 15 days during the final 4 months. All seedlings received Peters® 20:20:20 (N:P:K) plus micronutrients water-soluble fertilizer at a rate of 50 ppm in irrigation water every other watering cycle to prevent mortality of controls due to nutrient stress.

The experiment was arranged as a randomized complete block design with 4 CRF rate treatments (0, 8, 16, and 24 g), 6 blocks, and 384 seedlings. Each of six benches in the greenhouse was designated as one block due to variations in light, temperature, and air circulation. Within a block, 64 pots (16 pots/treatment) were randomly distributed and rearranged monthly. For each sampling, the experimental unit was the group of randomly selected pots per treatment in each block and the sampling unit was the individual seedling.

Measurements

Four seedlings in each treatment from each block (96 total at each sampling) were randomly selected for harvest at 2-month intervals (16 August 2000, 17 October 2000, 15 December 2000, and 17 February 2001) during the 8-month experiment. Seedlings were measured for height, stem diameter, shoot and root volume using water displacement (Burdett 1979), and shoot and root dry mass. At the final harvest (February 2001), two blocks were not sampled because of poor seedling health associated with heat stress resulting from insufficient air circulation in these blocks.

Root architecture within soil zones relative to the placement of the fertilizer layer was examined for each harvested seedling at each sampling time. Seedling roots were first washed free of media and then clipped 2.5 cm below cotyledon scar, which represents the position at which lateral roots tend to initiate. Roots were then sliced into three 8-cm sections with the middle (S2) section representing the portion

of roots where the fertilizer layer was present and the top (S1) and bottom (S3) being above and below the fertilizer layer, respectively. At initial seedling transplant, the cotyledon scar was always positioned approximately 3 cm below the top of the 30.5-cm pot (with 1 cm excluded for drainage screen), and the fertilizer layer was always 17.5 cm below the top of the pot. Thus, the fertilizer layer was always in the middle of the S2 section and division of roots into zones was consistent among sampling units. Roots in each section were divided into taproot and lateral roots, although the taproot rarely extended into S2. Within each root section, the number of active root tips (white tips >1 mm in length), the number of first-order lateral roots (>1 cm length), first-order lateral root length (cm), and dry taproot and lateral root masses were assessed.

At 4 and 8 months following transplanting, a composite foliar sample of the four seedlings sampled in each treatment for four blocks was created by removing needles from all positions on the seedling and drying at 70°C for 72 h. Approximately 2 g of foliage was collected randomly from the dried foliage of each seedling, creating an 8-g composite sample for each treatment replication. Foliage was then ground in a Wiley mill (40-mesh screen) and nutrient concentrations were determined (USAg Analytical Services, Inc., Pasco, Wash.) using methodology in Gavlak et al. (1994). Total Kjeldahl nitrogen was determined using the macro-Kjeldahl method and concentrations of P, K, Ca, Mg, and S were determined using wet ash extraction (nitric–perchloric acid).

Chlorophyll fluorescence (Opti-Sciences Pulse Modulated Chlorophyll Fluorometer OS5-FL) was sampled (13 September 2000, 16 October 2000, 14 December 2000, and 16 February 2001) on a subsample of four seedlings from each treatment in each block. A single needle from both the top and middle portion of the terminal seedling shoot was removed from each seedling. Within 3 min following removal, each needle was exposed to a pulse of light from a light emitting diode passing through a short pass filter. Measurements of Fs (steady state fluorescence), Fms (maximal fluorescence), and the quantum yield (QY) of photochemical energy conversion ((Fms – Fs)/Fms) (Opti-Sciences, Inc. 1997) were then recorded.

To determine estimated rates of nutrient release by mass over time, 24 g of CRF (the highest treatment rate) were sealed in nylon fabric and positioned at the same soil depth as the fertilizer layer in 24 pots (four pots/block) with no seedling. These pots were watered on the same schedule as other pots in the experiment. Every 2 months during the 8-month study, the CRF in six pots was removed, dried at 70°C for 72 h, and weighed. A temperature data recorder (R-2100, Telog Instruments, Inc.) was positioned at the approximate depth of the fertilizer layer in one randomly selected pot to monitor soil temperature.

Electrical conductivity (EC) was measured (Con-100, Oakton Instruments) on soil sampled from pots of seedlings harvested at 6 months following transplant. For each treatment within a block, soil from each of the three root zones was dried at 70°C for 72 h and sifted with a 2 mm sieve. A 5-g sample of dry soil was then added to 50 mL of distilled water and mixed thoroughly. After 24 h, the solution was decanted from the residual soil, and the EC of the solution was measured.

Statistical analysis

Data from each sampling were independently subjected to analysis of variance (ANOVA) for a randomized complete block design. Tests for normality, linearity, and constant variance were performed, and transformations were made when necessary to ensure the validity of these assumptions. Transformations were only necessary for portions of data associated with root architecture, and these instances are reported in the Results. When significant ($p = 0.05$ in F test) differences were detected among means for any parameter, Fisher's protected least significant difference procedure was used to detect significant differences among CRF treatments. Needle position was used as a source of variation in the ANOVA for chlorophyll fluorescence data and the significance of needle position and CRF rate was determined only if the needle position × fertilizer rate interaction was nonsignificant. Regression analyses were performed to determine the relationship between CRF rate and measurements of root morphology. Orthogonal contrasts were used to determine the statistical significance of higher-order regression models for explaining variability associated with the data. When more than one regression model was statistically significant, the model with the best fit for the data was selected based on adjusted R^2 values and analysis of the residuals. SAS® software (SAS Institute Inc., Cary, N.C.) was used for analysis of all data.

Results

Fertilizer release

The initial 24 g dropped to 15.8 ± 0.16 g after 2 months and 11.3 ± 0.26 g after 4 months, which coincided with the warmest soil temperature range recorded in the experiment (mean soil temperature 0–4 months, 22.4°C). The initial 24 g had dropped to 8.3 ± 0.18 g by February 2001, which was 8 months following application (mean soil temperature 4–8 months, 17.8°C).

There were significant differences in EC measured 6 months following transplant among CRF rates in the middle soil zone (S2) where the fertilizer layer was present ($p = 0.0011$) (Fig. 1). The 8-, 16-, and 24-g treatments had significantly higher EC levels than the control in S2, although there were no significant differences among these three rates. Electrical conductivity did not differ among treatments in the upper (S1) ($p = 0.1283$) or lower (S3) ($p = 0.1332$) soil zones (Fig. 1).

Whole-plant morphology

Six months following transplant, treatments differed for height growth ($p = 0.0029$), diameter growth ($p = 0.0046$), shoot volume ($p = 0.0004$), root volume ($p = 0.0026$), shoot dry mass ($p = 0.0059$), root dry mass ($p = 0.0021$), and total lateral root length ($p = 0.0002$), although not for total number of active root tips ($p = 0.1605$) (Table 1). Seedlings grown with 24 g of CRF were significantly smaller than other treatments for many whole-plant morphological pa-

Table 1. Mean values and SE for whole-plant morphological parameters by fertilizer rate in December 2000 (6 months following transplant).

	Morphological variable							
CRF rate (g)	Height growth (cm)	Diameter growth (mm)	Shoot volume (cm^3)	Root volume (cm^3)	Shoot dry mass (g)	Root dry mass (g)	Lateral root length (cm)	No. of active root tips
0	16.0*b*	3.26*b*	16.0*ab*	8.9*ab*	3.0*ab*	1.4*ab*	188*a*	136*a*
8	18.2*a*	3.83*a*	18.4*a*	10.2*a*	3.6*a*	1.7*a*	161*a*	135*a*
16	17.0*ab*	3.89*a*	13.8*b*	8.2*b*	2.9*bc*	1.4*b*	113*b*	105*a*
24	13.7*c*	3.06*b*	10.5*c*	6.1*c*	2.3*c*	1.0*c*	96*b*	100*a*
SE	0.70	0.17	0.99	0.61	0.21	0.10	11.5	13.0

Note: For each parameter, means followed by the same letter in a column did not differ significantly at $\alpha = 0.05$.

Fig. 1. Electrical conductivity (EC) of soil from three soil zones for treatments 6 months following transplant. Data points are means, and errors bars are SEs. Statistical differences were detected only in the middle soil zone (S2), with the EC of the 8-, 16-, and 24-g CRF rates significantly greater than the control at $\alpha = 0.05$. No significant differences occurred between the 8-, 16-, and 24-g rates.

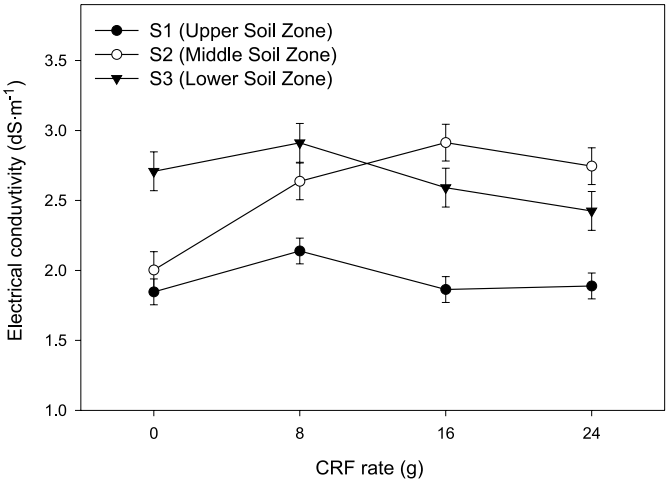
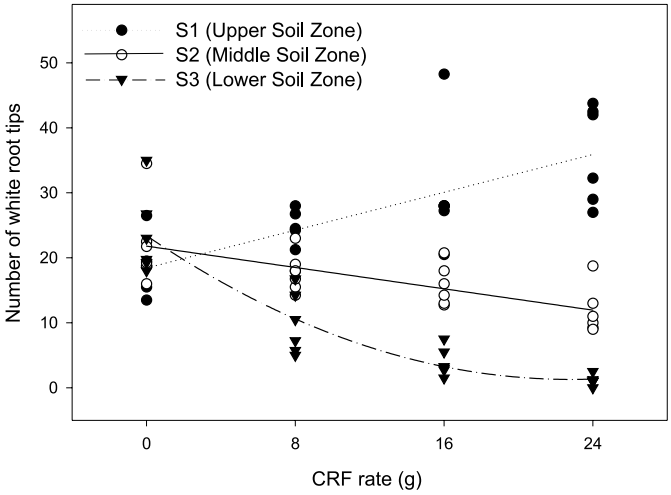


Fig. 2. Number of white root tips in the three soil zones (S1–S3) 2 months following transplant versus fertilizer rate. Data for S3 are presented on original scale prior to transformation. Regression equations are as follows: root tips = 18.425 + 0.728(rate), adjusted $R^2 = 0.51$, $p < 0.0001$ for S1; root tips = 21.80 – 0.410(rate), adjusted $R^2 = 0.43$, $p = 0.0003$ for S2; and log(root tips + 1) = 3.11 – 0.173(rate) + 0.024(rate)^2, adjusted $R^2 = 0.95$, $p < 0.0001$ for S3.



rameters, while seedlings in the 8-g fertilizer rate had the highest mean values for most parameters (Table 1).

Root architecture

The presence of a CRF layer affected root architectural development among soil zones, and this effect was more pronounced with increasing fertilizer rate. Two months following transplant, the distribution of active root tips was affected by fertilizer rate in S1 ($p = 0.0007$), S2 ($p = 0.0122$), and S3 ($p < 0.0001$). The number of active root tips increased with increasing fertilizer rate in S1 but decreased with fertilizer rate in S2 and S3 (Fig. 2).

Distinct differences in lateral root penetration into the lower soil zones were evident throughout the experiment. After 6 months, treatments affected lateral root dry mass in S1 ($p = 0.0127$), S2 ($p < 0.0001$), and S3 ($p = 0.0010$). Regression analyses showed quadratic trends for lateral root dry mass in each zone with little lateral root penetration into S2 and S3 at the highest CRF rates (Fig. 3). At the 16- and 24-g fertilizer rates, roots that did penetrate to the lowest soil zone tended to be large in diameter and oriented along the periphery of the pots.

Nutrients

Foliar N ($p = 0.0198$) and P ($p = 0.0277$) concentrations differed among treatments at 4 months following transplant (Table 2), although not at 8 months ($p = 0.1642$ and 0.1190 for N and P, respectively) (data not shown). Mean N concentrations increased, while mean P concentrations generally decreased with increasing fertilizer rate (Table 2). Treatments also affected Ca concentrations ($p = 0.0006$) at 4 months (Table 2), generally increasing with fertilizer rate, though not at 8 months ($p = 0.0537$). Concentrations of K, Mg, and S did not differ at the 4- (Table 2) or 8-month sampling.

Chlorophyll fluorescence

The Fms of needles sampled from the middle of the shoot was significantly higher than those from the top of the shoot for all samplings (p ranging from <0.0001 to 0.0065) (Ta-

Table 2. Mean values for foliar macronutrient concentrations and SE by fertilizer rate in October 2000 (4 months following transplant).

	Nutrient concentration (%)					
CRF rate (g)	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulfur
0	2.79*b*	0.63*a*	3.47*a*	0.79*b*	0.26*a*	0.15*a*
8	3.01*ab*	0.51*ab*	3.36*a*	0.98*b*	0.22*a*	0.12*a*
16	3.14*a*	0.44*b*	3.42*a*	1.43*a*	0.24*a*	0.11*a*
24	3.27*a*	0.44*b*	3.46*a*	1.36*a*	0.25*a*	0.11*a*
SE	0.09	0.04	0.13	0.08	0.01	0.01

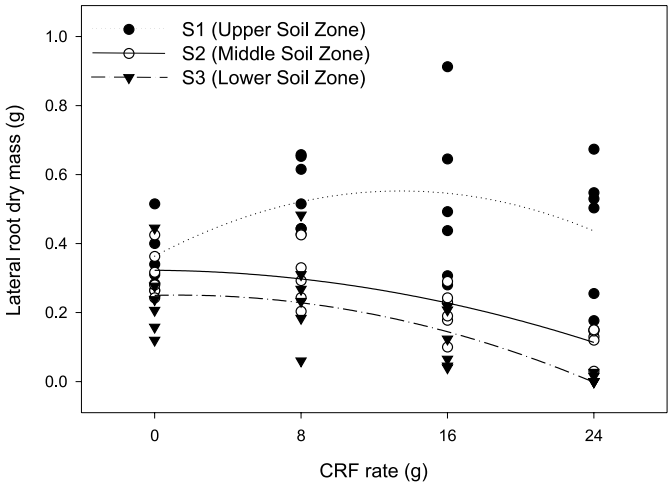
Note: For each nutrient, means followed by the same letter within a column did not differ significantly at $\alpha = 0.05$.

Table 3. Mean values and SE for chlorophyll fluorescence parameters (Fs (steady-state fluorescence), Fms (maximal fluorescence), and QY (quantum yield of photochemical energy conversion ((Fms – Fs)/Fms))) at four sampling points for fertilizer rate and needle position.

	Sampled on 9/13/2000			Sampled on 10/16/2000			Sampled on 12/14/2000			Sampled on 2/16/2001		
	Fs	Fms	QY	Fs	Fms	QY	Fs	Fms	QY	Fs	Fms	QY
CRF rate (g)												
0	127*a*	411*b*	0.67*b*	85*a*	468*a*	0.82*a*	186*a*	774*a*	0.75*a*	176*a*	747*a*	0.72*a*
8	142*a*	520*a*	0.71*a*	84*a*	440*a*	0.80*a*	194*a*	800*a*	0.75*a*	194*a*	853*a*	0.77*a*
16	134*a*	491*a*	0.72*a*	92*a*	478*a*	0.81*a*	185*a*	776*a*	0.75*a*	206*a*	866*a*	0.75*a*
24	151*a*	538*a*	0.71*a*	85*a*	421*a*	0.80*a*	188*a*	676*a*	0.66*a*	211*a*	764*a*	0.70*a*
SE	6.3	22.6	0.013	4.5	20.1	0.013	12.4	41.7	0.051	13.8	49.8	0.036
Needle position												
Middle	152*a*	553*a*	0.71*a*	90*a*	479*a*	0.81*a*	189*a*	793*a*	0.74*a*	196*a*	873*a*	0.76*a*
Top	124*b*	427*b*	0.70*a*	83*a*	425*b*	0.80*a*	187*a*	721*b*	0.71*a*	198*a*	742*b*	0.71*b*
SE	3.8	16.5	0.010	3.2	16.8	0.010	8.1	24.0	0.030	7.9	30.4	0.019

Note: At each sampling point for either fertilizer rate or needle position, means for each parameter followed by the same letter within a column did not differ significantly at $\alpha = 0.05$.

Fig. 3. First-order lateral root dry mass (RDM) in three soil zones (S1–S3) versus fertilizer rate 6 months following transplant. Data are presented on original scale prior to transformation. Regression equations are as follows: $\log(\text{RDM} + 0.01) = -1.30 + 0.095(\text{rate}) - 0.0034(\text{rate})^2$, adjusted $R^2 = 0.27$, $p < 0.0260$ for S1; $\log(\text{RDM} + 0.01) = -1.21 + 0.016(\text{rate}) - 0.0026(\text{rate})^2$, adjusted $R^2 = 0.59$, $p < 0.0001$ for S2; and $\log(\text{RDM} + 0.01) = -1.68 + 0.032(\text{rate}) - 0.0060(\text{rate})^2$, adjusted $R^2 = 0.72$, $p < 0.0001$ for S3.



ble 3). The QY of photochemical energy conversion was significantly higher for middle needles at the 16 February 2001 sampling date only ($p = 0.0024$).

Three months following transplant (13 September 2000), seedlings treated with CRF had significantly greater Fms ($p = 0.0037$) and QY ($p = 0.0389$) than controls (Table 3). In subsequent samplings, this trend was no longer present, and mean values for QY were reduced at the 24-g rate as compared with all other treatments, although the differences were not statistically significant (Table 3).

Discussion

Root architectural development

Roots tend to proliferate in areas of high nutrient supply without increasing overall root growth, particularly when the remainder of the plant is nutrient stressed (Friend et al. 1990). The placement of CRF as a single layer beneath the transplanted root system stimulated root proliferation above the fertilizer layer (S1) with increasing fertilizer rate. However, root penetration into the soil zone of fertilizer application (S2) and lower zone (S3) was severely restricted at the higher CRF rates. This was based on both the numbers of active roots tips, which are anatomically suited for efficient ion uptake (Peterson et al. 1999), and lateral root dry mass.

No satisfactory mechanism has been identified to explain the proliferation of roots in localized sources of nutrient sup-

ply (Granato and Raper 1989; Robinson 1994). The plant may preferentially allocate assimilates and carbohydrates to the nutrient-rich area of the root system (Drew and Saker 1975; Barta 1976), thus shifting growth to a portion of the roots rather than increasing growth in the entire root system (Friend et al. 1990). Granato and Raper (1989) attributed the proliferation of corn roots in localized sources of nutrient supply to in situ reduction and utilization of absorbed NO_3^-. Root response to localized nutrient sources may also be under hormonal control (Coutts and Philipson 1976).

In this experiment, root penetration into S2 and S3 at the higher CRF rates was likely restricted because of the accumulation of inhibitory levels of fertilizer salts in S2. Seedlings of woody plants, and conifers in particular, are extremely sensitive to salt injury (Landis et al. 1989). Although salt tolerance in roots varies with stage of seedling development (Zekri 1993), the recommended standard for salinity (EC) in water when growing conifers is 1.5 $dS \cdot m^{-1}$ (Landis et al. 1989). Phillion and Bunting (1983) found that black spruce (*Picea mariana* (Mill.) BSP) and white spruce (*Picea glauca* (Moench) Voss) seedlings were healthy at an EC up to 2.5 $dS \cdot m^{-1}$, but above 4.0 $dS \cdot m^{-1}$, conditions were lethal. Growth of blue spruce (*Picea pungens* Engelm.) and Douglas-fir seedlings, conifers highly sensitive to excessive EC levels, may be reduced by 10% with an increase in EC from 1.0 to 1.4 $dS \cdot m^{-1}$ and as much as 50% at 2.5 $dS \cdot m^{-1}$ (Landis et al. 1989).

Electrical conductivity levels were significantly greater in S2 for seedlings treated with CRF as compared with the control. Because this sampling was taken after the majority of the CRF had released (6 months), it is likely that EC in S2 for the CRF-treated seedlings reached levels higher than that recorded and increased with CRF rate. Root apical meristems are subject to death becaue of the buildup of toxic levels of ion concentrations and resulting decreases in soil osmotic potential (Drew 1975). Thus, elevated salt concentrations in the S2 zone of seedlings fertilized at high CRF rates injured elongating root tips and acted to limit root penetration below the fertilizer layer. This contributed to the proliferation of roots in S1, where conditions were favorable.

Seedling morphology

Six months following transplant, seedlings in the 8-g CRF treatment had higher mean values for most morphological parameters than any other treatment, while seedlings in the 24-g rate generally had the lowest values. Other studies have shown improved growth rates for forest tree seedlings fertilized with CRF compared with water-soluble fertilizer or unfertilized treatments (Walker and Kane 1997; Krasowski et al. 1999). Few experiments have documented growth reductions using CRF with forest tree seedlings despite the many reports of toxicities associated with high rates of fertilization with nutrients in solution. Ingestad (1979) distinguished critical N levels of 50 $mg \cdot L^{-1}$ and toxicity at 400 $mg \cdot L^{-1}$ for Scotch pine (*Pinus sylvestris* L.). Concentrations of 40–60 $mg \cdot L^{-1}$ of N in solution increased height growth of slash pine (*Pinus elliottii* Engelm.), but concentrations above 180 $mg \cdot L^{-1}$ reduced growth (Dewald et al. 1992). Lu et al. (1998) found that root and shoot dry mass of Douglas-fir decreased when N concentrations were increased from 50 to 200 ppm and attributed this to possible toxicities to roots associated with NH_4^+ ions. The lack of literature citing negative responses associated with CRF may be because CRF releases nutrients gradually. This may minimize the chance for accumulation of toxic nutrient levels, particularly with adequate irrigation. Despite improved nutrient release technologies, however, excessive levels of CRF clearly acted to reduce plant growth in this experiment.

Nutrients

Mean foliar N concentrations increased with increasing CRF rate at 4 months. This response has been previously reported for container-grown Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.) seedlings fertilized with different CRF types (Walker and Kane 1997; Walker and Huntt 2000). Mean foliar concentrations of P, however, tended to decrease with increasing fertilizer rate. This effect has been observed for Douglas-fir seedlings fertilized with N in the field (Roth and Newton 1996) and nursery (van den Driessche 1980). It is difficult to find a satisfactory explanation for this response. De Visser and Keltjens (1993) found that when N fertilizer was applied primarily as NH_4^+, less P was taken up than when N fertilizer was applied as NO_3^- and attributed this to acidification of the rhizosphere when N was taken up as NH_4^+. Phosphorus is generally far less mobile in the soil than N, and increasing root absorptive area, particularly through the production of root hairs (Marschner 1995), is important for efficient P uptake. Thus, it may be difficult to obtain a P response from fertilization if root growth is suppressed. The restrictions in root penetration noted at the higher CRF rates may explain the tendency for decreased P concentration with increasing CRF rate, as the plants could not effectively extract P from the soil.

Chlorophyll fluorescence

Chlorophyll fluorescence parameters were consistently higher in needles sampled from the middle of the plant as compared with the top of the plant. Researchers have reported higher photosynthetic rates in current-year foliage as compared with foliage from previous seasons (Teskey et al. 1984; Kajimoto 1990). Needles sampled from seedlings in this experiment, however, were all from the same growth flush. Radoglou and Teskey (1997) stated that actively growing foliage generally exhibits a low photosynthetic capacity and high respiration rates and found that the highest photosynthetic rates in loblolly pine (*Pinus taeda* L.) occurred when the foliage was more than 90% fully expanded. This helps explain the reduced chlorophyll fluorescence values of needles that were still actively elongating prior to the December 2000 sampling.

Mean values for Fms and QY were significantly higher for seedlings in all CRF treatments 3 months following transplant compared with controls. This coincided with the time period in which the majority of CRF had released. Increased nutrient availability for seedlings fertilized with CRF may have resulted in greater chlorophyll efficiency at this point. Birchler et al. (2001) found that chlorophyll fluorescence (F_v/F_m) of nursery-grown Douglas-fir seedlings fertilized with N and K in fall was consistently higher than

unfertilized seedlings. Although not statistically significant, mean values for QY at later sampling points were lowest for the 24-g CRF treatment, which may have been associated with stress due to fertilizer toxicities.

Conclusions

Although previous studies have shown that roots may proliferate in areas of localized fertilizer supply to enhance nutrient uptake, root development in these zones may also be restricted at high fertilizer application rates. This is a function of excessive initial nutrient release and subsequent increases of fertilizer salt concentrations in the soil solution. Thus, despite potential for improvements in forest tree seedling fertilization with CRF, these fertilizers may create conditions that adversely affect seedling development. This illustrates the importance of using conservative CRF rates and mixing fertilizer uniformly in soil media when growing seedlings in containers. Manufacturers of CRF must continue to develop fertilizer technology to provide a gradual and consistent release of nutrients that coincides with developmental needs of forest tree seedlings. This will help to maximize nutrient uptake and plant growth, while minimizing negative effects associated with excessive CRF rates. Although more growing space for roots is available in the field, it is possible that similar restrictions in subsoil root penetration may occur when CRF is applied at high rates to the planting hole, which may have implications regarding drought resistance. Differences in chlorophyll efficiency at the most active time of CRF nutrient release were detectable using chlorophyll fluorescence. It is necessary to sample foliage for chlorophyll fluorescence in a systematic manner to avoid discrepancies in data based on the developmental stage of foliage.

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References

Barta, A.L. 1976. Transport and distribution of $^{14}CO_2$ assimilates in *Lolium perenne* in response to varying N supply to halves of a divided root system. Physiol. Plant. **38**: 48–52.

Birchler, T.M., Rose, R., and Haase, D. 2001. Fall fertilization with N and K: effects on Douglas-fir seedling quality and performance. West. J. Appl. For. **16**: 71–79.

Brockley, R.P. 1988. The effects of fertilization on the early growth of planted seedlings: a problem analysis. Forestry Canada, Ottawa, Ont. For. Resour. Dev. Agree. Rep. 0835-0752:011.

Burdett, A.N. 1979. A nondestructive method for measuring the volume of intact plant parts. Can. J. For. Res. **9**: 120–122.

Carlson, W.C. 1981. Effects of controlled-release fertilizers on the shoot and root development of outplanted western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) seedlings. Can. J. For. Res. **11**: 752–757.

Carlson, W.C., and Preisig, C.L. 1981. Effects of controlled-release fertilizers on the shoot and root development of Douglas-fir seedlings. Can. J. For. Res. **11**: 230–242.

Coutts, M.P., and Philipson, J.J. 1976. The influence of mineral nutrition on the root development of trees: I. The growth of Sitka spruce with divided root systems. J. Exp. Bot. **27**: 1102–1111.

Coutts, M.P., and Philipson, J.J. 1977. The influence of mineral nutrition on the root development of trees: III. Plasticity of root growth in response to changes in the nutrient environment. J. Exp. Bot. **28**: 1071–1075.

De Visser, P.H.B., and Keltjens, W.G. 1993. Growth and nutrient uptake of Douglas fir seedlings at different rates of ammonium supply, with or without additional nitrate and other nutrients. Neth. J. Agric. Sci. **41**: 327–341.

Dewald, L., White, T.L., and Duryea, M.L. 1992. Growth and phenology of seedlings of four contrasting slash pine families in ten nitrogen regimes. Tree Physiol. **11**: 255–269.

Donald, D.G.M. 1991. Nursery fertilization of conifer planting stock. *In* Mineral nutrition of conifer seedlings *Edited by* R. van den Driessche. CRC Press, Boca Raton, Fla. pp. 135–167.

Drew, M.C. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system and the shoot, in barley. New Phytol. **75**: 479–490.

Drew, M.C., and Saker, L.R. 1975. Nutrient supply and the growth of the seminal root system in barley: II. Localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. J. Exp. Bot. **26**: 79–90.

Fisker, S.E., Rose, R., and Haase, D.L. 1995. Chlorophyll fluorescence as a measure of cold hardiness and freezing stress in 1 + 1 Douglas-fir seedlings. For. Sci. **41**: 564–575.

Friend, A.L., Eide, M.R. and Hinckley, T.M. 1990. Nitrogen stress alters root proliferation in Douglas-fir seedlings. Can. J. For. Res. **20**: 1524–1529.

Gavlak, R.G., Horneck, D.A., and Miller, R.O. 1994. Plant, soil, and water reference methods for the western region. Western Rural Development Center, Corvallis, Oreg.

Gleason, J.F., Duryea, M., Rose, R., and Atkinson, M. 1990. Nursery and field fertilization of 2 + 0 ponderosa pine seedlings: the effect on morphology, physiology, and field performance. Can. J. For. Res. **20**: 1766–1772.

Granato, T.C., and Raper, D. 1989. Proliferation of maize (*Zea mays* L.) roots in response to localized supply of nitrate. J. Exp. Bot. **40**: 263–275.

Haase, D.L., and Rose, R. (*Editors*). 1997. Symposium Proceedings: Forest Seedling Nutrition from the Nursery to the Field, 28–29 Oct. 1997. Nursery Technology Cooperative, Oregon State University, Corvallis, Oreg.

Hauck, R.D. 1985. Slow-release and bioinhibitor-amended nitrogen fertilizers. *In* Fertilizer technology and use. *Edited by* O.P. Engelstad. Soil Science Society of America, Inc., Madison, Wis. pp. 293–322.

Hawkins, C.D.B., and Lister, G.R. 1985. In vivo chlorophyll fluorescence as a possible indicator of the dormancy stage in Douglas-fir seedlings. Can. J. For. Res. **15**: 607–612.

Huett, D.O., and Gogel, B.J. 2000. Longevities and nitrogen, phosphorus, and potassium release patterns of polymer-coated controlled-release fertilizers at 30°C and 40°C. Commun. Soil Sci. Plant Anal. **31**: 959–973.

Ingestad, T. 1979. Nitrogen and plant growth: maximum efficiency of nitrogen fertilizers. Ambio, **6**: 146–151.

Kajimoto, T. 1990. Photosynthesis and respiration of *Pinus pumila* needles in relation to needle age and season. Ecol. Res. **5**: 333–340.

Khan, S.R., Rose, R., Hasse, D.L., and Sabin, T.E. 1996. Soil water stress: its effects on phenology, physiology, and morphology of containerized Douglas-fir seedlings. New For. **12**: 19–39.

Khan, S.R., Rose, R., Haase, D.L., and Sabin, T.E. 2000. Effects of shade on morphology, chlorophyll concentration, and chlorophyll fluorescence of four Pacific Northwest conifer species. New For. **19**: 171–186.

Kochba, M., Gambash, S., and Avnimelech, Y. 1990. Studies on slow release fertilizers: 1. Effects of temperature, moisture, and water vapor pressure. Soil Sci. **149**: 339–343.

Kozlowski, T.T. 1987. Soil moisture and absorption of water by tree roots. J. Arboric. **13**: 39–46.

Krasowski, M.J., Owens, J.N., Tackaberry, L.E., and Massicotte, H.B. 1999. Above- and below-ground growth of white spruce seedlings with roots divided into different substrates with or without controlled-release fertilizer. Plant Soil, **217**: 131–143.

Landis, T.D., Tinus, R.W., McDonald, S.E., and Barnett, J.P. 1989. The container tree nursery manual. Vol. 4. Seedling nutrition and irrigation. U.S. Dep. Agric. Agric. Handb. 674.

Lu, S., Mattson, K.G., Zaerr, J.B., and Marshall, J.D. 1998. Root respiration of Douglas-fir seedlings: effects of N concentration. Soil Biol. Biochem. **30**: 331–336.

Marschner, H. 1995. Nutrient availability in soils. *In* Mineral nutrition of higher plants. 2nd ed. Academic Press, New York. pp. 483–507.

Opti-Sciences, Inc. 1997. Opti-Sciences OS5-FL modulated chlorophyll fluorometer operation manual. Opti-Sciences, Inc., Tyngsboro, Mass.

Peterson, C.A., Enstone, D.E., and Taylor, J.H. 1999. Pine root structure and its potential significance for root function. Plant Soil, **217**: 205–213.

Phillion, B.J., and Bunting, W.R. 1983. Growth of spruce seedlings at various soluble fertilizer salt levels. USDA for. Serv. Tree Plant. Notes 34. pp. 31–33.

Radoglou, K., and Teskey, R.O. 1997. Changes in rates of photosynthesis and respiration during needle development of loblolly pine. Tree Physiol. **17**: 485–488.

Roberts, D.R., Toivonen, P., and McInnis, S.M. 1991. Discrete proteins associated with overwintering of interior spruce and Douglas-fir seedlings. Can. J. Bot. **69**: 437–441.

Robinson, D. 1994. Tansley review No. 73. The responses of plants to non-uniform supplies of nutrients. New Phytol. **127**: 635–674.

Roth, B.E., and Newton, M. 1996. Survival and growth of Douglas-fir relating to weeding, fertilization, and seed source. West. J. Appl. For. **11**: 62–69.

Teskey, R.O., Grier, C.C., and Hinckley, T.M. 1984. Change in photosynthesis and water relations with age and season in *Abies amabilis*. Can. J. For. Res. **14**: 77–84.

van den Driessche, R. 1980. Effects of nitrogen and phosphorus fertilization on Douglas-fir nursery growth and survival after outplanting. Can. J. For. Res. **10**: 65–70.

Vidaver, W., Binder, W., Brooke, R.C., Lister, G.R., and Toivonen, P.M.A. 1989. Assessment of photosynthetic activity of nursery-grown *Picea glauca* seedlings using an integrating fluorometer to monitor variable chlorophyll fluorescence. Can. J. For. Res. **19**: 1478–1482.

Walker, R.F., and Huntt, C.D. 2000. Production of containerized Jeffrey pine planting stock for harsh sites: growth and nutrition as influenced by controlled-release fertilization. West. J. Appl. For. **15**: 86–91.

Walker, R.F., and Kane, L.M. 1997. Containerized Jeffrey pine growth and nutrient uptake in response to mycorrhizal inoculation and controlled release fertilization. West. J. Appl. For. **12**: 33–40.

Zekri, M. 1993. Seedling emergence, growth and mineral concentration of three citrus rootstocks under salt stress. J. Plant Nutr. **16**: 1555–1568.