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Original article

Fall fertilization of *Pinus resinosa* seedlings: nutrient uptake, cold hardiness, and morphological development

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Abstract

• Fall fertilization may increase plant nutrient reserves, yet associated impacts on seedling cold hardiness are relatively unexplored.

• Bareroot red pine (*Pinus resinosa* Ait.) seedlings in north-central Minnesota, USA were fall fertilized at the end of the first growing season with ammonium nitrate (NH_4NO_3) at 0, 11, 22, 44, or 89 kg N ha^{-1}. Seedling morphology and cold hardiness [assessed by freeze induced electrolyte leakage (FIEL)] were evaluated six weeks after fertilization and following the second growing season.

• Seedling height and number of needle primordia increased with fertilizer rate for both sampling years. Seedlings fertilized with 44 and 89 kg N ha^{-1} attained target height (15 cm) after the second growing season. Shoot and root N concentration increased after the first growing season in fall fertilized seedlings compared to controls. Fall fertilized seedlings had lower FIEL (i.e., increased cold hardiness) compared to controls when tested at –40 °C after the first growing season, but no significant differences in FIEL of control and fertilized seedlings were observed after the second growing season.

• Results suggest that fall fertilization of red pine seedlings can help render desired target height in the nursery, while maintaining or increasing cold hardiness levels.

Mots-clés :
rusticité au froid /
croissance /
fertilisation azotée /
primordiums d'aiguilles

Résumé – Fertilisation automnale des plants de *Pinus resinosa* : absorption des éléments nutritifs, rusticité au froid, et développement morphologique.

• La fertilisation automnale peut augmenter les réserves en éléments nutritifs des plants, mais les répercussions sur la rusticité au froid des semis sont encore relativement inexplorées.

• Des plants à racines nues de *Pinus resinosa* Ait.dans le centre-nord du Minnesota (USA) ont été fertilisés à l'automne à la fin de la première saison de croissance avec du nitrate d'ammonium (NH_4NO_3) à 0, 11, 22, 44, ou 89 kg N ha^{-1}. La morphologie des plants et la rusticité au froid [estimée par la fuite d'électrolyte (FIEL) induite par le gel] ont été évaluées six semaines après la fertilisation et à la suite dans la deuxième saison de croissance.

• La hauteur des plants et le nombre de primordiums d'aiguilles ont augmenté avec le taux de fertilisation pour les deux années d'échantillonnage. Les plants fertilisés avec 44 et 89 kg N ha^{-1} ont atteint l'objectif de hauteur (15 cm) après la deuxième saison de croissance. La concentration en N des tiges et des racines a augmenté après la première saison de croissance chez les plants fertilisés à l'automne par rapport aux témoins. Les plants fertilisés à l'automne ont eu un plus faible FIEL (c'est-à-dire, une augmentation de rusticité), comparativement aux témoins lors du test à –40 °C après la première saison de croissance, mais aucune différence significative de FIEL entre plants fertilisés et témoins n'a été observée après la deuxième saison de croissance.

• Les résultats suggèrent que la fertilisation d'automne des plants de *Pinus resinosa* Ait. peut aider à obtenir l'objectif de hauteur souhaité dans la pépinière, tout en maintenant ou en augmentant les niveaux de rusticité au froid.

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1. INTRODUCTION

Fertilization is an integral component of nursery culture for production of high quality seedlings for afforestation and reforestation because it can enhance plant growth, nutrient storage reserves, and resistance to biotic and abiotic stresses (Landis, 1985). While fertilizer is conventionally applied during spring and summer in accordance with the active growing period, fall fertilization has also been used to further enhance seedling quality in black spruce (*Picea mariana* (Mill.) BSP.) (Boivin et al., 2004), loblolly pine (*Pinus taeda* L.) (Sung et al., 1997; VanderSchaaf and McNabb, 2004), slash pine (*Pinus elliottii* var. elliottii [Engelm.]) (Irwin et al., 1995), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Margolis and Waring, 1986; van den Driessche, 1985, 1988). Although seedlings are undergoing the hardening process for winter dormancy at this time, fall fertilization still promotes nutrient exploitation for storage reserves.

Biomass accumulation during hardening can lead to nutrient dilution in seedlings. This can be averted by applying fertilizer at a higher rate or exponentially (Boivin et al., 2004). Fall fertilization may significantly increase foliar nitrogen (N) levels (Boivin et al., 2004; van den Driessche, 1985, 1988), enhance root growth potential (van den Driessche, 1988), promote earlier bud break (Margolis and Waring, 1986; Thompson, 1983; van den Driessche, 1985), and improve survival and growth compared to conventionally fertilized seedlings (van den Driessche, 1988).

Despite anticipated benefits, there is potential for high N applications to delay onset of dormancy and cold hardiness. The relationship between fall N fertilization and seedling cold hardiness, however, is relatively unexplored and results reported thus far contrast widely. For example, excess N decreased cold hardiness in young and adult Scots pines (*Pinus silvestris* L.), adult Norway spruce (*Picea abies* L.), and black spruce seedlings (Aronsson, 1980; Soikkeli and Karenlampi, 1984; Bigras et al., 1996). Fløistad and Kohmann (2004) reported that first-year Norway spruce seedlings with foliar N concentration between 0.9 and 1.8% exhibited variable frost hardiness in spring. In contrast, N or NPK fertilization enhanced cold hardiness in red spruce (*Picea rubens* Sarg.) (DeHayes et al., 1989; L'Hirondelle et al., 1992) and white spruce (*Picea glauca* (Moench) Voss) seedlings (Calmé et al., 1993). Additionally, N application during fall increased cold hardiness in Douglas-fir, whereas phosphorus (P) applied without N decreased hardiness (Thompson, 1983). Furthermore, 2-year old ponderosa pine (*Pinus ponderosa* C. Lawson) seedlings showed enhanced cold hardiness with increasing N concentrations (Gleason et al., 1990). Cold hardiness was unaffected by three different rates of N and P application in Douglas-fir and western redcedar (*Thuja plicata* Donn ex D. Donn) seedlings (Hawkins et al., 1995). Birchler et al. (2001) also reported similar findings for Douglas-fir seedlings. These results suggest that cold hardiness responses to fall fertilization are species specific and may be influenced by rate of nutrient application, nutrient balance, or timing of assessment (Hawkins et al., 1995).

In forest tree nurseries located in northern latitudes, such as in the northeastern USA, 2+0 spruce and pine seedlings grown under conventional bareroot nursery culture may not attain desired target height during their second year due to relatively short growing seasons. In north-central Minnesota, USA, target height for 2 + 0 bareroot red pine (*Pinus resinosa* Ait.) seedlings is 15 cm, but seedlings that receive conventional spring and summer fertilization often fail to attain target height (Islam et al., 2008). The present fall fertilization study was undertaken primarily to determine the potential to augment second year height growth of 2 + 0 red pine seedlings without compromising cold hardiness. A limited number of published studies (e.g., Martz et al., 2006; Sutinen et al., 1992) have examined cold tolerance of red pine, but none investigated effects of fall fertilization on corresponding cold hardiness. Our study objectives were to evaluate if fall fertilization applied at the end of the first growing season would: (i) increase the number of needle primordia, resulting in greater shoot elongation and biomass in the following season; and (ii) affect cold hardiness as measured by freeze-induced electrolyte leakage (FIEL).

2. MATERIAL AND METHODS

2.1. Plant material

Red pine seeds were collected from north-central Minnesota, USA and sown (200 seedlings m^{-2}) into a bareroot nursery bed (182 m long and 1.2 m wide) in October 2004 at the Badoura state forest Nursery near Akeley, Minnesota, USA (46° 56′ N, 94° 43′ W). Seedlings emerged in May 2005; their shoots elongated until terminal buds formed during the last week of August and first week of September 2005. This phenology is typical for first year (1 + 0) seedlings, while seedlings in their second year of growth form buds in mid July. The nursery soil was a sandy loam, routinely amended with peat moss to maintain organic matter content around 3%. Soil pH was 5.5 with a buffer index of 6.5. Soil analyses in September 2005 (before application of fall fertilization) indicated a nitrate NO_3-(N) ppm of 0.9, a Bray 1 phosphorus (P) ppm of 30, and potassium (K) ppm of 74. Seedlings were watered 2 to 3 times each week (May through August) in 2005 and 1 to 2 times (May through September) in 2006. Weeds were controlled with Fusilade® (Syngenta Crop Protection, Inc., Greensboro, NC, USA) and hand-weeding.

2.2. Fertilizer treatments

Regular fertilizer applications in 2005 included: 6 top-dress applications of granular ammonium nitrate (NH_4NO_3; 34-0-0) at 33 kg N ha^{-1} on June 27, July 5, 11, 18, 25, and August 1; 3 foliar applications of liquid 20-20-20 at 1.12 kg N ha^{-1} on June 20, 27, and July 5; and 3 foliar applications of 20-20-20 at 1.8 kg N ha^{-1} on July 20, 28, and August 12. Fall fertilization was applied to a single nursery bed on 16 September 2005 when most seedlings had already formed terminal buds. Fall fertilizer (NH_4NO_3;34-0-0), was applied at 5 levels of N: 0, 11, 22, 44, and 89 kg N ha^{-1} on a clear day and no rainfall occurred immediately after fertilizer applications. Each level of fertilizer was randomly applied within the bed (replicated 4 times) with buffers between each treatment. Regular fertilizer applications

for 2006 included: 1 top-dress application of granular NH_4NO_3(34-0-0) at 67 kg N ha^{-1} on June 5; and 5 top-dress applications of granular 10-10-10 at 30.8 kg N ha^{-1} on June 26, July 10, 17, 24, and 31. Because seedlings are typically harvested during late fall of the second growing season, fall fertilization was not repeated during the second season.

2.3. Sampling and measurements

A total of 200 seedlings (10 seedlings per treatment replication) were randomly excavated using shovels from the nursery beds during the first week of November 2005 and 2006. Seedlings were placed inside plastic bags and packed into shipping boxes alongside cold refrigerant gel packs to ensure that seedlings maintained their physiological state during overnight shipment to Purdue university, West Lafayette, Indiana, USA. We measured height (root collar to tip of terminal bud), root collar diameter (RCD), number of needle primordia, and cold hardiness by freeze induced electrolyte leakage (FIEL) on 5 seedlings per treatment replication. The remaining 5 seedlings per treatment replication were used to determine shoot, root, and total plant dry mass and N analysis for shoot and root components.

2.4. Needle primordia

Number of needle primordia was determined following methods of Templeton et al. (1993). The shoot (at least 10 cm) was excised from the seedling, needles were excised carefully, and the acropetal end observed under magnification. Bud scales were carefully removed to expose the needle primordia; the cap of bud scales was loosened by using a hypodermic needle to make a 0.5-mm deep circumferential incision through the bark at the base of the lower most bud scales. Once bud scales were removed, the embryonic shoot was viewed under 10 × magnification with a dissecting microscope (Nikon SMZ-U Stereoscopic microscope, Melville, NY, USA), and the needle primordia were estimated using the average number counted per row multiplied by the number of columns.

2.5. Cold hardiness

Cold hardiness was determined with a freeze-induced electrolyte leakage (FIEL) test of needles. Compared to the whole plant freeze test, FIEL of needle samples yields a more precise, quick, sensitive, and objective predictor of changes or differences in tissue cold hardiness (Burr et al., 1990). Because one-year-old red pine seedlings have a small quantity of needles, needles were randomly selected along the entire length of the shoot, carefully detached from seedlings, and rinsed with distilled water. Two-year-old seedlings were sampled similarly. For each fertilizer treatment replication, a sufficient number of needles from 5 seedlings (following Martz et al., 2006) were obtained and cut into approximately 1-cm segments. From each group of 5 seedlings, 7 segments were placed into 7 separate vials (each vial corresponding to different test temperatures: 2 [control], –5, –10, –20, –25, –30, and –40 °C). We tested the needle samples to –40 °C because red pine is native to areas where the average annual minimum temperatures range from –23° to –40 °C (Rudolf, 1990). Each vial contained 1 mL of deionized (DI) water. To avoid excessive super cooling during freezing, only 1 mL of DI water was added to the vial prior to the freezing tests.

The vials were placed in a programmable freezer (Ultra-Low Freezer; CH40-13, SO-LOW Environmental Equipment, Cincinnati, OH, USA) for 1.5 h at 2 °C, after which time the control treatment vials were removed. The temperature was then decreased at a rate of –5 °C h^{-1}. Upon reaching each successive test temperature, the temperature was held for 30 min before decreasing again. After all the vials were removed and thawed, 9 mL of DI water was added to each vial to aid in measurement of electrical conductivity with an EC/TDS meter (Seven Easy, Mettler Toledo, SevenEasy Co. Mettler Toledo GmbH, Switzerland). Following initial measurements, vials and their contents were autoclaved at 121 °C for 20 min to obtain maximum EL. After cooling overnight at room temperature, the maximum EL valued were recorded. For each sample, FIEL was calculated using the following formula (Jacobs et al., 2008):

$$FIEL\,(\%) = \left(\frac{Freeze - induced\ EL}{Maximum\ EL}\right) \times 100$$

where, freeze-induced EL = EC of the sample measured after exposure to the test temperature and maximum EL = EC after autoclaving.

2.6. Nitrogen analysis

The remaining 5 seedlings (sampling described previously) were partitioned into roots and shoots and dried for 72 h at 68 °C. After determining biomass, oven-dried samples were ground to pass through a 2-mm sieve and sent to A&L Great Lakes Laboratories (Fort Wayne, IN, USA) for nutrient analysis. Total N was determined by combustion "Dumas" procedure (Association of Official Analytical Chemist (AOAC) 968.06) using a LECO nitrogen analyzer (LECO Corporation, St. Joseph, MI, USA). Shoot and root N contents were calculated by multiplying respective N concentration with dry mass.

2.7. Experimental design and data analysis

We used a completely randomized design with 5 levels of fall fertilization (0 [control], 11, 22, 44, and 89 kg N ha^{-1}) × 4 replications applied to a single nursery bed. Each fertilizer-replication combination was separated with a buffer zone. Tests for normality and constant variance were performed to ensure validity of the assumptions of analysis of variance (ANOVA) and no transformations were necessary. Data were analyzed with SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) using general linear model (GLM). When ANOVA indicated significant ($P < 0.05$) treatment effect, Least Significant Difference (LSD) was used to identify significant differences among fall fertilization treatments at $\alpha = 0.05$.

To test the effects of 7 test temperature levels and 5 fertilizer levels, and their interactions on FIEL, we used the GLM procedure in SAS. FIEL data met the assumptions of normality and homogeneity of variance. When ANOVA indicated significant ($P < 0.05$) treatment effect, LSD was used to identify significant differences among fall fertilization treatments under each test temperature level ($\alpha = 0.05$).

3. RESULTS

3.1. Nitrogen uptake

Fall fertilization significantly ($P = 0.0008$) affected shoot N concentration after the first growing season (Tab. I); in fertilized seedlings, shoot N concentration was increased 22–40%

Table I. Mean values (±SE) of morphological parameters of red pine seedlings after the first growing season. Seedlings were fall fertilized with a single application of ammonium nitrate (NH_4NO_3) at rates of 0, 11, 22, 44 and 89 kg N ha^{-1}. For each parameter, values in the same row with different letters differed statistically according to Fisher's Least Significant Difference test at $\alpha = 0.05$.

	Fall fertilizer application rate (kg N ha^{-1})				
	0	11	22	44	89
Height (cm)	6.07 (0.57)*b*	5.70 (0.48)*b*	5.95 (0.42)*b*	6.4 (0.26)*ab*	7.45 (0.15)*a*
RCD (mm)	1.70 (0.07)*a*	1.50 (0.04)*a*	1.55 (0.18)*a*	1.70 (0.14)*a*	1.80 (0.13)*a*
Shoot dry mass (g)	0.64 (0.05)*ab*	0.47 (0.09)*b*	0.61 (0.20)*ab*	0.81 (0.13)*ab*	0.98 (0.10)*a*
Root dry mass (g)	0.19 (0.02)*a*	0.21 (0.03)*a*	0.18 (0.03)*a*	0.20 (0.02)*a*	0.25 (0.01)*a*
Shoot N conc. (%)	1.66 (0.12)*c*	2.06 (0.08)*b*	2.04 (0.08)*b*	2.15 (0.04)*ab*	2.33 (0.04)*a*
Root N conc. (%)	0.92 (0.09)*b*	1.18 (0.03)*ab*	0.97 (0.32)*b*	1.34 (0.02)*ab*	1.55 (0.14)*a*
Shoot N cont. (mg)	10.61 (0.48)*b*	9.74 (2.01)*b*	12.64 (4.41)*b*	17.47 (2.97)*ab*	22.98 (2.05)*a*
Root N cont. (mg)	1.87 (0.40)*b*	2.51 (0.32)*ab*	1.74 (0.73)*b*	2.79 (0.37)*ab*	3.86 (0.27)*a*
Number of needle primordia	17.0 (0.8)*c*	19.2 (0.7)*c*	17.7 (0.9)*c*	25.0 (1.4)*b*	31.7 (1.0)*a*

Table II. Mean values (±SE) of morphological parameters of red pine seedlings after the second growing season. Seedlings were fall fertilized with a single application of ammonium nitrate (NH_4NO_3) at rates of 0, 11, 22, 44 and 89 kg N ha^{-1}. For each parameter, values in the same row with different letters differed statistically according to Fisher's Least Significant Difference test at $\alpha = 0.05$.

	Fall fertilizer application rate (kg N ha^{-1})				
	0	11	22	44	89
Height (cm)	13.02 (0.73)*a*	14.23 (0.68)*a*	14.87 (0.80)*a*	15.49 (0.55)*a*	16.28 (0.68)*a*
RCD (mm)	3.43 (0.17)*c*	3.63 (0.18)*bc*	4.17 (0.19)*abc*	4.27 (0.22)*ab*	4.88 (0.22)*a*
Shoot dry mass (g)	4.20 (0.46)*b*	4.84 (0.53)*b*	6.19 (0.69)*ab*	6.08 (0.76)*ab*	8.18 (0.76)*a*
Root dry mass (g)	0.98 (0.09)*b*	1.13 (0.08)*b*	1.49 (0.16)*ab*	1.42 (0.11)*ab*	1.91 (0.21)*a*
Shoot N conc. (%)	1.31 (0.10)*a*	1.37 (0.06)*a*	1.32 (0.03)*a*	1.35 (0.09)*a*	1.25 (0.04)*a*
Root N conc. (%)	0.75 (0.06)*a*	0.71 (0.02)*a*	0.71 (0.03)*a*	0.73 (0.04)*a*	0.68 (0.04)*a*
Shoot N cont. (mg)	51.57 (7.37)*b*	69.50 (10.54)*ab*	77.96 (11.22)*ab*	84.83 (13.02)*ab*	103.40 (10.06)*a*
Root N cont. (mg)	6.64 (0.68)*b*	8.50 (0.86)*b*	9.69 (1.70)*ab*	10.64 (1.04)*ab*	13.56 (2.09)*a*
Number of needle primordia	100.8 (8.3)*cd*	82.7 (3.7)*d*	109.7 (3.6)*bc*	126.2 (7.5)*b*	172.5 (5.2)*a*

compared to the control. Although root N concentration of seedlings fertilized with 89 kg N ha^{-1} was 68% greater than the control after the first growing season (Tab. I), fertilization was not significant ($P = 0.0866$). Fertilizer had no effect on either shoot ($P = 0.4417$) or root ($P = 0.7802$) N concentration after the second growing season (Tab. II).

Fall fertilization significantly affected shoot ($P = 0.0188$) and root ($P = 0.0326$) N content after the first growing season (Tab. I). Shoot and root N content increased 19–116% and 34–106%, respectively, in fertilized seedlings compared to the control (Tab. I). Similarly, a significant fertilization effect was observed for shoot ($P = 0.0070$) and root ($P = 0.0080$) N content after the second growing season (Tab. II).

3.2. Height growth and needle primordia

Although seedlings fertilized at the highest rate (89 kg N ha^{-1}) were 23% taller than control seedlings after the first growing season, fertilization did not have a significant ($P = 0.0649$) effect (Tab. I). Fertilization did, however, significantly ($P = 0.0020$) affect seedling height after the second growing season (Tab. II). Height increment in seedlings fertilized with 89 kg N ha^{-1} was 25% greater than the control after the second growing season (Tab. II), but differences were not statistically significant ($P = 0.0646$).

Despite not yielding a significant ($P = 0.4739$) effect on seedling root collar diameter (RCD) after the first growing season (Tab. I), fall fertilization did significantly ($P < 0.0001$) affect RCD after the second growing season (Tab. II). Shoot and root dry mass followed a similar trend: fertilization was not significant ($P = 0.0941$ for shoots and $P = 0.5054$ for roots) after the first growing season, but was ($P = 0.0001$ for shoots and $P < 0.0001$ for roots) after the second season (Tabs. I and II).

Fall fertilization significantly ($P < 0.0001$) affected number of needle primordia after each growing season (Tabs. I and II). In each year, more needle primordia were formed as fertilizer rate increased, resulting in longer buds (Fig. 3). Seedlings fertilized with 89 kg N ha^{-1} had 86% and 71% more needle primordia compared to the control after the first and second growing seasons, respectively (Tabs. I and II).

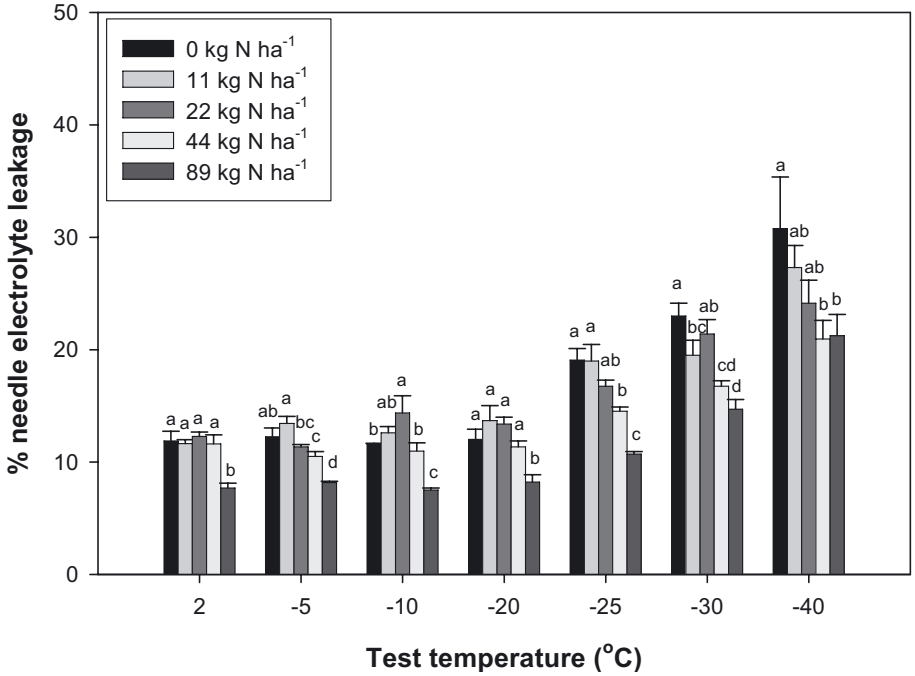

Figure 1. Mean needle electrolyte leakage values for each test temperature after the first growing season. Red pine seedlings were fall fertilized with a single application of ammonium nitrate (NH_4NO_3) at rates of 0, 11, 22, 44 and 89 kg N ha^{-1}. Each bar represents mean (n = 5) ± SE. Bars with different letters within each test temperature are significantly different at $\alpha = 0.05$.

3.3. Cold hardiness

Cold hardiness of red pine seedlings was significantly (P = 0.0001) affected by fall fertilization after both the first and second growing season. Test temperatures also significantly ($P < 0.0001$) affected seedling FIEL in both seasons. The fertilizer × temperature interaction was not significant (P = 0.0543) after the first growing season; however, a significant (P = 0.0031) interaction was observed after the second growing season. Values of FIEL generally increased regardless of fertilizer treatment as test temperature decreased, except that seedlings fertilized with 89 kg N ha^{-1} had significantly lower FIEL compared to the control (0 kg N ha^{-1}) (Fig. 1). The FIEL values ranged between 8–12% until the test temperature reached –25 °C. While FIEL values for all fertilizer treatments increased sharply as test temperature declined thereafter, seedlings fertilized with 89 kg N ha^{-1} had significantly lower FIEL across test temperature ranges compared to the control. For example, at a test temperature of –40 °C, FIEL for control seedlings increased to 31% whereas fertilized (44 and 89 kg N ha^{-1}) seedlings had only 21% leakage (Fig. 1). Similar trends in FIEL were observed after the second growing season (Fig. 2), although at the –40 °C test temperature seedling FIEL was not significantly (P = 0.3290) affected by fall fertilization (Fig. 2).

4. DISCUSSION

Fall fertilization caused both acute (observed at the end of the first growing season) and persistent (observed after two growing seasons) changes in red pine seedling development. Although our fall fertilizer rates (0, 11, 22, 44 and 89 kg N ha^{-1}) were relatively low compared to Birchler et al. (2001) (0, 80, 160 and 320 kg N ha^{-1}) and Irwin et al. (1998) (57 kg N ha^{-1} and 3 × 57 kg N ha^{-1}), addition of N increased shoot and root N concentrations, height, and number of needle primordia in terminal buds, while also increasing cold hardiness after the first growing season.

Application of N to dormant slash pine (Irwin et al., 1998), ponderosa pine (Gleason et al., 1990), and Douglas-fir (Margolis and Waring, 1986) seedlings increased N concentration. In our study, six weeks after applying fertilizer to red pine seedlings with dormant terminal buds, shoot and root N concentrations in seedlings receiving 89 kg N ha^{-1} were 40% and 68% greater than for the control. Control seedlings had shoot N around 1.7%, whereas 89 kg N ha^{-1} seedlings had shoot N around 2.3%. This increase in shoot and root N concentration may enhance free amino acid concentration for protein synthesis (Margolis and Waring, 1986), which led to an increase in growth reflected by the average 15% increase in height of fertilized seedlings compared with nonfertilized cohorts (Tabs. I and II). Similarly, significant height growth was observed four weeks after initiation of hardening in black spruce seedlings that were fertilized during hardening compared to nonfertilized seedlings (Colombo et al., 2003). In contrast, Irwin et al. (1998) found no significant differences in seedling height in control and fall fertilized slash pine seedlings. The relative increment in height with increased fertilizer application in the present study suggests that even during October, under relatively low temperatures (average October high 13 °C and low

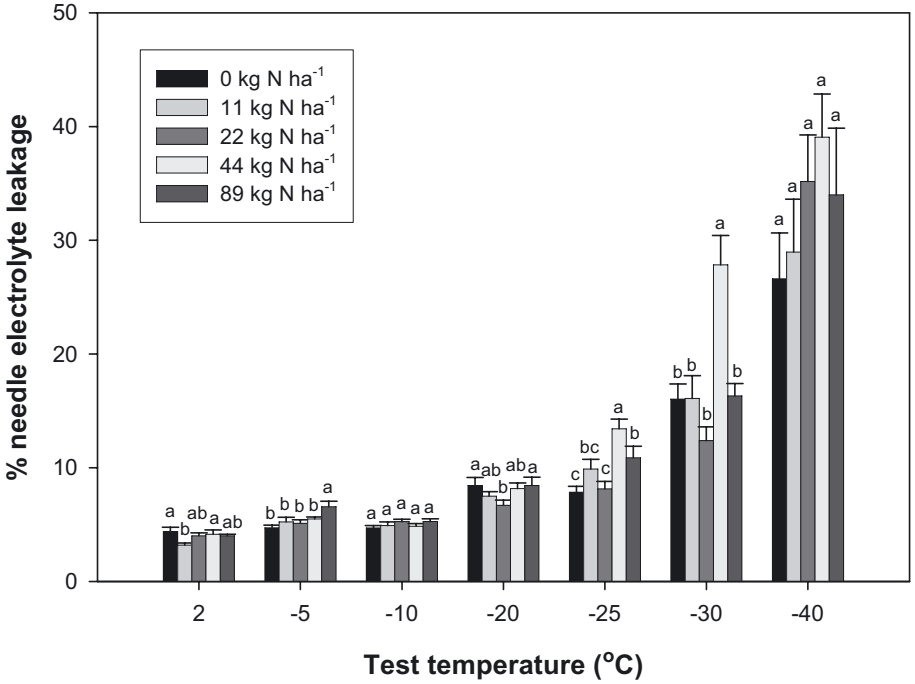

Figure 2. Mean needle electrolyte leakage values for each test temperature after the second growing season. Red pine seedlings were fall fertilized with a single application of ammonium nitrate (NH_4NO_3) at rates of 0, 11, 22, 44 and 89 kg N ha^{-1}. Each bar represents mean (n = 5) ± SE. Bars with different letters within each test temperature are significantly different at α = 0.05.

1 °C), seedlings were physiologically active. Colombo et al. (2003) noted that black spruce seedlings required 3 weeks to initiate budscales at the tip of apical meristem when seedlings were exposed to 8 h day length during hardening. Margolis and Waring (1986) observed a significant rise of free amino acid levels in needles one month after fall application of ammonium nitrate, suggesting that a substantial amount of N was mobilized or transported to foliage that was still photosynthetically active.

Moreover, higher N concentrations were also associated with an increased number of needle primordia in terminal buds (Tab. I). This is presumably due to continued assimilation and partitioning of carbon (VanderSchaaf and McNabb, 2004). A similar increase in number of needle primordia was observed in black spruce seedlings fall fertilized with 150 ppm of N compared to non-fertilized seedlings (Colombo et al., 2003). They also reported that initiation of needle primordia in terminal buds continued 4 weeks longer in fertilized seedlings compared to non-fertilized seedlings, but no significant differences were observed in cold injury.

Several studies have shown the benefit of improved mineral nutrition on cold hardiness. For example, Timmis (1974) reported Douglas-fir seedlings with low foliar N concentration (i.e., 0.8%) had a LT_{50} of –13 °C and those with a higher concentration (i.e., 1.6%) had a LT_{50} of –30 °C. Gleason et al. (1990) reported that fall fertilized ponderosa pine seedlings with a N concentration of 1.55% were more cold hardy than control seedlings with 1.47% N. Fernández et al. (2007) observed that N fertilized plants with more than 1.25% N better tolerated freezing than those that had less than 1%. Similarly, Luoranen et al. (2008) observed poor autumn cold hardiness for *Picea abies* seedlings with lower (i.e., 1.1%) compared to higher (i.e., > 1.6%) foliar N concentrations. However, Hawkins et al. (1995) found no correlations between foliar N and cold hardiness in Douglas-fir seedlings containing 1.2 to 2.6% N. Additionally, Birchler et al. (2001) did not detect any effect of fall fertilization on cold hardiness of Douglas-fir seedlings. Moreover, Aronsson (1980) and Hellergren (1981) reported decreased cold hardiness with increasing N concentrations in Scots pine seedlings.

In the present study, we observed that increasing fertilizer rate improved mineral nutrition (higher shoot N concentrations) and this was associated with greater cold hardiness after the first growing season (Tab. I). For example, FIEL of control seedlings at –40 °C was 30%. FIEL values for seedlings that received 11, 22, 44 and 89 kg N ha^{-1} were 24, 22, 22 and 20%, respectively (Fig. 1). It is not surprising that red pine seedlings, regardless of fertilizer treatment and when tested at –40 °C, exhibited less than 30% tissue electrolyte leakage. Extreme winter temperatures at north-central Minnesota are severe and could decline to –46 °C, suggesting that seedlings grown from local seed sources are well adapted to such low temperatures. Martz et al. (2006) reported a LT_{50} (i.e., test temperature that causes injury or death to 50% of the test population) of –148 °C for red pine needles measured in January following cold acclimation, but they visually estimated the extent of needle freezing injury (i.e., browning). Furthermore, Sutinen et al. (1992) found that electrolyte leakage of red pine needles never exceeded 30% when needles were exposed to –80 °C or lower temperatures or subjected to a slow

Figure 3. Red pine terminal bud size increased with increasing fall fertilizer rates. Buds represent (from left to right) 0, 11, 22, 44 and 89 kg N ha^{-1} fertilizer treatments for two-year-old seedlings.

freeze-thaw stress of –196 °C. However, rapid freezing of red pine needles to –196 °C (with liquid nitrogen) resulted in over 80% electrolyte leakage.

Improved N concentration of fall fertilized seedlings may have provided necessary resources for seedlings to increase the amount of organic compounds, such as amino acid or other organic acids, which woody plants accumulate when exposed to low temperatures (Kontunen-Soppela (2001). Cell plasma membranes become permeable during freezing (Colombo et al., 2003; Kontunen-Soppela 2001) and higher FIEL values reflect damaged cell membranes. During cold acclimation the lipid-protein ratio of cell membranes and the soluble protein concentration change in a manner that the threshold temperature of cell damage is lowered compared to non-acclimated plants (Kontunen-Soppela 2001). Nitrogen fertilization may increase the concentration of these proteins, which include apoplastic proteins having antifreeze activity, cryoprotective proteins, and dehydrins (Kontunen-Soppela 2001).

Indeed, Sutinen et al. (1992) attributed very low electrolyte leakage in winter-hardy red pine needles to changes in cell wall properties and their ability to develop tolerance towards extracellular ice formation as needles were severely injured after rapid freezing. In addition, Fuchigami and Nee (1987) attributed increased cold hardiness to higher overall stress resistance, which also reduces susceptibility of nursery stock to other stresses (e.g., drought).

Benefits observed at the end of first growing season (i.e., increased height, number of needle primordia) persisted throughout the second growing season. For fall fertilized seedlings, increased height attained at the end of the first growing season, along with additional needle primordia in buds, resulted in an average 22% increase in second year height compared to control seedlings. Along with increased height growth, fall fertilization also significantly increased RCD and the number of needle primordia in terminal buds (Tab. II). Shoot:root also increased in fall fertilized seedlings (4.5) compared to the control (4.0) after the second growing season as more N was mobilized to above ground tissues. This is possibly due to greater diameter growth observed in red pine seedlings as well as greater needle formation and retention. Similar increases in shoot:root were also observed in late-season fertilized black spruce seedlings (Boivin et al., 2004) and in *Eucalyptus globulus* Labill. (Fernandez et al., 2007).

To further augment height growth in red pine seedlings, we suggest two possible options: (i) application of fertilizer earlier in fall to take advantage of warmer soil temperatures and atmospheric conditions that are conducive to seedling growth; and, (ii) modifying the current summer fertilizer pattern to an exponential application regime, which should increase nutrient

uptake efficiency (Dumroese et al., 2005) and thereby minimize leaching losses in sandy soils with low nutrient retention. These factors could further promote height growth as Birge et al. (2006) observed that exponentially fertilized northern red oak (*Quercus rubra*) and white oak (*Q. alba*) under bareroot cultivation were 99–162% and 5–66% taller, respectively, than unfertilized seedlings.

5. CONCLUSIONS

Our data showed that applying 44 or 89 kg N ha^{-1} to 1 + 0 red pine seedlings during the fall of the first growing season improved height growth of 2 + 0 seedlings by approximately 20%. Fall fertilization resulted in buds with more needle primordia after the first growing season, and these primordia contributed to increased height growth during the second growing season. The effect of fall fertilization persisted through the second year of growth, with the 44 and 89 kg N ha^{-1} treatments again yielding taller seedlings containing buds with more needle primordia. Fall fertilization helped facilitate reaching target specifications for 2 + 0 red pine seedlings without reducing cold hardiness. Because red pine exhibits relatively low genetic variability and heterogeneity in morphological characteristics, and occurs natively in a narrow north-south geographic range, results of the present study may have applicability to a wide range of red pine populations.

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