Effects of Fall Fertilization on Morphology and Cold Hardiness of Red Pine (*Pinus resinosa* Ait.) Seedlings

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ABSTRACT
Red pine (*Pinus resinosa* Ait.) seedlings were topdress-fertilized with granular ammonium nitrate (NH_4NO_3) at the rate of 0, 11, 22, 44, or 89 kg/ha (0, 10, 20, 40, or 80 lb N/ac) during fall of 2005 in Badoura State Forest Nursery, Akeley, Minnesota. Seedlings received either a single (September 16) or double (September 16 and 23) application of fall fertilizer. Seedling morphology and cold hardiness were evaluated in November of 2005

(1+0s) and 2006 (2+0s). Seedling morphological attributes were the same regardless of application method (single versus double). Seedling height and number of needle primordia increased significantly with increased fertilizer rate at the end of both growing seasons. In general, cold hardiness (measured by freeze-induced electrolyte leakage [FIEL] test) increased at the end of the 1+0 season in seedlings that received fall fertilization as either a single or double application. At the end of the 2+0 season, however, cold hardiness decreased (7% to 30%) with increased fertilizer rate in seedlings that received a single application of fall fertilization, but increased (15% to 50%) with fertilizer rate in seedlings that received double applications compared to controls. We are following these seedlings after outplanting to verify potential benefits of fall fertilization on seedling field performance.

KEYWORDS
electrolyte leakage, height, nitrogen fertilization, needle primordiaseed collection, seed testing, conifers, hardwoods

Introduction

Proper and regular fertilization is an integral component during nursery culture for the production of high quality seedlings for afforestation and reforestation programs. Fertilization during nursery culture can enhance plant growth, nutrient storage reserves, and resistance to stresses and diseases (Landis 1985; Rook 1991; van den Driessche 1991). In addition to conventional spring and summer fertilization, fall fertilization has been successfully used to improve overall seedling quality in loblolly pine (*Pinus taeda*) (Sung and others 1997; VanderScaaf and McNabb 2004), slash pine (*P. elliottii*) (Irwin and others 1998), and Douglas-fir (*Pseudotsuga menziesii*) (Margolis and Waring 1986; van den Driessche 1985, 1988). Fall fertilization allows roots to exploit nutrients for subsequent use while the terminal buds become dormant. Application of fall fertilization has significantly increased foliar nitrogen (N) levels (Simpson 1985; van den Driessche 1985, 1988). Additionally, fall fertilization resulted in significant increases in root growth potential (Simpson 1985; van den Driessche 1988), earlier bud break (Thompson 1983; van den Driessche 1985; Margolis and Waring 1986), and greater survival and growth rates than conventionally fertilized seedlings (Anderson and Gessel 1966; van den Driessche 1988).

Seedling cold hardiness in response to N fertilization in general, and fall fertilization in particular, showed contrasting results. For example, an excess of N decreased frost hardiness in young and adult Scots pines (*Pinus sylvestris*) and in adult Norway spruce (*Picea abies*) (Aronsson 1980; Soikkeli and Karenlampi 1984). In contrast, N addition enhanced cold hardiness in red spruce (*Picea rubens*) (DeHayes and others 1989). Additionally, N applied 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*) seedlings showed enhanced cold hardiness with increasing N concentrations (Gleason and others 1990). Cold hardiness was unaffected by 3 different rates of N and P application in Douglas-fir and western redcedar (*Thuja plicata*) seedlings (Hawkins and others 1995). Therefore, it appears that the cold hardiness responses to fall fertilization are species specific, and amount of fertilizer might have played an important role because too much application of N may delay onset of dormancy.

At Badoura State Forest Nursery, 2+0 red pine (*Pinus resinosa* Ait.) seedlings grown under conventional nursery culture often do not attain target height growth in their second year. This study was undertaken primarily to augment their second year growth. Our study objectives were to evaluate if fall fertilization applied at the end of the 1+0 growing season would: 1) increase the number of needle primordia, resulting in greater shoot elongation and biomass in the following season; and 2) affect cold hardiness as measured by the freeze-induced electrolyte leakage (FIEL) test.

Study Procedure

Plant Material and Fertilizer Treatments

Seeds of red pine were collected from north-central Minnesota during fall 2000, and subsequent seedlings were grown in Badoura State Forest Nursery (46°56'N, 94°43'W) in Akeley, Minnesota. Seeds were sown in October 2004, allowed to stratify naturally, and germinated in May 2005. The nursery bed had sandy loam soil, which was altered by incorporating peat to increase organic matter content and moisture retention. The soil pH was 5.5, with a buffer index of 6.5, and 3% organic matter content. Soil tests conducted in September 2005 showed a nitrate NO_3^-(N) ppm of 0.9, a Bray 1 P ppm of 30, and potassium (K) ppm of 74.

The nursery beds were watered 2 to 3 times every week (May through August) in 2005 and 1 to 2 times every week (May through September) in 2006. Fertilizer application in 2005 (the 1+0 year) was as following: 6 topdress applications of granular ammonium nitrate (NH_4NO_3; 34N:0P_2O_5:0K_2O) at 33 kg N/ha (30 lb N/ac) on June 27, July 5, 11, 18, 25, and August 1; 3 foliar applications of liquid fertilizer (20N:20P_2O_5:20K_2O) at 5.5 kg N/ha (5 lb N/ac) on June 20, 27, and July 5; and 3 foliar applications of liquid fertilizer (20N:20P_2O_5:20K_2O) at 9 kg N/ha (8 lb N/ac) on July 20, 28, and August 12. For the experiment, we topdressed 2 beds with granular NH_4NO_3 (34N:0P_2O_5:0K_2O) at 5 levels of N: 0 (control), 11, 22, 44, and 89 kg/ha (0, 10, 20, 40, and 80 lb/ac). These applications were applied systematically down each bed; each rate was replicated 4 times within each bed. One bed received all of the fertilizer on September 16 (single application). The other bed received one application of fall fertilizer (same dose as applied to the first bed) on September 16 and a second dose (double application) on September 23. The fertilizer application schedule for summer 2006 (the 2+0 year) was as following: 1 topdress application of granular NH_4NO_3 (34N:0P_2O_5:0K_2O) at 67 kg N/ha (60 lb N/ac) on June 5; and 5 topdress applications of granular fertilizer (10N:10P_2O_5:10K_2O) at 30.8 kg N/ha (28 lb N/ac) on June 26, July 10, 17, 24, and 31. Weeds were controlled with Fusilade® and hand-weeding.

Seedlings were randomly hand-dug from the nursery beds during the first week of November 2005 and 2006 and shipped to Purdue University. Seedling morphological attributes (height [root collar to tip of terminal bud]; root collar diameter; shoot, root, and total plant dry weight after oven drying for 72 hours at 68 °C [154 °F]; needle primordia [described below]); and a physiological attribute (cold hardiness [described below]) were measured.

Needle Primordia

Number of needle primordia was determined following Tampleton and others (1993). The shoot (at least 10 cm [4 in]) was excised from the seedling and needles were carefully removed. The excised shoot was observed at a magnification of about 10X using a dissecting microscope. Bud scales were carefully removed to expose the needle primordia by making an incision using a hypodermic needle at a depth of 0.5 mm through the bark at the base of the lower-most bud scales. The tip of the hypodermic needle was used to cut around the circumference of the shoot, which loosened the cap of bud scales. Once the bud scales were removed, the embryonic shoot was viewed under the bright-field microscope, and the needle primordia were counted using the average number in a row multiplied by the number of columns.

Cold Hardiness

We used the freeze-induced electrolyte leakage (FIEL) test (Burr and others 1990) to determine seedling cold hardiness. Needles (randomly from the crown) were carefully detached from seedlings and rinsed with distilled water to remove ions from the surface. For each fertilizer treatment and replication, a sufficient number of needles from 5 seedlings were obtained and cut into 1-cm (0.4-in) segments. From each group, 7 segments were placed into 7 separate vials (each vial for corresponding test temperatures; that is, 2 [control], -5, -10, -20, -25, -30, and -40 °C [36, 23, 14, -4, -13, -22, and -40 °F]). Each vial contained 1 ml of deionized water. To avoid excessive super-cooling during freezing, only 1 ml of deionized water was added to the vial prior to the freezing tests.

The vials were placed in a programmable freezer for approximately 1.5 hours at 2 °C (36 °F), after which the control treatment vials were removed. The temperature was then decreased at a rate of approximately -5 °C/hour (-9 °F/hour). Upon reaching each successive test temperature, the temperature was held for 30 minutes before decreasing again. Once all the vials were removed and thawed, which was usually done on the next day, 9 ml of deionized water were added to each vial to aid in measurement of electrical conductivity. After the initial measurements were taken, the vials and their contents were autoclaved at 121 °C (250 °F) for 20 minutes. After cooling overnight at room temperature, the second measurements were taken, which represent total electrolytes. Electrolyte leakage is expressed as percentage of the total electrolytes.

Data Analysis

Seedling height, needle primordia, and cold hardiness data underwent analysis of variance using SAS (SAS Institute Inc, Cary, North Carolina), and an alpha level of 0.05.

Results and Discussion

We found no significant effects between single or double application of fall fertilization on any seedling morphological attributes, so we will discuss those results based on the total amount of N applied. Fall fertilization had a significant effect on seedling height after the 1+0 and 2+0 growing seasons (Table 1). Seedlings that were fall-fertilized with 44 and 89 kg/ha (40 and 80 lb N/ac) exhibited 8% and 21% more height growth compared to the control (0 kg N/ha) at the end of the 1+0 season, and 23% and 20% more height growth after the 2+0 year. Irwin and others (1998) found no significant differences in heights of slash pine seedlings fall-fertilized with zero, low (57 kg N/ha [50 lb N/ac), or high (171 kg N/ha delivered in 3 applications of 57 kg/ha [3X at 50 lb N/ac]) rates. Red pine seedlings reached target height due to the extra height growth attained by providing the highest fertilizer rate (89 kg/ha [80 lb/ac]). All seedlings were subsequently sold (VanSickle 2006). The relative increment in height with increased fertilizer application suggests that even in the month of October, when ground temperature was declining and low, red pine seedlings growing in nursery beds were physiologically active (VanderSchaaf and McNabb 2004). All other morphological attributes were similar across fertilizer treatments (data not shown).

Fall fertilization also had a significant effect on numbers of needle primordia after the 1+0 and 2+0 growing seasons (Table 1). In each year, more needle primordia were formed as fertilizer rate increased, resulting in longer buds (Figure 1). This is possibly due to the continued assimilation and partition of carbon (VanderSchaaf and McNabb 2004). Seedlings that were fall-fertilized with 44 and 89 kg N/ha (40 and 80 lb N/ac) had 41% and 76% more primordia compared to the control (0 kg N/ha) at the end of the 1+0 season, and 29% and 73% more primordia after the 2+0 year.

Fall fertilization rates significantly affected cold hardiness. At the end of the 1+0 season, cold hardiness (determined by FIEL; lower EL values indicate enhanced cold hardiness; all values presented represent those collected at -40 °C [-40 °F]) was similar between seedlings that received either a single or double application of fall fertilizer. However, fertilized seedlings were more cold hardy than control seedlings (Table 1). At the end of the 2+0 season, however, cold hardiness decreased with increased fertilizer rate in seedlings that received the single application, but increased with increased fertilizer rate in seedlings that received the double application.

Although Timmis (1974) found that Douglas-fir seedlings that received more fertilizer (N concentration was 1.6%) were much more cold hardy (-30 °C [-22 °F] versus -13 °C [9 °F] than those

Table 1. Heights, numbers of needle primordia, and electrolyte leakage percentages of fall-fertilized red pine seedlings.

		Fertilizer rate in kg N/ha (lb N/ac)				
		0	**11 (10)**	**22 (20)**	**44 (40)**	**89 (80)**
Height[z]	1+0	6.2 (2.4)[y]	6.0 (2.4)	5.9 (2.4)	6.7 (2.6)	7.5 (3.0)
	2+0	13.4 (5.3)	14.2 (5.6)	14.9 (5.9)	16.4 (6.4)	16.0 (6.3)
Primordia[x]	1+0	17	19	18	24	30
	2+0	96	92	114	124	166
FIEL—single dose	1+0	31[w]	27	24	21	21
FIEL—double dose	1+0	28	20	19	22	19
FIEL—single dose	2+0	27	29	35	39	24
FIEL—double dose	2+0	39	26	35	30	26

[z] Average heights for single and double dose seedlings combined.
[y] cm (in)
[x] Average number for single and double dose seedlings combined.
[w] The percentage leakage. Lower values indicate higher levels of cold hardiness.

receiving less fertilizer (N concentration was 0.8%), Birchler and others (2001) found that fall fertilization did not affect cold hardiness in Douglas-fir seedlings. While single or double application of fall fertilizer did not significantly affect morphological parameters, it appeared that double application significantly increased cold hardiness in 2+0 seedlings. No visible winter damage, however, was observed on control 2+0 seedlings. Therefore, from a grower's perspective, a single application of fall fertilizer would probably suffice to ensure the extra, desired height growth.

Our fall fertilizer rates were relatively low considering the rates Birchler and others (2001) and Irwin and others (1998) have used. Our low rates, coupled with rapidly declining day length and the short duration of time before the ground froze in northern Minnesota, may be why we observed only a modest height increase in red pine seedlings in response to fall fertilization. We feel that 2 possible options may further augment height growth in red pine seedlings. First, the current summer fertilizer application pattern can be modified to an exponential application regime, as demonstrated by Birge and others (2006) with bareroot red and white oak seedlings. Exponential application of fertilizer would increase uptake efficiency of the seedlings (Dumroese and others 2005) and minimize leaching losses because sandy soil has low retention of nutrients. Second, application of significantly more fall fertilizer than used in our current study may provide additional benefit.

Conclusions

We were able to increase the heights of 2+0 red pine approximately 20% by applying 44 or 89 kg N/ha (40 or 80 lb N/ac) during the fall of the 1+0 season. Although fall fertilization did not affect some seedling morphological attributes, increasing amounts of fertilizer yielded longer buds with more needle primordia. More needle primordia after the 1+0 season may be the cause for increased height growth during the second season. The effect of fall fertilization continued through the second year of growth, with the 44 or 89 kg N/ha (40 and 80 lb N/ac) treatments yielding longer buds and more needle primordia at the end of the 2+0 season as well. The extra growth provided by fall fertilization helped 2+0 red pine at Badoura State Forest Nursery better meet target specifications without reducing cold hardiness. We have outplanted these seedlings and will evaluate them to see if the effect carries over to field performance.

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Figure 1. Red pine bud size increases with fall fertilizer rate, indicating potential for shoot growth the following season. Buds represent (from left to right) 0 and 89 kg N/ha (0 and 80 lb N/ac) fertilizer treatments, respectively. Buds shown are from 2+0 seedlings.

References

Anderson HW, Gessel SP. 1966. Effects of nursery fertilization on outplanted Douglas-fir. Journal of Forestry 64:109-112.

Aronsson A. 1980. Frost hardiness in Scots pine (*Pinus sylvestris* L.). II. Hardiness during winter and spring in young trees of different mineral nutrient status. Studia Forestalia Suecica 155:1-27.

Birchler TD, Rose R, Haase DL. 2001. Fall fertilization with N and K: Effects on Douglas-fir seedling quality and performance. Western Journal of Applied Forestry. 16(2):71-79.

Birge Z, Salifu KF, Jacobs DF. 2006. Modified exponential nitrogen loading to promote morphological quality and nutrient storage of bareroot-cultured *Quercus rubra* and *Q. alba* seedlings. Scandinavian Journal of Forest Research 21:306-316.

Burr KE, Tinus RW, Wallner SJ, King RM. 1990. Comparison of three cold hardiness tests for conifer seedlings. Tree Physiology 6:351-369.

DeHayes DH, Ingle MA, Waite CE. 1989. Nitrogen fertilization enhances cold tolerance of red spruce seedlings. Canadian Journal of Forest Research 19:1037-1043.

Dumroese RK, Page-Dumroese DS, Salifu KF, Jacobs DF. 2005. Exponential fertilization of *Pinus monticola* seedlings: nutrient uptake efficiency, leaching fractions, and early outplanting performance. Canadian Journal of Forest Research 35:2961-2967.

Gleason JF, Duryea ML, Rose R, Atkinson M. 1990. Nursery and field fertilization of 2+0 ponderosa pine seedlings: the effect on morphology, physiology, and field performance. Canadian Journal of Forest Research 20:1766-1772.

Hawkins BJ, Davradou M, Pier D, Shortt R. 1995. Frost hardiness and winter photosynthesis of *Thuja plicata* and *Pseudotsuga menziesii* seedlings grown at three rates of nitrogen and phosphorus supply. Canadian Journal of Forest Research 25:18-28.

Irwin KM, Duryea ML, Stone EL. 1998. Fall-applied nitrogen improves performance of 1+0 slash pine nursery seedlings after outplanting. Southern Journal of Applied Forestry 22:111-116.

Landis TD. 1985. Mineral nutrition as an index of seedling quality. In: Duryea ML, editor. Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Corvallis (OR): Forest Research Laboratory, Oregon State University. p 29-48.

Margolis HA, Waring RH. 1986. Carbon and nitrogen allocation of Douglas-fir seedlings fertilized with nitrogen in autumn. I. Overwinter metabolism. Canadian Journal of Forest Research 16:897-902.

Rook DA. 1991. Seedling development and physiology in relation to mineral nutrition. In: van den Driessche R, editor. Mineral nutrition of conifer seedlings. Boca Raton (FL): CRC Press. p 85-111.

Simpson DG. 1985. Late season fertilizer effects on 2+0 bareroot seedlings [unpublished data]. Vancouver (BC): British Columbia Ministry of Forests.

Soikkeli S, Karenlampi L. 1984. The effects of nitrogen fertilization on the ultrastructure of mesophyll cells of conifer needles in northern Finland. European Journal of Forest Pathology 14:129-136.

Sung SS, Black CC, Kormanik TL, Zarnoch SJ, Kormanik PP, Counce PA. 1997. Fall nitrogen fertilization and the biology of *Pinus taeda* seedling development. Canadian Journal of Forest Research 27:1406-1412.

Tampleton CWG, Odlum KD, Colombo SJ. 1993. How to identify bud initiation and count needle primordia in first-year spruce seedlings. Forestry Chronicle 69:431-437.

Thompson B. 1983. Why fall fertilize? In: Proceedings of the Western Nurseryman s Conference; 1982 August 10-12; Medford, OR. Ashland (OR): Southern Oregon Regional Services Institute, Southern Oregon State College. p 85-91.

Timmis R. 1974. Effect of nutrient status on growth, bud set, and hardiness in Douglas-fir seedlings. In: Tinus RW, Stein WI, Balmer WE, editors. Proceedings of the North American containerized forest tree seedling symposium. Great Plains Agricultural Council Publication No 68. p 187-193.

van den Driessche R. 1985. Late season fertilization, mineral nutrient reserves, and retranslocation in planted Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings. Forest Science 31:485-496.

van den Driessche R. 1988. Nursery growth of conifer seedlings using fertilizers of different solubilities and application time, and their forest growth. Canadian Journal of Forest Research 18:172-180.

van den Driessche R. 1991. Effects of nutrients on stock performance in the forest. In: van den Driessche R, editor. Mineral nutrition of conifer seedlings. Boca Raton (FL): CRC Press. p 229-260.

VanderScaaf C, McNabb K. 2004. Winter nitrogen fertilization of loblolly pine seedlings. Plant and Soil 265:295-299.

VanSickle C. 2006. Personal communication. Akeley (MN): Badoura State Forest Nursery. Nursery Manager.