

Effect of Fall-applied Nitrogen on Growth, Nitrogen Storage, and Frost Hardiness of Bareroot *Larix olgensis* Seedlings

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Nursery response of evergreen trees to fall fertilization has been studied widely, but little attention has been given to deciduous trees. Bareroot Olga Bay larch (*Larix olgensis* Henry) seedlings were fertilized in the nursery with urea at four rates (0, 30, 60, 90 kg N ha⁻¹), with half of each rate applied on two dates (September 16 and October 1, 2009). The seedlings were excavated for evaluation on October 15. In the unfertilized (control) treatment, root and shoot dry mass increased by 100% and 57% respectively, while N concentration in the roots and shoots increased by 43% and 40% during the 30 day period. This indicated that substantial biomass growth during this period did not lead to internal nutrient dilution. Root dry mass increased when fall fertilization rates were ≥ 60 kg N ha⁻¹. Fall fertilization increased N concentrations in root tissue by 48–73%. Compared with the control, shoot tissues of fall fertilized seedlings had slightly higher N concentration and content and significantly higher frost hardiness.

Keywords deciduous trees, fall fertilization, frost hardiness, Olga Bay larch

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1 Introduction

Until newly planted seedlings grow roots into the surrounding soil, they are mainly dependent on their internal nutrient reserves (van den Driessche 1985, Rikala et al. 2004). Consequently, proper seedling nutrition at the nursery is a critical cultural practice for subsequent field performance (Birchler et al. 2001). If nutrient uptake is limited in the nursery without supplemental fertilization, biomass accumulation during hardening may lead to an internal nutrient dilution (Boivin et al. 2004); nutrient dilution can be averted by applying fertilizer in the fall (Boivin et al. 2002, 2004, Islam et al. 2009).

Thus, fall fertilization has been widely used worldwide as a cultural practice for several evergreen species, such as *Picea abies* (L.) Karst. (Rikala et al. 2004, Heiskanen et al. 2009), *Picea mariana* (Mill.) B.S.P. (Boivin et al. 2002, 2004), *Pinus taeda* L. (South and Donald 2002, Vander Schaaf and McNabb 2004), *Pinus elliotii* Engelm. (Duryea 1990, Irwin et al. 1998), *Pinus resinosa* Ait. (Islam et al. 2009), *Pinus ponderosa* Dougl. ex Laws. (Gleason et al. 1990), *Pseudotsuga menziesii* (Mirb.) Franco (van den Driessche 1985, Birchler et al. 2001), *Eucalyptus globulus* Labill. (Fernández et al. 2007), and *Quercus ilex* L. (Oliet et al. 2011, Andivia et al. 2011, 2012). Fall fertilization at the nursery can build nutrient reserves in seedlings, especially in the foliage (van den Driessche 1985).

In contrast to evergreen trees, retranslocation of nitrogen (N) from leaves prior to abscission can contribute a larger proportion of the N within deciduous seedlings; this N can subsequently contribute to growth the next season (Aerts 1996). For example, *Larix occidentalis* Nutt. retranslocated 87% of its foliar elemental N (Gower et al. 1995). However, the effects of fall fertilization on seedling quality have not been explored for deciduous conifer species. Little information is available on whether the accepted fall fertilization theory for evergreen tree species is shared by deciduous conifers.

Fall fertilization may increase plant nutrient reserves, though the relationship between fall N fertilization and seedling frost hardiness is relatively unexplored, and results reported to date

contrast widely (Islam et al. 2009). The interaction of fertilization and frost hardiness has focused on evergreen tree species, such as *Pinus resinosa* (Islam et al. 2009), *Picea abies* (Fløistad and Kohmann 2004), *Picea mariana* (Bigras et al. 1996), *Pseudotsuga menziesii* (Birchler et al. 2001), and *Quercus ilex* (Andivia et al. 2011, 2012), with little attention paid to deciduous tree species. In most forest nurseries, frost hardiness is commonly evaluated using freeze-induced electrolyte leakage (FIEL) (Bigras and Colombo 2001) because, compared to the whole-plant freeze test, FIEL yields a more rapid, sensitive, and objective predictor of differences in tissue frost hardiness (Burr et al. 1990). Frost hardiness is typically evaluated on the terminal shoot tissues of deciduous species (McKay 1994) and the needles of evergreens (Aronsson 1980, McKay 1994, Islam et al. 2009). Although some research has examined fall fertilization and FIEL of evergreen needle foliage (Islam et al. 2009), the relationship between FIEL of shoot tissue of deciduous trees and fall fertilization remains unknown.

Olga Bay larch (*Larix olgensis* Henry) is one of the most important timber species planted in northeastern China. Compared with other softwoods, it is valued for its rapid early growth, dense cellular structure, and for its characteristic strength and resistance to rot. The predominant nursery stocktypes for this species are 1+0 and 1+1 bareroot seedlings. The 1+0 seedlings are sown and grown in outdoor seedbeds for 1 year at a density of 400–600 seedlings m⁻². The 1+1 seedlings are produced by transplanting 1+0s into another bed for a second year of growth at a lower growing density of 180–220 seedlings m⁻² (Lu 1989, Lian et al. 1992, Li 2009). N availability is one key factor limiting seedling development. Owing to the variation of standard nursery practice regimes and nursery soil characteristics, the amount of N applied to 1+0 seedlings can vary widely. In general, seedlings are top dressed with equal amounts of N at 3 to 5 regular intervals during July (or from July to early September) so that total N applied is 60–250 kg N ha⁻¹ (Li 2009, Ma 2010, Jiang et al. 2011).

Our study objectives were to investigate, for the deciduous conifer Olga Bay larch, if 1) growth decreased tissue N concentration in the fall and 2) if N additions in the fall increased seedling

growth, tissue N concentrations, and frost hardiness.

2 Materials and Methods

2.1 Plant Materials

Seedlings for this study were grown at the Jiangmifeng nursery near Jilin City, Jilin Province, China (126°48'E, 43°57'N, elevation 233 m), which has been producing Olga Bay larch seedlings since 1952. The area is characterized by a temperate continental monsoon climate. Mean annual air temperature is 6.4 °C, ranging from -14.1 °C in the coldest month (January) to 23.9 °C in the hottest month (July), with 130 frost-free days. Monthly average temperatures in September and October are 15.6 and 7.5 °C, respectively. Average annual precipitation is 645 mm, of which 63% occurs during the rainy season between June and August. The date of first frost varies from 4 to 17 October, with the date of first snow occurring between October 10 and November 17. In mid April, 2009, initial soil samples (0–15 cm) were collected and analyzed to characterize native fertility. The soil is 79% sand, 11% silt, and 10% clay (a sandy loam) with a pH of 5.1 and soil organic carbon of 0.8%. Average total N, available P, and available K were 770, 37, and 77 mg kg⁻¹ respectively. After soil sampling, 150 kg ha⁻¹ of P₂O₅ (65 kg ha⁻¹ of P) as triple superphosphate fertilizer was operationally broadcast on the experimental area and the nursery bed was cultivated to a depth of 15 cm.

Seeds, collected earlier from the Baishishan seed orchard (127°35'E, 43°28'N, elevation 520 m) and appropriate for use in this nursery (Tian et al. 1994), were operationally broadcast sown by one crew and covered with a thin layer of coarse silica grit on May 10, 2009. In mid June seedlings were thinned to a final density of 500 seedlings m⁻². Weeds were controlled by hand. During seedling establishment it rarely rained and light irrigation, applied with a micro-sprinkler system, was applied once a day to prevent germinants from being injured by high surface temperatures (Hao 1975, Li 1979). Subsequently, seedlings were irrigated as needed and depending on the

frequency of the monsoon rain. From July to the beginning of September, a total of 150 kg ha⁻¹ of N was applied in 5 increments of 30 kg ha⁻¹ at 15 day intervals. Urea (46-0-0) was weighed and dissolved in water and the solution was applied to the seedbeds manually with a sprayer. After a 10 minute interval, seedlings were rinsed with pure water to avoid potential damage from fertilizer burn. Beginning in September, the amount and frequency of irrigation were reduced. Bud set is induced by ambient cool temperatures, shortening of day lengths in autumn, and reduced irrigation. For most seedlings, terminal buds developed during the first two weeks of September. Needles turned yellow during the last week of September and began abscising from the basipetal portion of the seedlings during the first week of October.

2.2 Experimental Design

A randomized complete block design was used to test 4 fall fertilization rates of 0 (control), 30, 60, and 90 kg N ha⁻¹; the design was replicated in three blocks, with each block 12 m long and 1.2 m wide. Half of the N (15, 30, and 45 kg ha⁻¹) was applied using a sprayer on September 16, with the remaining half applied on October 1. Including the operationally applied N added before the fall application, the total amounts of N applied during the entire growing season for the four treatments were 150, 180, 210, and 240 kg ha⁻¹.

2.3 Sampling and Measurements

Forty-five seedlings (15 seedlings per replicate of 0 kg N ha⁻¹) were randomly excavated from the nursery beds on September 15 to determine morphological attributes and mineral nutrient status before fall fertilization. Similarly, 180 seedlings (15 seedlings per treatment replicate) were excavated on October 16 to determine the effect of fall fertilization on seedling morphology. An additional 480 seedlings (40 seedlings per treatment replication) were sampled to measure frost hardiness by FIEL (5 seedlings per treatment replication for the 8 induced test temperatures). In order to minimize edge effects on growth, excavated seedlings were selected randomly in an

area 0.2 m wide and 0.6 m long from the center of the nursery beds. Once excavated, seedlings were rinsed with fresh water 3–4 times followed by a 5 min soak in distilled water. Seedlings were packed in plastic bags, placed inside shipping boxes alongside cold refrigerant gel packs to maintain their physiological state, and shipped overnight to Beijing Forestry University.

For each seedling, height was determined from the ground line to the terminal bud, and root collar diameter (hereafter diameter) was measured with a caliper at ground level. Relative increment of height and diameter was calculated by the following formula:

$$\text{Relative increment (\%)} = \frac{\text{size 2} - \text{size 1}}{\text{size 1}} \times 100$$

where size 1 and size 2 were the height or diameter prior to fall fertilization, and after fall fertilization, respectively.

For each treatment replicate, seedlings were separated into shoots and roots, composited by tissue type, and oven-dried at 65°C for 48 h to determine dry mass. After determining biomass, samples were ground and sieved through a 0.25 mm screen for N nutrient analysis. Plant material was wet-digested in a block digester using the $\text{KMnO}_4\text{-Fe-H}_2\text{SO}_4$ method modified to recover NO_3 (Bremner and Mulvaney 1982). A standard Kjeldahl digestion with water distillation was used to measure total N by a distillation unit (UDK-152, Velp Scientifica, USA). Shoot and root N contents were calculated by multiplying respective N concentrations with dry mass.

Frost hardness of Olga Bay larch was determined with a FIEL test of shoots. For each treatment replicate, 40 seedlings were randomly separated into 8 groups of 5 seedlings. Two 0.5-cm segments were cut from the top of each leader shoot within each group, resulting in 10 segments. Each set of 10 segments was placed into one tube, and each tube corresponded to a different test temperature: 2 (control), –5, –10, –15, –20, –30, –40, and –50°C. A total of 96 tubes were obtained (4 fall fertilization treatments $\times$ 8 test temperatures $\times$ 3 replicates).

Each tube contained 1 ml of deionized water to prevent desiccation and to provide a nucleating agent to propagate ice into tissues (Bigras and

Colombo 2001). Blanks (tubes with deionized water only) were included to account for this source of ions. The tubes were placed in a programmable freezer (BYK-98, Shanghai Xiamei Biochemical Tech Development Ltd, Inc. China) for 1.5 h at 2°C, after which the control treatment tubes were removed. The temperature was then reduced at a rate of 5°C h^{–1}. Upon reaching each successive test temperature, the temperature was held for 30 min before decreasing again (Islam et al. 2009). Samples frozen from –5 to –20°C were thawed in refrigerators at 4°C for 24 h. Samples frozen from –30 to –50°C were first brought to –20°C and then transferred to 4°C to complete their thaw (Sutinen et al. 1992). All samples were kept in darkness during freezing and thawing (Bigras and Colombo 2001).

After thawing, 9 ml of deionized water was added to each tube to aid in measurement of electrical conductivity (EC_1) and samples were shaken for 24 h at 4°C to promote steady diffusion. Initial conductivity (EC_1) was read in $\mu\text{mho cm}^{-1}$ with a meter (DDS-307A, Shanghai Precision & Scientific Instrument Co., Ltd, China). Tissue samples were then exposed to a 100°C water bath for 20 min to release remaining electrolytes. Final conductivity (EC_2) was determined the same way as EC_1 . For each sample, FIEL was calculated from EC_1 and EC_2 , corrected for blanks as follows:

$$\text{FIEL(\%)} = \frac{(EC_1 - B_1)}{(EC_2 - B_2)} \times 100$$

where B_1 and B_2 were the blanks measured before and after the water bath.

2.4 Data Analysis

Means and standard errors of the variables were calculated for the treatment groups using the SPSS Win 16.0 program (Chicago, USA). After fall fertilization, morphological and nutritional data were evaluated using a univariate analysis in general linear model (GLM) by a linear mixed model. Treatment means computed from individual seedlings for each replicate were used for height and diameter analysis. For the biomass and nutrient calculations, data were analyzed from a

composite sample for each treatment from a replicate. For each analysis, when the fixed effect of fertilization treatment was significant ($\alpha = 0.05$), a Tukey's multiple comparison test was conducted to test the significant differences among treatments.

To test the effects of 8 test temperature levels and 4 fertilizer rates, and their interactions on FIEL, we used the general linear model (Eq. 1):

$$Y_{ijk} = \mu + S_i + T_j + ST_{ij} + R_k + e_{ijk} \quad (1)$$

where Y_{ijk} is the mean observation in fall fertilization treatment i , temperature j , replicate k ; μ is the total mean; S_i is the fixed effect of fall fertilization rate i ($i = 1, 2, 3, 4$); T_j is the fixed effect of freezing temperature j ($j = 1, 2, 3 \dots 8$); ST_{ij} is the effect of fall fertilization S_i and temperature T_j ; R_k is the random effect of the replicate k ($k = 1, 2, 3$), and e_{ijk} is the random error. Our data met the assumptions of normality and homogeneity of variance. When ANOVA indicated significant differences ($P < 0.05$) among treatments, Tukey's test was used to identify significant differences among fall fertilization treatments under each test temperature level ($\alpha = 0.05$).

3 Results

3.1 Seedling Growth

In one month, from September 15 to October 16, the height and diameter increment for unfertilized seedlings was 7% and 3%, respectively (Fig. 1). For height, only seedlings fall fertilized with 90 kg N ha⁻¹ were significantly taller than the unfertilized seedlings (Fig. 1), whereas for diameter, all fall fertilized seedlings were significantly greater than the control. Fall fertilization of 30, 60, and 90 kg N ha⁻¹ increased diameter 11%, 16%, and 19% compared with the control.

More biomass was allocated to roots than to shoots during the September 15 to October 16 test period (Fig. 2); root biomass doubled while shoot biomass increased by only 57%. Fall fertilization with ≥ 60 kg N ha⁻¹ significantly increased root biomass, whereas no significant differences were observed in shoot biomass (Table 1).

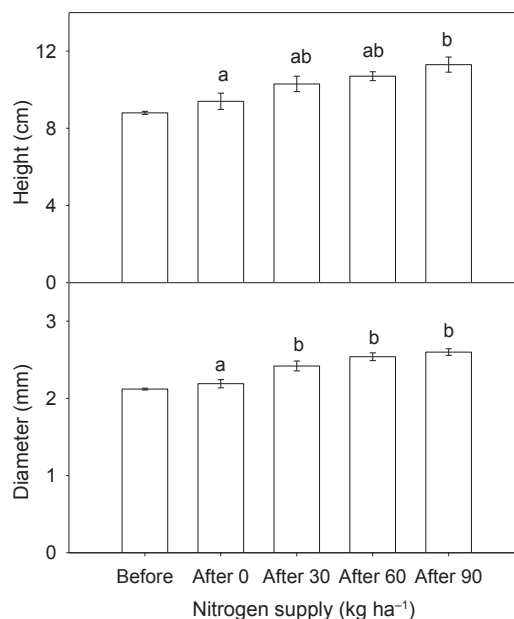


Fig. 1. Height and diameter before and after fall fertilization in *Larix olgensis* seedlings at the end of first growing season. Each bar represents mean $\pm$ SE ($n = 3$). Bars with different letters are significantly different according to Tukey's HSD test ($\alpha = 0.05$).

3.2 Nutrient Status

N concentrations in root and shoot tissues of unfertilized seedlings (Fig. 2) increased by 43% and 40%, respectively, from September 15 to October 16. At the end of the nursery assay, N concentration in the root tissue was significantly greater in the 60 kg N ha⁻¹ treatment than in control, but shoot N concentrations were unaffected by fall fertilization (Table 1 and Fig. 2).

As shown in Fig. 3, N content in root and shoot tissues of the unfertilized seedlings increased by 198% and 108% from September 15 to October 16. Similar to the results for N concentrations (Fig. 2), fall fertilization yielded a significant effect on the N content of roots but not of shoots (Table 1).

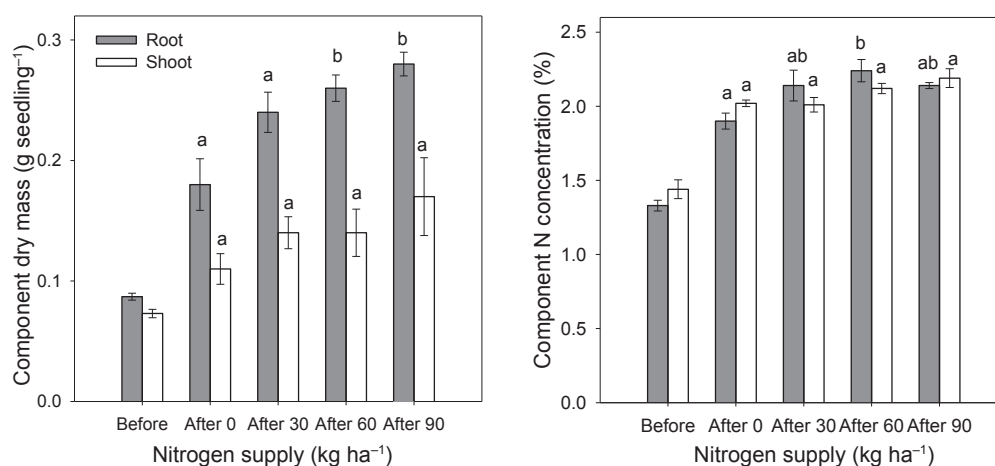


Fig. 2. Component dry mass (left) and N concentration (right) before and after fall fertilization (mean±SE, $n = 3$) of *Larix olgensis* seedlings at the end of the first growing season. For each component, bars with different letters are significantly different according to Tukey's HSD test ($\alpha = 0.05$).

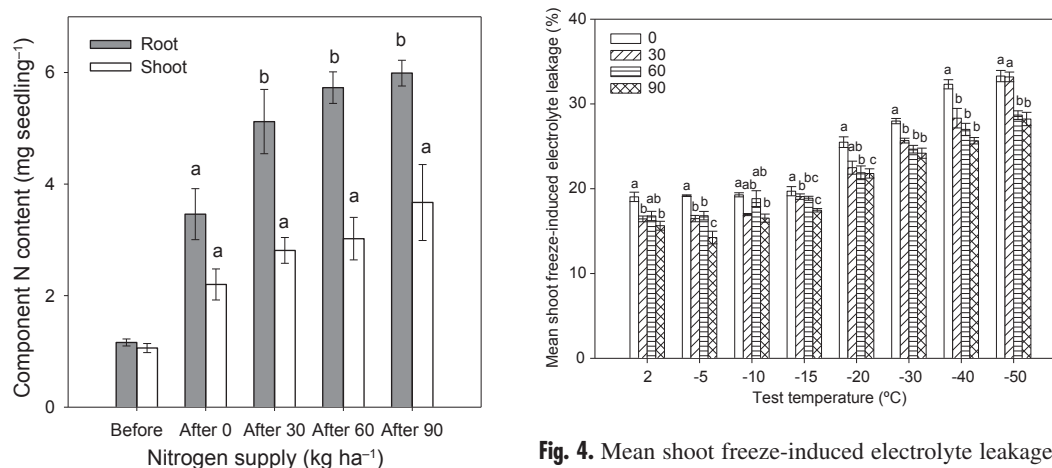


Fig. 3. Component N content before and after fall fertilization (mean±SE) of *Larix olgensis* seedlings at the end of the first growing season. For each component, bars with different letters are significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Fig. 4. Mean shoot freeze-induced electrolyte leakage (FIEL) values for each test temperature in *Larix olgensis* seedlings after the first growing season. Seedlings were fall fertilized with nitrate at rates of 0, 30, 60 and 90 kg N ha⁻¹. Each bar represents mean ($n = 3$) ±SE. Within each test temperature, bars with different letters are significantly different according to Tukey's HSD test ($\alpha = 0.05$).

3.3 Frost Hardiness

Frost hardiness, seedling FIEL, and fertilizer × temperature interaction were all affected significantly by fall fertilization (Table 2). The FIEL values generally increased as test temperatures

decreased regardless of N rates (Fig. 4). Maximum and minimum values of FIEL were observed at a fall fertilizer N rate of 0 and 90 kg N ha⁻¹, respectively. Compared to the 0 kg N ha⁻¹ treatment, FIEL was significantly lower in the 90 kg N ha⁻¹ treatment at all test temperatures, in the

Table 1. ANOVA results for the effects of fall fertilization on the morphology and N status of *Larix olgensis* seedlings after the first growing season. d.f. – mean degrees of freedom; MS – mean square

Source	d.f.	Height MS	P	Diameter MS	P	Shoot dry mass MS	P	Root dry mass MS	P	Shoot concentration MS	P	Root concentration MS	P	Shoot content MS	P	Root content MS	P
Fertilization	3	27.923	0.004	1.436	0.005	0.002	0.221	0.005	0.006	0.022	0.116	0.063	0.039	1.093	0.095	3.893	0.004
Replicate	2	7.726	0.083	0.674	0.034	0.003	0.133	0.001	0.107	0.002	0.779	0.022	0.237	1.249	0.083	1.210	0.064
Error	6	1.989		0.108		0.001		0.000		0.007		0.012		0.323		0.269	

Table 2. ANOVA results for the effects of fall fertilization and temperature on freeze-induced electrolyte leakage (FIEL) of the shoots of *Larix olgensis* seedlings after the first growing season. d.f. – mean degrees of freedom; MS – mean square

Source	d.f.	FIEL of the shoot MS	P
Fertilization	3	361.451	<0.001
Temperature	7	65.182	<0.001
Fertilization × Temperature	21	3.312	<0.001
Replicate	2	1.785	0.178
Error	62	1.004	

60 kg N ha⁻¹ treatment except at 2°C and -10°C, and in the 30 kg N ha⁻¹ treatment except at -10, -20, and -50°C. FIEL values ranged between 14–20% when the test temperature was warmer than -15°C, but once the test temperature was colder than -20°C, FIEL values increased sharply.

4 Discussion

The increases in seedling biomass we observed in our unfertilized control seedlings (root, 100%; shoot, 57%) were similar to those reported for *Picea mariana* seedlings (root, 104%; shoot, 42%) during late-season hardening (Boivin et al. 2004). Miller and Timmer (1997) found that *Picea mariana* seedlings are capable of gaining as much as 794% in root dry mass and 142% in shoot dry mass during hardening. Thus, substantial growth, particularly in the roots, occurs during hardening despite the reduction of temperature and shortening of day-length (Miller and Timmer 1997). Without additional nutrient supply in the late-season, however, concentration of N, P, and K declined in *Picea mariana* seedlings (Boivin et al. 2002, 2004). Hence, fall fertilization is considered necessary to avert nutrient dilution effects for most evergreen tree species (Duryea 1990, Irwin et al. 1998, Boivin et al. 2002, 2004, Rikala et al. 2004, Fernández et al. 2007, Islam et al. 2009, Heiskanen et al. 2009). However, in our Olga Bay larch seedlings, N concentrations in roots and shoots increased by more than 40% from September 15 to October 16 (c.f. Fig. 2);

nitrogen dilution because of growth did not occur.

For Olga Bay larch, lack of N dilution in roots and shoots may have been due to retranslocation of N from senescing foliage. As much as 87% of N was retranslocated from needle tissues for *L. occidentalis* (Gower et al. 1995) and *L. laricina* (Du Roi) K. Koch (Chapin and Kedrowski 1983). Trees with a low N status retranslocate a smaller proportion of leaf N prior to leaf abscission than do trees with a more favorable nutrient status (Chapin and Kedrowski 1983). Thus, the effect of fall fertilization on N recycling of seedlings should be experimentally determined.

Although fall fertilization failed to improve N concentration in shoot tissues (c.f. Fig. 2), it did enhance frost hardiness as measured by FIEL (c.f. Fig. 4). That is, minor increases in N concentration in shoot tissues (c.f. Fig. 2) could contribute to a significant increase in frost hardiness (c.f. Fig. 4). Many studies have demonstrated that N was the nutrient that determined frost tolerance (Rikala and Repo 1997, McKay and Morgan 2001, Fernández et al. 2007). The general consensus is that an optimum N concentration exists for hardening (Bigras et al. 1996), with values too low or too high impairing the frost hardening process. As an example, the optimal N concentration in the needles of *Picea abies* is considered to be about 2.0–2.5% N (Ingestad 1979), and freeze injury was observed when needle N concentration dropped to 1.79% (Fløistad and Kohmann 2004). In general, if deficiencies are present before application, fall fertilization can reestablish adequate mineral conditions, which could lead to an increase in frost hardiness (Bigras and Colombo 2001). In our study, shoot N concentration was 1.44% before fall fertilization and 2.02%, 2.01%, 2.12%, 2.19% at the end of the growing season for our rates of 0, 30, 60, 90 kg N ha⁻¹, respectively (c.f. Fig. 2). Therefore, considering only shoot frost hardiness (c.f. Fig. 4), it appears the optimal shoot N concentration for 1+0 Olga Bay larch seedlings is $\geq 2.19\%$. Although frost hardiness is more related to fall-applied N than phosphorus (P) and potassium (K) (Fernández et al. 2007, Rikala and Repo 1997), several studies have shown that the concentration ratio of other elements to N could account for changes in frost hardiness. For example, *Pseudotsuga menziesii* seedlings reached maximum frost

hardiness when the concentration ratio of K/N was 0.6 (Timmis 1974), and the type of fertilizer can also affect elemental uptake (Graciano et al. 2006). In this paper, seedlings were fall fertilized only with N, and tissue concentrations of elements other than N were not determined, so it remains unknown whether our frost hardiness values were affected by other critical elements.

5 Conclusions

Nutrient dilution caused by late-season growth did not occur for Olga Bay larch seedlings, rather, the concentration of N in root and shoot tissues of unfertilized control seedlings increased about 41% during the hardening period. Fall fertilization increased seedling quality in terms of height, diameter, root biomass, root N concentration and content. Minor increases in N concentration resulting from fall fertilization improved shoot frost hardiness. Our data suggest that applying 60 to 90 kg N ha⁻¹ to 1+0 Olga Bay larch seedlings become a standard nursery operation to improve seedling quality.

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