Effects of Foliar Urea Fertilization on Nitrogen Status of Containerized 2+0 Black Spruce Seedlings Produced in Forest Nurseries

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Abstract

A 7-day study of foliar fertilization was carried out after fall bud set of containerized 2+0 black spruce (Picea mariana [Mill.] B.S.P.) to assess if one application of urea (U), alone or with surfactant (US), can lead to a rapid increase of foliar nitrogen (N) concentration. Two washing treatments of seedling shoots (W: 15 sec washing [control], WS: W + 5 min soaking) were also performed to evaluate their efficiency to remove urea residues from the needle surface before foliar N concentration analysis. At day 0 (2 hours after application), fertilized seedlings already had significantly greater foliar N concentration than unfertilized seedlings (NF) and after 7 days, it had increased 7 and 12 percent for U and US seedlings, respectively. The addition of a surfactant did not significantly improve N status. Foliar N concentration of fertilized seedlings was not significantly affected by washing treatments. These results indicate that foliar urea fertilization after bud set is an effective tool for rapidly increasing foliar N concentration without affecting seedling shoot height.

Introduction

Of the 128 million containerized and bareroot forest seedlings that were produced in the 19 forest nurseries (13 privately owned and 6 government owned) in Québec (Canada) in 2013, 94 percent were grown in containers, one-half of which were black spruce (Picea mariana [Mill.] B.S.P.) (Arsenault, pers. comm.). These nurseries follow a nutritional approach developed in the 1980s (Langlois and Gagnon 1993) and applied operationally using PLANTEC software (Girard et al. 2001). Using this approach, containerized seedlings are fertilized weekly to satisfy their nutrient demands (nitrogen [N], phosphorous [P], and potassium [K]) for growth, while taking into account their phenological phases (active or dormant). Containerized conifer seedlings in Québec nurseries must not only meet morphological quality criteria (e.g., height, diameter, height/diameter), but also must have a minimum foliar N concentration of 1.6 percent for seedlings grown in cavities with volumes smaller than 200 cm³ (12 in³) and 1.8 percent for seedlings grown in cavities equal to or larger than 200 cm³ before delivery for outplanting (Veilleux et al. 2014). Québec forest nurseries assess whether seedlings have met the minimum foliar N concentration target after autumn bud formation and again before delivery for outplanting. Before this analysis, the Québec governmental laboratory washes the seedling shoots for 15 seconds to remove the fertilizer residues from the needle surface. To date, with the exception of our preliminary study (Gagnon 2011), no other study has evaluated the efficiency of this washing method to remove fertilizer residues.

During the period between fall bud set and the evaluation of foliar N concentration, foliar N fertilization could be a useful tool for increasing the foliar N concentration of containerized conifer seedlings to the desired level without affecting their shoot height growth. Foliar N fertilization of containerized conifer seedlings can also be used at any time during the growing season to complement soil fertilization and rapidly increase foliar N concentration, thus permitting nursery growers to attain target foliar N levels throughout the season. Foliar sprays, which are primarily used to correct micronutrient deficiencies, such as iron chlorosis, can also be used with N to provide a quick “green-up” before seedlings are shipped to the planting site (Landis et al. 1989). According to Dumroese (2003), foliar fertilization can be used to quickly recharge nutrient-depleted containerized seedlings or to add high doses of nutrients for luxury consumption (nutrient loading). Because foliar N fertilization is applied to the foliage rather than to the soil, it can also contribute to a reduction in the quantity of nutrients leached from container-grown seedlings and, consequently, the pollution of groundwater by nitrate.

Foliar N application has been largely used in agriculture and horticulture during the past 50 years (Handreck and Black 1984, Alexander and Schroeder 1987, Gooding and Davies 1992, Wojcik 2004). Only a few studies have been carried
out, however, with conifer seedlings grown in containers under forest nursery conditions: Monterey pine (Pinus radiata D. Don.) (Coker et al. 1987, Coker 1991), black spruce (Gagnon 2011), ponderosa pine (Pinus ponderosa Laws.), and Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) (Montville and Wenny 1990, Montville et al. 1996). This lack of research may be explained by the small absorptive surface of conifer needles relative to leaves of broadleaved plants and by the waxy cuticular surface of the needles, which slows nutrient absorption (Landis et al. 1989, Marschner 1995, Mengel and Kirkby 2001, Lamhamedi et al. 2003). In autumn, after bud formation, the wax load on the needle surface increases (Landis et al. 2010) and the cuticle becomes thicker.

Surfactants are often used with foliar spray solutions to ensure diffusion of nutrients across the cuticle, which, because of its hydrophobic nature, impedes the diffusion of hydrophylic ions (Mengel and Kirkby 2001). According to Landis et al. (1989), a surfactant is often used with foliar fertilization to ensure uniform distribution of the fertilizer solution over the needle surface. Indeed, by reducing the surface tension of water droplets, the surfactant permits a thin layer to adhere to the needle surface, thus improving nutrient absorption (Wittwer and Teubner 1959, Mengel and Kirkby 2001, Wojcik 2004).

Among the three N sources that can be used for foliar N fertilization (ammonium [NH₄⁺], nitrate [NO₃⁻], and urea [CO(NH₂)₂]), urea is the most readily absorbed by the leaves of most crops. Several studies showed that after its rapid absorption by leaves, urea is then rapidly metabolized and translocated by plants (Handreck and Black 1984, Alexander and Schroeder 1987, Gooding and Davies 1992, Wojcik 2004). Urea can also be applied at relatively high concentrations without damaging needles because of its low-phytotoxicity potential (Alexander and Schroeder 1987, Gooding and Davies 1992, Wojcik 2004). Urea is also considered to be the most suitable form of N for foliar applications because of its nonpolarity and its high solubility in water and oil (Wittwer et al. 1963, Yamada et al. 1965, Swietlik and Faust 1984). Being a neutral molecule, urea is absorbed more quickly by needles than either NH₄⁺ or NO₃⁻ because it rapidly diffuses through the waxy cuticle (Wittwer et al. 1963). Using these three N sources in a foliar fertilization study with containerized Pinus radiata seedlings, Coker et al. (1987) showed that urea was absorbed 10 times more rapidly than NO₃⁻ and three times faster than NH₄⁺. Given these advantages of urea for foliar fertilization, this N source was successfully tested in a preliminary study with containerized 2+0 black spruce seedlings (Gagnon 2011).

The objectives of this study were to evaluate (1) the effects of a single foliar application of urea after seedling budset on N concentration of containerized 2+0 black spruce seedling needles, (2) the addition of a surfactant to the urea solution on the efficiency of urea foliar fertilization, and (3) the efficiency of washing treatments of seedling shoots to remove urea residues from the needle surface for subsequent accurate determination of foliar N concentration after a foliar urea fertilization.

**Materials and Methods**

**Seedlings**

Large 2+0 black spruce seedlings (seedlot: EPN-V2-PLU-1-0) grown in 25-310 containers (25 cavities with a volume 310 cm³ [19 in³] each, IPL 25-310, Saint-Damien, Québec, Canada) at Normandin nursery were used for this experiment. This governmental forest nursery (ministère des Forêts, de la Faune et des Parcs, MFFP du Québec) is located in the Saguenay-Lac St. Jean region of Québec (48°48′48″ N, 72°45′00″ W), Canada.

In Quebec, seedlings produced in cavity volumes more than 300 cm³ [18 in³] are deemed large seedlings and are grown under forest nursery conditions for 2 years. During their first growing season, 1+0 seedlings are produced under white, unheated polyethylene tunnels (figure 1), the covers of which are removed in October, at the end of the season. Thereafter, seedlings are moved outside the tunnels and placed directly on the ground until spring (April). The thick snow cover and lack of air circulation under the containers protect the seedlings against frost damage during the winter. During the second growing season, 2+0 seedlings are cultivated outdoors (figure 1). All 2+0 container seedlings are irrigated by sprinklers arranged in a square pattern and fertilized using a tractor-mounted boom sprayer.

**Figure 1.** In Québec forest tree nurseries, containerized seedlings are grown for 2 years: 1+0 seedlings are produced in unheated white polyethylene tunnels (left) and 2+0 seedlings are grown outdoors (right). These seedlings are large 2+0 black spruce seedlings produced in 25-310 containers at Normandin nursery (Québec, Canada) in July. (Photo by Jean Gagnon 2013)
Before the experiment, seedlings were fertilized biweekly from May 18 to September 27 according to the seedlings’ weekly nutritional needs (Langlois and Gagnon 1993) determined by Plantec 2 software, a new version of PLANTEC (Girard et al. 2001). Fertilization totalled 81 mg (0.0027 oz) N (41 percent NH$_4^+$, 49 percent NO$_3^-$, and 10 percent urea), 21 mg (0.0007 oz) P, and 42 mg (0.0014 oz) K. The seedlings also received small amounts of calcium and magnesium as well as micronutrients present in commercial soluble fertilizers. No fertilizer was applied between September 28 and the beginning of the foliar fertilization study on October 12. Irrigation of these seedlings was managed using IRREC irrigation software (Girard et al. 2011). Directly before application of the foliar fertilization treatments, substrate fertility was determined on one composite sample from each treatment (72 root plugs per composite sample). The average substrate concentration of mineral N was 0.4 ppm and the concentration of urea-N was 0 ppm. This analysis was performed by the laboratoire de chimie organique et inorganique (ISO/CEI 17025) de la Direction de la recherche forestière (DRF), MFFP du Québec. This laboratory carried out all other N analyses (tissue and water) described in this article.

**Foliar Fertilization Treatments**

A completely randomized design with two factors (foliar urea fertilization and washing of seedling shoots), each with three levels of treatments and eight replicates (2 containers per replicate), was installed at Normandin nursery on October 12, 2011. A total of 600 containers received one of the three treatments of foliar urea (46-0-0) fertilization on day 0 (October 12): (1) Urea (U), (2) Urea + surfactant (US), and (3) no fertilization (NF: control). For the US treatment, the surfactant used was Agral 90 (Norac Concepts Inc. 2009, Guelph, Ontario, Canada), a nonionic surfactant containing 90 percent of nonylphenoxypolyethyloxy ethanol (NPE). Agral 90 was mixed with the urea solution in the following proportions: 1 ml per L (0.11 oz per gal). Because the addition of Agral 90 to urea solution leads to foam formation, a 12.5 percent antifoaming/defoaming agent (Fighter-F® 12.5 antifoaming/defoaming agent, Loveland Products, Inc. Greeley, CO) was added at a dose of 15 ml (0.51 oz) to the 32-L (8-gal) mix of urea and surfactant (US treatment).

For the two foliar urea fertilization treatments (U, US), an application of 15 mg (0.0005 oz) N per seedling or 29 kg N per ha (26 lb per ac) was applied, corresponding to a dose of 33 mg (0.0011 oz) urea per seedling or 68 kg per ha [60 lb per ac]. For each urea treatment, 7 kg (15 lb) of 46-0-0 was mixed in 48 L (13 gal) of water, producing a solution with a concentration of 145.8 g urea per L [1.2 lb per gal]. U and US treatments were applied at a rate of 518 L per ha (57 gal per ac) using a tractor-mounted boom sprayer (Model Multi 33, Timm Enterprises Inc., Oakville, Ontario, Canada) equipped with a 720-L (191-gal) reservoir and two rails of nine-nozzle irrigation (Model Teejet XR 11002, TeeJet Technologies, Spraying Systems Co., Wheaton, IL) (figure 2). At the time of fertilization, air temperature was 14ºC (57ºF) and relative humidity was 68 percent. No irrigation to rinse the foliage was applied either following foliar urea fertilization or during the 7-day study.

![Figure 2. For the urea foliar fertilization study carried out in mid-October at Normandin nursery, urea treatments were applied using tractor-mounted booms to 2+0 black spruce seedlings grown in 25-310 containers.](image)

Immediately after application of the fertilization treatments, 16 containers per treatment (8 replicates of 2 containers) were randomly selected. These containers were then moved into an unheated warehouse with open doors for 7 days, thus exposing the seedlings to outside temperatures while protecting them from rainfall. A total of 72 seedlings per fertilization treatment (9 seedlings per replicate by 8 replicates) was randomly harvested on day 0 (October 12) and at 1, 3, 5, and 7 days (October 13–19) after foliar urea fertilization. On each harvest date, the seedlings were severed at the root collar and placed into 24 bags, each containing either 3 shoots or 3 roots. The shoots were then subjected to washing treatments.

**Washing Treatments and Water Analyses**

The harvested seedling groups (three seedlings per replicate by eight replicates) were randomly subjected to one of three shoot washing treatments before analysis of foliar N
concentration: (1) washing (W: control): washing for 15 seconds using a sink-mounted vegetable sprayer (standard washing method of the laboratoire de chimie organique et inorganique de la DRF, MFFP du Québec, for analyses of nutrients in seedling tissues); (2) washing + soaking (WS): same as the W treatment but followed by soaking for 5 minutes; or (3) no washing or soaking (NWS). These three washing treatments were done 0, 1, 3, 5, and 7 days after foliar fertilization to evaluate their efficiency in removing urea residues from the needle surfaces and their effect on foliar N concentration. Washing treatments on day 0 were carried out 1 hour after fertilization.

After washing seedling shoots, the water used for washing or for soaking (3.2 L [0.8 gal] for W, 2 L [0.5 gal] for WS) was transferred to 250 ml (10 oz) plastic bottles. The bottles from all six treatments (two washing treatments by three fertilization treatments) were frozen and sent to the laboratory for analysis. Following filtration of the water samples (PVDF filters of 0.45 µm), urea-N concentration was determined by high-performance liquid chromatography with diode array detector (model 1200, Agilent Technologies, Waldbronn, Germany) using a Sugar-Pak I column (Waters, Milford, MA). Because the two washing treatments were carried out using a composite sample of three seedling shoots for each fertilization treatment, the urea-N concentration in the washing water was then calculated for one seedling. Thereafter, the amount of urea-N in each water sample was obtained by multiplying the volume by its concentration.

**Seedling Measurements**

Following the washing treatments, shoot and root tissues of the nine treatments (three fertilization treatments by three washing treatments) were oven-dried for 48 hr at 65 °C (149 °F). In addition, seedlings harvested on day 7 were measured for height, root-collar diameter, shoot, root, and total dry mass (24 seedlings per treatment by 9 treatments) and a visual assessment of foliar color or burning damage. The average morphology (± standard error: SE) for all treatments at day 7 was height (26.7 ± 0.3 cm, [10.7 in]), diameter (3.43 ± 0.03 mm, [0.14 in]), shoot dry mass (3.47 ± 0.05 g, [0.12 oz]), root dry mass (1.73 ± 0.02 g, [0.06 oz]), and total dry mass (5.20 ± 0.06 g, [0.18 oz]).

Needle, stem, and root samples (n = eight composite samples of three seedlings per replicate per treatment) were analyzed for total N concentrations (N_{tot}) using a LECO Nitrogen Determinator (model TruMac N, LECO Corporation, St. Joseph, MI). On day 0, these tissues were placed in the oven 2 hours after foliar urea fertilization; therefore N_{tot} tissue concentration on day 0 corresponds to 2 hours. Nitrogen content of each seedling part (needles, stem, roots, and total) was calculated (concentration by dry mass) to accurately reflect nitrogen uptake and accumulation (Timmer and Miller 1991).

**Statistical Analyses**

In this experiment, the first factor (foliar urea fertilization) was applied on day 0 (October 12) while the second factor (washing of seedling shoots) was carried out 0, 1, 3, 5, and 7 days (October 12–19) after fertilization. Because the washing treatments were applied on each of the five harvest dates, their effects are confounded in part with the effects of days, so two different approaches were used to analyze the data: (1) a linear mixed-effects model for repeated measurements (days 0 to 7) and (2) a linear mixed-effects model for each day.

First, a linear mixed-effects model for repeated measurements was carried out to determine the effects, over time, of the three foliar urea fertilization treatments on several variables using a variance-covariance matrix to account for the correlation between measurements done on the same experimental units. This matrix was chosen to minimize the likelihood value of the model while using as few parameters as possible. Thus, for all the variables presented in the results section (N concentrations and contents in needles, stems, shoots, roots, and seedlings), the selected variance-covariance matrix was variance components (VC), except for root N content where heterogeneous compound symmetry (CSH) was chosen. Fertilization treatments, days, and their interaction were introduced in the model as fixed-effect factors, whereas the replicates of fertilization treatments were considered as a random-effect factors. The three washing treatments (considered as subreplicates) and their interaction with days were also considered as random-effect factors. When the interaction between the fertilization treatments and the days was significant, comparisons between the fertilization treatments were performed for each of the five harvest dates (0, 1, 3, 5, and 7 days after fertilization).

Second, a linear mixed-effects model for each day was carried out to compare the washing treatments and to determine if an interaction occurred between them and the fertilization treatments. Fertilization and washing treatments, as well as their interaction, were introduced in the model as fixed-effect factors, whereas the replicates of fertilization treatments were considered to be random-effect factors. When the interaction
between the fertilization and the washing treatments was significant, comparisons were first carried out between the fertilization treatments for each washing treatment and second between the washing treatments for each fertilization treatment.

All of the statistical analyses were performed using the MIXED procedure of SAS (version 9.2, SAS Institute, Cary, NC, United States). When required, a simulation-based approach was used to assess differences (Westfall et al. 1999). Normality of the residuals was confirmed using the Shapiro-Wilk’s statistic, whereas the homogeneity of variance was validated using standard graphical methods. Differences were deemed significant when \( p < 0.05 \).

**Results**

**Nitrogen Concentration and Content**

The interaction between fertilization treatments and day was significant for foliar N concentration (\( p = 0.0177 \)) and content (\( p = 0.0009 \)). At day 0 (2 hours after fertilization), foliar N concentrations of U and US seedlings were 8 and 10 percent, respectively, higher than that of NF seedlings (figure 3a). After 3, 5, and 7 days, U and US seedlings continued to have significantly greater foliar N concentrations than NF seedlings, although no significant differences existed between U and US seedlings (figures 3a and 4a). After 7 days, N content was 23 and 27 percent higher than NF seedlings for U and US seedlings, respectively (figure 4b). Also, U and US seedlings appeared greener than the controls and had no burning damage from urea or from urea plus surfactant on their needles.

Although the interaction between the fertilization treatments and day was not significant for shoot N concentration (\( p = 0.1577 \)), it was significant for shoot N content (\( p = 0.0013 \)). During the 7-day study, U and US seedlings had significantly greater shoot N concentrations than NF seedlings, but these two fertilized treatments did not differ significantly (figure 3b). After 7 days, shoot N concentration of U and US seedlings were 9 and 13 percent higher, respectively, compared with unfertilized seedlings, (figures 3b and 4a), and their N contents were each increased 27 percent (figure 4b).

The interaction between the fertilization treatments and days was significant for root N concentration (\( p = 0.0016 \)) and content (\( p = 0.0028 \)). At day 0, root N concentration of U and US seedlings was 7 and 8 percent higher, respectively, compared with NF seedlings, (results not shown). After 1, 3, and 5 days (results not shown) and at day 7 (figure 4a), however, root N concentration did not differ significantly among the
three fertilization treatments. The root N content did not differ significantly among fertilization treatments after 0, 1, 3, and 5 days (results not shown) or at day 7 (figure 4b).

Although the interaction between the fertilization treatments and days was not significant for seedling N concentration ($p = 0.1187$), it was significant for seedling N content ($p = 0.0059$). During the 7-day study, U and US seedlings had a significantly greater seedling N concentration than NF seedlings, but these two fertilization treatments were not significantly different (figure 3c). At day 0, compared with NF seedlings, seedling N concentration of U and US seedlings had increased 8 and 11 percent, respectively (figure 3c). After 7 days, compared with NF seedlings, seedling N concentration of U and US seedlings had increased 5 and 7 percent, respectively (figures 3c and 4a), and seedling N content of U and US seedlings had increased 15 and 17 percent, respectively (figure 4b).

**Effect of Seedling Shoot Washing Treatments on Foliar Nitrogen Concentration**

Shoot washing treatments and fertilization treatments had significant interaction for foliar N concentration ($p \leq 0.0077$). Foliar N concentrations were significantly lower for seedlings from either washing treatment compared with the control treatment (figure 5). U and US seedlings had no significant difference in foliar N concentration between washed (W) seedlings and washed and soaked (WS) seedlings after 0, 1, and 3 days (figure 5). The same trend was also observed after 5 and 7 days (results not shown).

**Amount of Urea Removed by the Washing Treatments**

Washing and fertilization treatments had significant interaction on days 0 ($p = 0.0261$), 1 ($p = 0.0056$), 3 ($p < 0.0001$), and 5 ($p < 0.0001$) and very close to being significant on day 7 ($p = 0.0506$). As expected, water used for washing treatments of unfertilized seedlings contained no urea-N (results not shown). For each fertilization treatment, however, urea-N content in the water used for washing was significantly greater (approximately 90 percent of the total) over time than that used for soaking (figure 6). In addition, more urea-N was removed by washing seedlings from the U treatment than those from the US treatment (figure 6). The urea-N content in washing water for each fertilization treatment decreased over time indicating foliar urea absorption by U and US seedlings during the 7-day study (figure 6).

![Figure 5](image-url)  
**Figure 5.** Effect of seedling shoot washing treatments on 2+0 black spruce seedling foliar nitrogen concentration 0, 1, and 3 days after foliar fertilization with (a) urea or (b) urea + surfactant. For each day, bars with different letters differ significantly at the 5-percent level ($n = 8$ composites samples ± SE).

![Figure 6](image-url)  
**Figure 6.** Urea-N content in the water used for washing or water used for soaking after washing 2+0 black spruce seedling shoots 0, 1, 3, 5, and 7 days after foliar fertilization with (a) urea or (b) urea + surfactant ($n = 8$ composites samples ± SE).
Discussion

The use of foliar urea fertilization after fall budset of containerized 2+0 black spruce seedlings promoted a rapid (within 2 hours) increase in foliar N concentration under forest nursery conditions compared with unfertilized seedlings. This effect was still observed after 7 days. With *Pinus radiata* seedlings, all foliar-applied 15N urea was taken up within 6 hours (Coker et al. 1987, Coker 1991), and with apple (*Malus domestica* Borkh) trees, most foliar uptake of 15N urea occurred within 2 days (Dong et al. 2002).

Rapid urea absorption and increased foliar N concentration following foliar urea fertilization have also been observed in agriculture and horticulture studies (Handreck and Black 1984, Alexander and Schroeder 1987, Gooding and Davies 1992, Wojcik 2004). In our experiment, however, using surfactants (Alexander and Schroeder 1987, Gooding and Davies 1992, Wojcik 2004). To our knowledge, this experiment and a preliminary one conducted by Gagnon (2011) are the first to test the effects of washing treatments on foliar N concentration. Our results showed that washed seedlings had significantly reduced foliar N concentration compared with those that were not washed.

Foliar N concentration of seedlings that were washed and soaked, however, was not significantly lower than those that were only washed. It is likely that most of the urea residue on the needle surface was removed by the washing treatment, which occurred before the soaking treatment, as evidenced by the significantly higher N content in water used for the washing treatment compared with water used for the soaking treatment. Because foliar N concentration of fertilized seedlings was not significantly affected by these two washing treatments, we conclude that the current method for washing seedling shoots without soaking is appropriate to remove most (90 percent) of the fertilizer residues on the needles. Urea-N content in water collected from the W treatment decreased rapidly over time indicating foliar urea absorption of U and US seedlings, which was confirmed by the rapid increase of their foliar N concentration during 7 days.

Conclusion

The results of this 7-day study with containerized 2+0 black spruce seedlings showed that foliar fertilization of urea applied after fall budset is a useful tool for rapidly increasing the foliar N concentration of conifer seedlings without affecting their shoot height growth. This tool can help Québec nursery growers to meet the physiological quality criteria (minimum of 1.6 or 1.8 percent N concentration depending on container size) for container-grown conifer seedlings.

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