Boron retranslocation in Scots pine and Norway spruce

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Summary We previously traced10B-enriched boric acid from shoots to roots to demonstrate the translocation of boron (B) in Scots pine (Pinus sylvestris L.) and Norway spruce (Picea abies (L.) Karst.) seedlings. To gain a more detailed understanding of B translocation, we sought: (1) to demonstrate B retranslocation directly, by showing that foliar-applied 10B is located in the new growth after dormancy; and (2) to assess whether shoot-applied B affects growth in the long term.

We applied 10B-enriched boric acid to needles of Scots pine and Norway spruce seedlings. After a dormancy period and 9 weeks of growth, small but significant increases in the 10B isotope were found in the new stem and needles of both species. In Scots pine, the total B concentration of the new stem was also increased. Both species contained polyols, particularly pinitol and inositol. Boron–polyol complexes may provide a mechanism for mobilizing B in these species.

To determine the long-term effects of applied B, seedlings were grown for two growing seasons after the application of 10B to shoots. In Norway spruce, the proportion of 10B in the root systems and current needles of the harvest year was slightly higher than in the controls, and in Scots pine root systems, marginally so. The B treatment had no effect on growth of Norway spruce seedlings. In Scots pine seedlings, the B treatment caused a 33% increase in total dry mass and significantly increased the number of side branches.

Keywords: mineral nutrients, Picea abies, Pinus sylvestris, stable isotopes.

Introduction

Retranslocation is a series of processes that, in deciduous species, involves translocation of nutrients to storage organs before winter dormancy and back from storage organs to growing sink organs at the onset of the growing season. In evergreen coniferous species, needles function as storage organs (Helmisaari 1992). Early studies provided some evidence for boron (B) retranslocation in Scots pine (Pinus sylvestris L.) and Norway spruce (Picea abies (L.) Karst.). In Scots pine, B concentrations ([B]) in the previous-year needles decreased during the development of current-year shoots, indicating mobility (Helmisaari 1990). In Norway spruce, [B] in needles of different ages is either relatively stable—particularly when the concentration is low (Lehto and Mälkönen 1994)—or decreases with needle age (Finér 1989), a characteristic of mobile nutrients. Recently, we obtained direct evidence of B mobility in Scots pine and Norway spruce seedlings in a stable isotope labeling experiment in which elevated 10B/11B ratios were found in the root systems of both species within 24 h of foliar application of 10B to shoots (Lehto et al. 2000), indicating that B can move in the direction opposite to that of the transpiration stream, a necessary condition for retranslocation.

However, Lehto et al. (2000) reported that the extent of the B translocation to roots from shoots of Scots pine and Norway spruce was slight compared with that in several deciduous trees (Brown and Hu 1996). Nevertheless, B retranslocation may be an important process in evergreen trees over the long term, because coniferous trees can sequester a major part of the total B pool of the ecosystem in their biomass, particularly at sites low in B (Aphalo et al. 2002).

Hu et al. (1997) showed that B is mobilized in the phloem as a complex with polyols, explaining why B is mobile in species that have significant translocation of polyols such as sorbitol and mannitol (Brown and Shelp 1997). Pinitol and mannitol have been found in Scots pine and Norway spruce (Aronsson et al. 1976, Theander 1982), and sorbitol is also present in Scots pine (Domisch et al. 2001). However, within a tree species, the occurrence of these compounds has not been studied in the context of B mobility. Most studies on B retranslocation in plant species rely on information about the occurrence of polyols from other studies (Brown and Hu 1998, Lehto et al. 2000). Because the translocation of polyols may vary with the phenology and age of plants (Aronsson et al. 1976), it is of interest to study the occurrence of polyols in parallel with B mobility (Perica et al. 2001).

The objectives of this study were: (1) to demonstrate B retranslocation directly, i.e., obtain evidence that foliar-applied B is located in the new growth after dormancy; and (2) to assess whether foliar-applied B can affect biomass accumulation in the long term.

Materials and methods

Short-term experiment

Scots pine and Norway spruce seedlings, each of a central Fin-
land provenance, were grown in containers in a commercial nursery according to standard nursery practice until the end of the first growing season. On September 9, they were transplanted with the peat plug intact to 0.5-l pots containing a 1:1 (v/v) mixture of quartz sand (particle size 0.1–0.6 mm) and unfertilized unlimed peat. The seedlings were then placed in a growth room that provided a 12-h photoperiod at a photosynthetic photon flux (PPF) of 250 µmol m\(^{-2}\) s\(^{-1}\), a day/night temperature of 18/15 °C and a relative humidity of 80%.

Five seedlings per species were harvested before transplanting to determine the initial needle B concentration ([B]). Shoots were severed at the soil surface, dried at 55 °C and the [B] of needles was determined as described below.

Ten days after transplanting, 12 seedlings per species were randomly allocated to the \(^{10}\)B tracer treatment (+B) and control treatment (–B). The +B treatment solution was 50 mM \(^{10}\)BO\(_3\) (99% \(^{10}\)B, Aldrich, St. Louis, MO) with 0.1% Sunoco 11E3 oil-based adjuvant (Sun Oil Company, Philadelphia, PA), and for –B, only Sunoco was used. After adding Sunoco, the solution was stirred continuously with a magnetic stirrer. Each plant was kept in a horizontal position such that the apical part of each needle, 1 cm away from the stem, was immersed in the solution for 20 s from one side of the plant. Subsequently the plant was turned 90° and the needles on that side were treated in the same way. Care was taken to ensure that the stem and buds were not in contact with the solution. Side branches were also not treated with the solution. The plants were left to dry overnight in a horizontal position. During treatment application and drying, the soil was protected from spills of \(^{10}\)B solution with Paraffilm and aluminum foil. After treatment application, a narrow tube was used for watering to ensure that no leaching from needle surfaces would occur, and fallen needles were removed with forceps.

Dormancy was induced in the treated plants by short-day and low-temperature treatments: 1 week in an 8-h photoperiod at about 60 µmol m\(^{-2}\) s\(^{-1}\) and a day/night temperature of 12/12 °C, followed by 5 weeks in a 6-h photoperiod and a day/night temperature of 12/9 °C, 4 weeks in continuous darkness at 6 °C and 2 weeks in continuous darkness in 0 °C. Subsequently, the growing season started with 3 weeks in an 8-h photoperiod at 60 µmol m\(^{-2}\) s\(^{-1}\) and a day/night temperature of 15/8 °C, and 4 days in a 12-h photoperiod at 160 µmol m\(^{-2}\) s\(^{-1}\) and a day/night temperature of 18/12 °C. For the remainder of the growing season, a 20-h photoperiod was maintained at 310 µmol m\(^{-2}\) s\(^{-1}\) and a day/night temperature of 20/15 °C, without fertilizer.

The plants were harvested after 9 weeks of long days, when the needles were fully extended. The new leader shoots were harvested, taking care to avoid contamination from the treated needles. Chemical analyses were made on seven replicate plants, chosen as representative of their species and treatment in terms of dry mass of new shoot growth. The leader shoot was separated into needles and stem.

Samples were dried at 55 °C, ground, ashed and digested in HNO\(_3\) (Suprapur). The \(^{10}\)B/\(^{11}\)B isotope ratio was determined by inductively coupled plasma-mass spectrometry (ICP-MS, Sciex Elan 6000, Perkin-Elmer, Wellesley, MA), and the [B] was measured with an inductively coupled plasma optical emission spectrometer (ICP-OES; Iris Advantage Duo High Resolution, Thermo Jarrell Ash, Franklin MA) at the Geological Survey, Espoo, Finland.

Five seedlings per species, similar to those used for the tracer study, were harvested and analyzed for polyols in the new growth. Current-year needles and the stem of the topmost whorl of the seedlings were dried at 40 °C and ground separately in a ball mill. Sugars and polyols were determined by capillary gas chromatography (GC) (Hewlett-Packard Model 5890 equipped with an HP 5971 series mass selective detector). The extraction procedure was modified from that of Chaplin (1994). Duplicate 5-mg subsamples were extracted for 2 min in 700 µl of 80% ethanol by stirring with a glass rod. The sample was then placed on ice for 15 min, before being re-extracted for 2 min and centrifuged (13,000 g for 3 min; Biofuge pico, Heraeus Instruments, Langenselbold, Germany). The extraction procedure was repeated and the two extracts were combined and evaporated to dryness under nitrogen. The dried sample was dissolved in 200 µl of 1-methylimidazole, followed by 1 ml of acetic anhydride and thorough mixing. After 10 min at room temperature, 2 ml of water was added and the sample was cooled on ice. After cooling, the peracetylated carbohydrates were extracted into the lower layer formed after vortex mixing the aqueous solution with 1 ml of dichloromethane. This layer was removed with a Pasteur pipette and identified by GC. The column used was BPX70 (70% cyanopropyl polysilphenylene-siloxane) ID 0.22 mm, length 25 m, film thickness 0.25 µm (SGE International, Ringwood, Australia). The temperature program was started at 190 °C and the temperature was then raised to 260 °C at a rate of 3 °C min\(^{-1}\). The interface and injector temperatures were 180 °C and 230 °C, respectively. The auto-injected volume was 2 µl and helium was the carrier gas. Electron ionization spectra were recorded at 70 eV. The identification of carbohydrates was based on retention times and on mass spectra (m/z) using selected ion monitoring (SIM) with reference compounds. Sugars were quantified against commercial standards of glucose, fructose (Merck, Darmstadt, Germany), pinitol, quebrachitol (Sigma, St. Louis, MO), maninitol, dulcitol, sorbitol, arabitol, xylitol and myo-inositol (Fluka, Neu-Ulm, Germany). Because the method does not provide a reliable quantitative analysis of glucose, glucose results are not reported.

**Long-term experiment**

Scots pine and Norway spruce seeds, each of a southern Finnish provenance from a seed orchard, were soaked in cold water overnight, and treated with 30% H\(_2\)O\(_2\) for 15 min. Seeds were sown directly in 65-ml Ray Leach Cone-tainers (Stuewe & Sons, Corvallis, OR) filled with a mixture of 7:3 (v/v) mix of quartz sand of particle size 0.5–1.5 mm and sieved mor humus from a Scots pine or Norway spruce dominated forest. The humus originated from a low-B site at Heinävesi (Lehto and Mäkkönen 1994). In a similar soil mix, the initial total [B] was less than 0.5 mg kg\(^{-1}\) (Möttönen et al. 2001). The seedlings were grown in a controlled-environment room, providing a 21-h photoperiod at 275 µmol m\(^{-2}\) s\(^{-1}\), a day/night temperature of 20/15 °C and a day/night relative humidity of 75/80%. Initially, there were three seedlings per pot. Complete nutrient so-
The labeling experiment started when the seedlings were 120 days old. The seedlings were thinned to one per pot, and randomly allocated to +B and –B treatments. In the +B treatment, the whole shoot was immersed for 60 s in a 50 mM H$_{10}$BO$_3$ solution, in which 99.0% of the B was $^{10}$B (Aldrich), with 0.1% Sunoco 11E/3 oil-based adjuvant. After adding Sunoco, the solution was stirred continuously with a magnetic stirrer. The –B plants were treated with adjuvant in water. After 24 h, all experimental plants were washed, first in water with 0.005% Triton-X100 for 15 s, stirring continuously, then rinsed in double-deionized water for 1 min. During the labeling and washing, the soil surface was covered with Parafilm that was wrapped around the stems. After the treatment and washing, the seedlings were held in a horizontal position for 3 h, until the foliage was dry.

The treated seedlings were kept in the controlled-environment chamber for 14 days in a 21-h photoperiod at 275 µmol m$^{-2}$ s$^{-1}$, a day/night temperature of 20/15 °C and a day/night relative humidity of 75/80%, without fertilization. Thereafter, a full annual cycle was simulated in the growth chamber as indicated in Table 1. Subsequent simulated seasons followed the same pattern until Summer 3, when the plants were harvested 14 weeks after the commencement of long days, at which time shoot extension and needle growth had terminated. During Summers 2 and 3, the plants were fertilized three times per week with nutrient solution (Riddoch et al. 1991) containing 1 mg N and all other nutrients except boron. Through the experiment, senescent and abscised needles were collected with forceps to prevent leaching of B from needle litter.

At harvest, the seedlings were severed at the soil surface and the total number of lateral branches was counted. In Scots pine, needle color was variable, from yellowish to green, and seedlings were classified based on needle color as ‘pale’ (value 1), ‘intermediate’ (value 2) or ‘normal’ (value 3). Current-year shoots (C) were separated from each seedling, and the needles and stems of age-class C+1 were combined with those from age-class C+2. The root systems were separated from the soil and cleaned in a small amount of double-deionized water. Total [B] and B isotope ratios of the current-year needles and the root systems were determined as described previously.

**Statistical analysis**

Data were analyzed by the $t$-test. The dry mass data were log transformed.

**Results**

**Short-term experiment**

The Scots pine and Norway spruce seedlings harvested at the beginning of the short-term experiment had mean [B] of 34.7 ± 2.3 and 23.5 ± 1.4 mg kg$^{-1}$, respectively. The proportion of $^{10}$B in new needles increased in the growing season following application of the +B treatment in both Scots pine ($P < 0.001$) and Norway spruce ($P = 0.030$; Figure 1). In Scots pine, the proportion of $^{10}$B was significantly elevated in new stems ($P = 0.004$), whereas the slight increase in the proportion of $^{10}$B in Norway spruce stems was not significant ($P = 0.117$). In response to the +B treatment, total [B] was 8% higher in Scots pine stems ($P = 0.082$), but there was no effect on Scots pine needles or on Norway spruce stems and needles (Table 2).

Fructose concentrations were less than 1 mg g$^{-1}$ in both species. Inositol and pinitol were the most abundant polyols in both Scots pine and Norway spruce. The inositol concentration was 31 mg g$^{-1}$ in Scots pine needles and 13 mg g$^{-1}$ in Norway spruce needles, whereas pinitol concentration was 12 and 7 mg g$^{-1}$, respectively (Table 3). An unidentified compound, resembling pinitol, was also found in both species. Sorbitol and mannitol were found in needles of both species, but mannitol concentrations were low. Dulcitol was found in low concentrations in both species and arabitol was found only in Norway spruce needles. No xylitol or quebrachitol was detected.

**Long-term experiment**

Two growing seasons after the treatments, the proportion of

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**Table 1. Simulated seasons and conditions in the controlled-environment chambers during the first simulated year. Conditions are indicated only if they differ from previous period. Abbreviations: PPF = photosynthetic photon flux; and RH = relative humidity.**

<table>
<thead>
<tr>
<th>Season</th>
<th>Length (days)</th>
<th>Day length (hours)</th>
<th>PPF (µmol m$^{-2}$ s$^{-1}$)</th>
<th>Day/night temperature (°C)</th>
<th>Day/night RH</th>
<th>Fertilizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before labelling</td>
<td>120</td>
<td>21</td>
<td>275</td>
<td>20/15</td>
<td>75/80</td>
<td>Complete</td>
</tr>
<tr>
<td>After labelling</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No fertilizer</td>
</tr>
<tr>
<td>Autumn 1</td>
<td>5</td>
<td>6</td>
<td>250</td>
<td>15/12</td>
<td>12/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>160</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter 1</td>
<td>14</td>
<td></td>
<td>4/2</td>
<td>80/80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26</td>
<td></td>
<td>4/0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring 1</td>
<td>5</td>
<td>18</td>
<td>10/5</td>
<td>75/80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>275</td>
<td>15/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer 2</td>
<td>130</td>
<td>310</td>
<td></td>
<td></td>
<td></td>
<td>Complete –B</td>
</tr>
</tbody>
</table>

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compared with about 12 mg kg\(^{-1}\) in Norway spruce. Root B

Table 2. Mean (± SE) total B concentration (mmol kg\(^{-1}\)) in current-year stems of Scots pine and Norway spruce in the growing season following application of \(^{10}\)B to needles (+B) and controls (–B) (n = 7). Significance of treatment differences: **** = \(P < 0.001\); ** = \(P < 0.01\); * = \(P < 0.05\); and ns = \(P \geq 0.05\). Error bars are equal to ± 1 SE.

Figure 1. Proportion of \(^{10}\)B as % of total B in current-year needles and current-year stems of Scots pine and Norway spruce in the growing season following application of \(^{10}\)B to needles (+B) and controls (–B) (n = 7). Significance of treatment differences: **** = \(P < 0.001\); ** = \(P < 0.01\); * = \(P < 0.05\); and ns = \(P \geq 0.05\). Error bars are equal to ± 1 SE.

\(^{10}\)B relative to total B in current-year needles of +B Norway spruce increased to 21.3% in response to the +B treatment (\(P < 0.001\); Figure 2). In Scots pine, the +B treatment did not increase the proportion of \(^{10}\)B in current-year needles. The +B treatment slightly (\(P < 0.001\)) increased the proportion of \(^{10}\)B in the root systems of both species. Although the proportion of \(^{10}\)B in root systems of +B-treated Scots pine was only 20.5% (natural abundance 19.9%), in root systems of +B-treated Norway spruce, it was 21.2%.

Mean [B] in current-year needles did not differ significantly between treatments in either species (Table 4). In Scots pine, the mean [B] in current-year needles was about 8 mg kg\(^{-1}\), compared with about 12 mg kg\(^{-1}\) in Norway spruce. Root B

Table 2. Mean (± SE) total B concentration (mmol kg\(^{-1}\)) in current-year needles and current-year stems of Scots pine and Norway spruce seedlings in the growing season following \(^{10}\)B treatment. The \(P\)-value indicates probability of a difference between treatments within each species and plant part in a \(t\)-test (n = 7).

<table>
<thead>
<tr>
<th></th>
<th>Total B concentration</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–B</td>
<td>+B</td>
</tr>
<tr>
<td>Scots pine needles</td>
<td>1.45 ± 0.097</td>
<td>1.43 ± 0.079</td>
</tr>
<tr>
<td>Scots pine stems</td>
<td>1.22 ± 0.045</td>
<td>1.32 ± 0.029</td>
</tr>
<tr>
<td>Norway spruce needles</td>
<td>1.16 ± 0.063</td>
<td>1.18 ± 0.062</td>
</tr>
<tr>
<td>Norway spruce stems</td>
<td>1.46 ± 0.097</td>
<td>1.49 ± 0.114</td>
</tr>
</tbody>
</table>

Discussion

In the short-term experiment, the proportion of \(^{10}\)B relative to total B was elevated in current-year needles and stems of both Scots pine and Norway spruce in response to a foliar application of \(^{10}\)B followed by a dormancy season and a growing season. These results indicate that B was retranslocated from the old needles to the new plant parts during growth.

Quantitative analyses of the amounts of B retranslocation have been made based on sequential samplings, taking into account changes in tissue mass resulting from transformations of storage compounds. Helmisari (1992) calculated that retranslocation in the aboveground plant parts accounted for 9–17% of the B requirements of the annual growth in Scots pine stands of different ages. Boron concentrations in current needles during dormancy in the Scots pine studied by Helmisari (1990) were between 8 and 15 mg kg\(^{-1}\). Boron concentrations less than 8 mg kg\(^{-1}\) are considered suboptimal (Veijalainen et al. 1984). At low tissue [B], a large proportion of B is immobile in the cell wall structure (e.g., Dannel et al. 1998). Therefore, more retranslocation of B could be expected at high internal [B] such as in our short-term experiment.

The amount of \(^{10}\)B found in the current-year needles and stems of Norway spruce and Scots pine was low, perhaps reflecting inefficient B retranslocation in these species. However, because the original peat plug was intact during the whole experiment, the roots were able to take up B with natural isotope ratios from the soil, thereby diluting the added \(^{10}\)B. We have preliminary evidence that coniferous trees do not readily take up large quantities of H\(^3\)BO\(_3\) from needle surfaces, because there was no \(^{10}\)B uptake when a mild surfactant (Tween) was used in place of the oil-based surfactant (M. Räisänen and T. Lehto, unpublished observations). Brown and Hu (1998) found large differences in the amount of \(^{10}\)B in labeled leaves in a range of broad-leaved species, and suggested that differential B absorption of the added tracer accounted for these differences. An additional factor limiting the increment in \(^{10}\)B in sink organs could be displacement of the original \(^{11}\)B by applied \(^{10}\)B in the source leaves, resulting in increased mo-
bility of $^{11}$B as well. This phenomenon has been observed previously (Brown and Hu 1996, Lehto et al. 2000), and its effect will increase with increasing length of the experiments. Nevertheless, our results provide direct evidence that retranslocation of B occurred.

Mannitol and sorbitol function in long-distance phloem transport in many plant species (Zimmermann and Ziegler 1975, Noiraud et al. 2001), and the occurrence of inositol and pinitol in phloem sap suggests that these polyols have a similar role (Blevins and Lukaszewski 1998), although they have been much less studied. Pinitol is present in the phloem of Norway spruce at about 20% of the sucrose concentration on a per mass basis (Rohde et al. 1996). Mannitol has been shown to complex with B in the phloem sap, and sorbitol complexes with B in the extrafloral nectar of plant species that retranslocate B (Hu et al. 1997). Although we analyzed polyols in the needles and stems, we expect that they were also present in the phloem of the seedlings. Inositol is known to have a role in phosphorus metabolism, and its possible role in B translocation also deserves further study because inositol has been reported as the only polyol in the phloem of many plants (Zimmermann and Ziegler 1975). Inositol and pinitol have closely related biosynthetic pathways. In most gymnosperms, D-pinitol can be synthesized indirectly from myo-inositol by methylation and epimerization (Loewus and Dickinson 1982).

Pinitol was found at a concentration of about 1% of dry mass in this study, whereas mannitol concentrations were 100 times lower than pinitol concentrations in Norway spruce, and even less in Scots pine. This result differs from the findings of a field study by Theander (1982), where pinitol and mannitol were found in almost similar concentrations in Scots pine. Mannitol is a major carbohydrate in many fungi, and healthy needles can support a range of endophytic fungi: 15–85% of third-year needles were infected in a survey of different Norway spruce forests in Finland (Müller and Hallak-sela 1998). It is therefore possible that some of the mannitol in older needles in the study of Theander (1982) was of fungal origin. Aronsson et al. (1976) also found that the concentrations of mannitol and pinitol were in the same order of magnitude in Scots pine and Norway spruce growing in a phytotron. In contrast, Rohde et al. (1996) detected only pinitol in the phloem of field-grown Norway spruce. Seasonal variations in the concentrations of polyols may account for these discrepancies. In our study, polyols were analyzed at only one time, when the needles were already fully extended. At this stage of growth, some carbohydrate translocation from 1-year-old needles to current needles may still be occurring (Ericsson 1978).

In the long-term experiment, B concentrations in current-year needles were about 8 mg kg$^{-1}$ in Scots pine and 12 mg kg$^{-1}$ in Norway spruce, two growing seasons after B application (but with other nutrients supplied throughout the study). This suggests that B retranslocation was a major source of B for the new needles, although recycling of B through root death and their microbial decomposition was not excluded.

In the short-term experiment, there were no major differences between the species in the extent of $^{10}$B enrichment in

<table>
<thead>
<tr>
<th>Fructose</th>
<th>Sorbitol</th>
<th>Pinitol</th>
<th>Pinitol analog</th>
<th>Inositol</th>
<th>Mannitol</th>
<th>Dulcitol</th>
<th>Arabitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scots pine stems</td>
<td>nd</td>
<td>0.77 ± 0.09</td>
<td>4.06 ± 0.46</td>
<td>0.86 ± 0.09</td>
<td>3.83 ± 0.08</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Scots pine needles</td>
<td>0.52</td>
<td>3.94</td>
<td>12.10</td>
<td>1.87</td>
<td>31.20</td>
<td>0.050</td>
<td>0.080</td>
</tr>
<tr>
<td>Norway spruce stems</td>
<td>0.60 ± 0.13</td>
<td>1.29 ± 0.53</td>
<td>7.32 ± 0.87</td>
<td>0.58 ± 0.17</td>
<td>4.63 ± 1.52</td>
<td>0.065 ± 0.015</td>
<td>0.033 ± 0.015</td>
</tr>
<tr>
<td>Norway spruce needles</td>
<td>0.44</td>
<td>3.04</td>
<td>7.20</td>
<td>0.09</td>
<td>13.30</td>
<td>0.020</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Figure 2. Proportion of $^{10}$B as % of total B in current-year needles and whole root systems of Scots pine and Norway spruce two growing seasons after application of $^{10}$B to shoots (+B) and controls (–B) (n = 7). Significance of treatment differences: *** = P < 0.001; ** = P < 0.01; * = P < 0.05; and ns = P ≥ 0.05. Error bars are equal to ± 1 SE.
new growth. In contrast, in the long-term experiment, elevated \(^{10}B\) was found only in current-year needles of Norway spruce, whereas there was no elevation of \(^{10}B\) in the current-year needles of Scots pine beyond the first growing season after application of B. Most of the B in the root systems probably was bound to cell wall polysaccharides, because root \([B]\) was only 4 and 6 mg kg\(^{-1}\) in Scots pine and Norway spruce seedlings, respectively. Roots are often the plant part most sensitive to B deficiency (Dell and Huang 1997). Möttönen et al. (2001) found that root tip and mycorrhiza formation were reduced in Norway spruce when needle \([B]\) was 16–17 mg kg\(^{-1}\), and needle B concentrations in our study were below this value. Boron retranslocation for root growth could be of importance for trees during droughts or other periods of low B availability. The ability to redistribute B internally may be of particular importance for growth of new shoots in the spring. Shoot extension growth in boreal conifers occurs within a relatively short period, when both stomatal conductance and active nutrient uptake are limited by low soil temperatures (Domisch et al. 2002, Lahti et al. 2002). In these circumstances, shoot growth could be prevented by a temporary B deficiency, if B retranslocation did not occur.

Although there was no excess of \(^{10}B\) in new needles of +B-treated Scots pine after two growing seasons, total plant biomass was 33% higher than in controls, partly because of an increase in the number of side branches. Increased branching at the expense of the leader shoot has been identified as one of the first symptoms of growth disturbance associated with B deficiency in Scots pine trees (Veijalainen et al. 1984). Severe growth disturbances involve complete loss of apical dominance because of failure of bud development; however, we found no adverse effects of the +B treatment on the morphology of the leaders in our Scots pine seedlings. Similar branching effects in response to B fertilization have been reported for soybean (Schon and Blevins 1990).

One possible explanation for the +B-induced increase in growth in Scots pine is increased uptake of other nutrients, as a result of enhanced development of mycorrhizas. In contrast, in Norway spruce seedlings, we found no effects of B on growth and only slight effects of B status on N uptake (M. Möttönen, T. Lehto and P.J. Aphalo, unpublished observations). In a field study, B fertilization did not increase the concentrations of other nutrients in Norway spruce trees (Möttönen et al. 2003).

In conclusion, B is retranslocated in Scots pine and Norway spruce, in a process that is likely mediated by polyols. In Scots pine, foliar-applied B affected root \([B]\) and shoot biomass up to two growing seasons after application. The demonstration of B retranslocation provides an explanation for the long-lasting effect of B fertilization on needle B status in Norway spruce (Möttönen et al. 2003) and Scots pine stands (A. Rummukainen, S. Kaunisto and T. Lehto, unpublished observations).

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Table 4. Properties of Scots pine and Norway spruce control seedlings and seedlings treated with \(^{10}B\) two growing seasons earlier. The \(P\)-value indicates probability of a difference between treatments within each species and plant part in a t-test, except for needle color, which was analyzed by the Kruskal-Wallis test. Values are means ± SE of seven plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Scots pine</th>
<th>Norway spruce</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–B</td>
<td>+B</td>
</tr>
<tr>
<td>Needle B concentration mmol kg(^{-1})</td>
<td>0.80 ± 0.028</td>
<td>0.74 ± 0.050</td>
</tr>
<tr>
<td>Root B concentration mmol kg(^{-1})</td>
<td>0.34 ± 0.018</td>
<td>0.40 ± 0.019</td>
</tr>
<tr>
<td>Number of branches</td>
<td>3.00 ± 0.31</td>
<td>5.14 ± 0.40</td>
</tr>
<tr>
<td>Needle color</td>
<td>1.71</td>
<td>2.57</td>
</tr>
</tbody>
</table>

Figure 3. (a) Dry mass ratios (from bottom: root, stem and needle ratio) and (b) total dry mass in Scots pine and Norway spruce seedlings two growing seasons after application of \(^{10}B\) to shoots (+B) and controls (–B) \((n = 7)\). Significance of treatment differences: *** \(P < 0.001\); ** \(P < 0.01\); * \(P < 0.05\); and ns \(P \geq 0.05\). Error bars equal ± 1 SE.
References


Rohde, M., R. Waldmann and J. Lunderstädt. 1996. Induced defence reaction in the phloem of spruce (Picea abies) and larch (Larix decidua) after the attack by Ips typographus and Ips cembrae. For. Ecol. Manage. 86:51–59.


