Growth and nutrition of *Betula pendula* at different relative supply rates of zinc

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Summary Small birch plants (*Betula pendula* Roth) were cultivated in a hydroponic spray solution where the relative growth rate (*G*$_{rel}$; day$^{-1}$) was controlled by the relative supply rate of zinc (*Zn*$_{rel}$; day$^{-1}$). After an adjustment phase to steady-state growth, *G*$_{rel}$ equaled *Zn*$_{rel}$. The *Zn*$_{rel}$ treatments were 0.05, 0.125 and 0.20 day$^{-1}$ with free access to all other nutrients. In an additional treatment, there was free access to all nutrients, including zinc (FA treatment). The pH of the nutrient solution was approximately 4.5 and conductivity was 100 µS cm$^{-1}$. The duration of each treatment depended on *Zn*$_{rel}$ and ranged from 4 (FA treatment) to 10 weeks (at *Zn*$_{rel}$ = 0.05 day$^{-1}$). The plants showed persistent and typical zinc-deficiency symptoms at steady-state growth when *G*$_{rel}$ was 0.05 and 0.125 day$^{-1}$, whereas there were few symptoms when *G*$_{rel}$ was 0.2 day$^{-1}$. The Zn concentration of the plants ranged from 8 (at *Zn*$_{rel}$ = 0.05 day$^{-1}$) to 21 µg g$_{DM}$$^{-1}$ (DM = dry mass) (at *Zn*$_{rel}$ = 0.2 day$^{-1}$) and was approximately 42 µg g$_{DM}$$^{-1}$ in the FA treatment. Uptake rates of Zn, calculated per root growth rate (µmol g$_{DM, root}^{-1}$), were about 2.8 times higher at *Zn*$_{rel}$ = 0.20 day$^{-1}$ than at *Zn*$_{rel}$ = 0.05 day$^{-1}$. The root and stem biomass fractions were approximately constant at all supply rates of Zn, whereas the leaf biomass fraction tended to increase with increasing supply rate of Zn. Net assimilation rate was constant from FA to an leaf biomass fraction tended to increase with increasing supply rates. "Rosetting" of apple and peach, "mottle leaf" or "frenching" of citrus, and "white bud" of maize are examples of deficiency symptoms that are normally linked to low internal zinc content (Chapman 1966). Tree leaves that are low in zinc often exhibit chloroses, curled leaf margins and necrotic spots. The internal zinc concentration ([Zn]) required for plant growth is estimated to be in the range of 15 to 125 µg g$_{DM}$$^{-1}$ (Stone 1968, Van Hook et al. 1980), but information about the quantitative zinc requirement of forest trees is sparse. External concentration of nutrients in natural ecosystems are low, especially in forest systems (e.g., Boardman and McGuire 1990). In soil solutions, [Zn] is typically < 5 µM (Mengel and Kirkby 1987), and concentrations of 0.01 to 2.5 µM have been used in nutrient solution experiments (Carroll and Loneragan 1968).

Plant properties may vary with time and negative correlations between growth and internal nutrient concentration have been reported in experiments in which nutrients are added in initially well-defined external concentrations (Hewitt 1957, Smith 1962). In contrast to such experiments, Ingestad and co-workers (e.g., Ingestad 1982, Ingestad and Kähr 1985, Ericsson and Ingestad 1988, Ericsson and Kähr 1993, 1995, Göransson 1993, 1994, 1998) used relative supply rate of nutrients as the growth-controlling variable. By quantifying the amount of added nutrient over time and adding it in relation to the calculated plant requirement, plants can be grown at constant *R*$_{G}$. Basic methodology and related theoretical considerations regarding the technique of relative nutrient supply have been discussed in detail by Ågren (1985), Ingestad and Lund (1986) and Ingestad and Ågren (1988, 1992).

The present investigation focused on the nutrition and carbon partitioning of small birch plants for which Zn was the growth-controlling variable and which was supplied at exponentially increasing amounts, i.e., at different relative supply rates, *R*$_{Zn}$.

Materials and methods

Seeds of *Betula pendula* Roth were germinated and planted in growth units. A zinc-free nutrient solution was added every 10 minutes, to keep conductivity at about 100 µS cm$^{-1}$ and pH at 4.5. After 14 days, seedlings were harvested and analyzed for zinc. Sixty seedlings (in each treatment), with an individual fresh mass of approximately 50 mg, were divided into groups of four and their zinc content was calculated based on the data obtained from the Zn analyses. Thereafter, zinc was added as zinc nitrate, in relation to the calculated content and the estimated requirement for plants growing at relative growth rates (*G*$_{rel}$) of 0.05, 0.125 and 0.20 (day$^{-1}$). There was free access to all other nutrients. In an additional treatment, there was free access to all nutrient elements, including zinc, so that the plants were growing at their maximal *G*$_{rel}$. The conductivity was...
constant at > 100 µS cm\(^{-1}\) and the pH of the nutrient solution was 4.5 in all treatments.

When the \(R_G\) of the seedlings was similar to the relative supply rate of zinc (\(R_{Zn};\ day^{-1}\)), 10 plant groups were re-weighed and returned to the growth units. Five, three and two plant groups were harvested successively on three occasions during the experiments at intervals of 3 (FA) to 14 days (\(R_{Zn}=0.05\)) (cf. Ågren 1985). All treatments were repeated once. Nutrient elements and total nonstructural carbohydrates were analyzed. For a detailed description of methods, calculations and statistics, see Göransson (1994).

### Results

#### Visible symptoms

The plants developed zinc-deficiency symptoms in all of the growth-limiting \(R_{Zn}\) (0.05, 0.125 and 0.2 day\(^{-1}\)) treatments. The symptoms were pronounced in the 0.05 and 0.125 day\(^{-1}\) treatments, and the typical “little leaf” syndrome appeared. The leaf veins were dark green with light to normal green areas in between, and older leaves had curled margins, chloroses and necroses. Younger leaves, sometimes with very deep cut margins, were normal green with light green marginal meristems. The shoots were bushy, especially in the 0.05 day\(^{-1}\) treatment, resembling the “rosette” syndrome. In the 0.20 day\(^{-1}\) treatment, few chloroses and necroses appeared, although the oldest leaves were often malformed. Leaf mortality was low at all limiting supply rates of zinc. The roots appeared to be normal and the root system was dense with short roots in the 0.05 day\(^{-1}\) treatment.

#### Growth and nutrition

The duration of each treatment depended on \(R_{Zn}\), and was from 4 (free access to all nutrients (free access treatment)) to 10 weeks (0.05 day\(^{-1}\)). The fresh mass (FM) of the individual plants ranged between 7.2 and 12.8 g at the final harvest, regardless of \(R_{Zn}\). The relative growth rate was stable in each experiment and the coefficient of determination (\(r^2\)) was greater than 0.98 in the curve-fit determination of \(R_G\). The \(R_G\) of the different plant parts was close to the \(R_{Zn}\) value (Table 1) with, in a few cases, a somewhat lower coefficient of determination (\(r^2 \geq 0.96\)) than for plant \(R_G\). At stable \(R_{Zn}\), there was a linear and 1:1 relationship between plant \(R_G\) and \(R_{Zn}\) (Figure 1) with an \(r^2\) equal to 0.99.

The [Zn] of the plants and plant fractions was lower at Zn limitation than in the FA treatment (Figure 2, Table 1). Plant [Zn] was 8 and 21 µg g\(^{-1}\)DM at \(R_{Zn}\) of 0.05 and 0.20 day\(^{-1}\), respectively. The [Zn] in plants at FA was approximately 41 µg g\(^{-1}\)DM. Foliar [Zn] ranged from 7 to 19 µg g\(^{-1}\)DM at limitation. At a limitation of \(R_{Zn}=0.05\) day\(^{-1}\) (Figure 3), there were only minor differences in [Zn] between old and younger leaves. At \(R_{Zn}=0.125\) and 0.2 day\(^{-1}\) and in the FA treatment, young leaves had significantly higher [Zn] than fully developed and old leaves. In all treatments, [Zn] was greater in roots than in stems and leaves. Changes in the nutrient composition of the plants occurred at Zn limitation (Table 1). In the leaf, stem and root fractions there was a general trend toward higher concentrations of nitrogen, phosphorus, calcium, magnesium, iron and manganese at growth limiting supply rates of zinc (\(R_{Zn}=0.05, 0.125\) and 0.2 day\(^{-1}\)).

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The uptake rate of zinc per unit root growth rate (\(d\text{Zn}/d\text{DM}_{\text{root}}\)) increased with increasing \(R_{Zn}\) at different relative supply rates of zinc (\(R_{Zn};\ day^{-1}\)) at different relative supply rates of zinc (\(R_{Zn};\ day^{-1}\)). Free access to all nutrient elements was denoted by FA. In each experiment, analyses were performed at three consecutive harvests on leaf, stem and root fractions from five, three and two groups of four plants, respectively. Means followed by different letters are significantly different (\(P=0.01, \) Fisher’s LSD). The relative growth rate (\(R_G;\ day^{-1}\)) was calculated by linear regression based on four weight determinations; \(r^2\) is the coefficient of determination.

### Table 1. Internal concentration of macronutrients (mg g\(^{-1}\)DM) and iron, manganese and zinc (µg g\(^{-1}\)DM) at different relative supply rates of zinc (\(R_{Zn};\ day^{-1}\)). Free access to all nutrient elements is denoted by FA. In each experiment, analyses were performed at three consecutive harvests on leaf, stem and root fractions from five, three and two groups of four plants, respectively. Means followed by different letters are significantly different (\(P=0.01\), Fisher’s LSD). The relative growth rate (\(R_G;\ day^{-1}\)) was calculated by linear regression based on four weight determinations; \(r^2\) is the coefficient of determination.

<table>
<thead>
<tr>
<th>Growth parameters</th>
<th>Nutrient concentrations</th>
</tr>
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<tbody>
<tr>
<td>(R_{Zn})</td>
<td>(R_G)</td>
</tr>
<tr>
<td>Leaf</td>
<td></td>
</tr>
<tr>
<td>0.05 0.05 &gt; 0.99</td>
<td>0.125 0.11 &gt; 0.96</td>
</tr>
<tr>
<td>0.20 0.23 &gt; 0.99</td>
<td>43.7 c</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
</tr>
<tr>
<td>0.05 0.05 &gt; 0.99</td>
<td>0.125 0.15 &gt; 0.99</td>
</tr>
<tr>
<td>0.20 0.23 &gt; 0.99</td>
<td>28.4 c</td>
</tr>
<tr>
<td>Root</td>
<td></td>
</tr>
<tr>
<td>0.05 0.04 &gt; 0.97</td>
<td>0.125 0.10 &gt; 0.98</td>
</tr>
<tr>
<td>0.20 0.23 &gt; 0.99</td>
<td>45.5</td>
</tr>
</tbody>
</table>

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tively), and was 12.2 µmol g⁻¹ DM in the FA treatment. The [Zn] in the spray-nutrient solution was less than 105 nM at end of the experiments, regardless of RZn.

Carbon partitioning

Relatively less biomass was partitioned to leaves at growth-limiting Zn availability (RZn = 0.05–0.2 day⁻¹) (Figure 4) than in the FA treatment, and up to 26% of the leaves showed severe zinc-deficiency symptoms at RZn = 0.05 and 0.125 day⁻¹. No significant treatment effect on stem partitioning was recorded. Biomass partitioning to roots was constant at RZn = 0.05 and 0.125 day⁻¹ at approximately 27% of total plant dry mass compared with 23% of the biomass in the FA treatment.

Both structure and function of the leaves were influenced by zinc limitation. The leaf area ratio (LAR; m² kg⁻¹ DM plant⁻¹) at RZn = 0.05 day⁻¹ was 44% of that at FA (Table 2) and specific leaf area (SLA; m² kg⁻¹ DM, leaf⁻¹) was about 50% lower at RZn = 0.05 day⁻¹ than in the FA treatment. At an RZn of 0.05 day⁻¹, net assimilation rate (NAR; kg DM m⁻² day⁻¹) was approximately 43% of that with free access to all nutrients. There were no significant treatment differences in LAR or NAR (except in the FA treatment) between the different age classes of the leaves, although the SLA of old leaves tended to be higher at RZn of 0.2 day⁻¹ than at RZn of 0.05 day⁻¹ (results not shown).

Figure 1. Relationship between relative growth rate (R_G; day⁻¹) of small birch plants and relative supply rate of zinc (R_Zn; day⁻¹). The equation of the fitted line is y = −0.007 + 1.009x with r² = 0.99. The symbols represent the mean R_G of each experiment, calculated from four determinations.

Figure 2. Dependence of R_G (day⁻¹) on the zinc concentration (µg g⁻¹ DM) of small birch plants. The equation of the fitted line is y = −0.011 + 0.009x with r² = 0.92. Open symbols represent plants at sub maximum relative growth rate and filled symbol are for plants with free access to all nutrients. Each symbol represents the mean value from three consecutive harvests.

Figure 3. Internal zinc concentration (µg g⁻¹ DM) in leaf fractions of small birch plants grown at different relative supply rates of zinc (R_Zn; day⁻¹). Free access to all nutrient elements is denoted by FA. The different fractions were: I = old leaves; II = fully developed leaves; and III = young leaves. Columns with different letters are significantly different (P = 0.01, Fisher’s LSD). Each bar represents the mean value of six analyses.

Figure 4. Dry matter partitioning to leaf (hatched), stem (solid) and root (open) fractions (percent of seedling dry mass) at different relative supply rates of zinc (R_Zn; day⁻¹). Free access to all nutrient elements is denoted by FA. Columns with different letters are significantly different (P = 0.01, Fisher’s LSD). Each bar represents the mean value of six analyses.
Table 2. Influence of R_{Zn} (day^{-1}) on leaf area ratio (LAR; m^{2} kg_{DM,plant}^{-1}), net assimilation rate (10^{-3})(NAR; kg_{DM} m^{-2} day^{-1}), and the specific leaf area (SLA; m^{2} kg_{DM,leaf}^{-1}). Values are means of five to seven measurements. Means within each column followed by different letters are significantly different (P = 0.01, Fisher's LSD). Abbreviation: FA = free access to all nutrients.

<table>
<thead>
<tr>
<th>R_{Zn}</th>
<th>LAR</th>
<th>NAR</th>
<th>SLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>8.1 a</td>
<td>5.6 a</td>
<td>15.2 a</td>
</tr>
<tr>
<td>0.125</td>
<td>11.6 b</td>
<td>10.4 b</td>
<td>22.1 b</td>
</tr>
<tr>
<td>0.20</td>
<td>17.0 c</td>
<td>11.7 bc</td>
<td>29.8 c</td>
</tr>
<tr>
<td>FA</td>
<td>18.5 c</td>
<td>12.9 c</td>
<td>30.3 c</td>
</tr>
</tbody>
</table>

At growth-limiting supply rates of zinc (R_{Zn} = 0.05–0.2 day^{-1}), the total nonstructural carbohydrate (TNC) concentration of the leaves was within the range 9.8 to 13.6% of dry mass (Figure 5), whereas with free access to all nutrients, the TNC concentration of old leaves was significantly higher at 17.4% of dry mass. In contrast, the FA treatment did not significantly increase the TNC concentration of young leaves. In roots, the TNC concentration was less than 2.1% of dry mass in all treatments with limited zinc supply rates and 4.9% of dry mass in the FA treatment. Foliar starch concentrations ranged between 5.0 and 9.2% of dry mass in all treatments. The starch concentration was significantly higher in old leaves and significantly lower in young leaves in the FA treatment compared to values at Zn limitation. The starch concentration in roots was less than 1.5% of total dry mass.

**Discussion**

In the presence of a limited zinc supply, birch displayed typical and persistent zinc-deficiency symptoms including “little leaf” and “rosette buds.” In earlier studies, where the same technique of nutrient supply was used, small *Betula* plants showed no specific visible deficiency symptoms at limited nitrogen or phosphorus supply, except during the adjustment phase to steady-state growth (Ingestad and Lund 1979, Ericsson and Göransson 1993, 1994, 1998). It seems to be a general pattern that severe and persistent deficiency symptoms do not occur at limitations that are less than 50% of the maximum relative growth rate.

Although plant relative growth rate can be controlled by regulating the relative supply rate of zinc, this can only be achieved by growing the plants in growth units free from contamination by Zn and by waiting until any initial excess Zn in the plants had been exhausted. Under these experimental conditions, plant R_{G} was approximately equal to the growth-limiting variable, R_{Zn} (Figure 1) and the coefficient of determination at limitation was generally high (≥0.96, Table 1), which is consistent with the observation that the proportion of plant biomass including damaged leaves tended to stabilize at steady-state growth within each treatment. The results suggest a positive, linear relationship (r^{2} = 0.92, Figure 2) between plant R_{G} and internal [Zn] when R_{Zn} is the growth-controlling variable. This is in agreement with results obtained when the same technique of steady-state nutrition was applied to other nutrients (e.g., Ingestad 1981, Ericsson and Ingelst 1988, Ericsson and Kähr 1993, Göransson 1993, 1994, 1998). The data do not support the negative correlation between internal nutrient status of plants and growth that is often reported (Steinbjer 1951, Hewitt 1956, Smith 1962, Bouma 1983).

Several explanations of this difference in findings have been suggested, the most plausible of which is that it is associated with differences in methodology and with non steady-state growth (Ågren 1985, Ingestad and Ågren 1992, Wikström 1994).

The calculated zinc requirement for maximum growth of small birch plants (about 0.23 day^{-1}) was 62% of that taken up when the supply was non-limiting (Figure 2, Table 1). The N:Zn ratio of 100:0.05 was somewhat different from that calculated by Ingestad (1981) to be optimal for growth of birch plants. The range between the calculated x-intercept for [Zn]
at maximum growth and the x-intercept at FA conditions (41 µg g DM⁻¹), is much smaller for zinc (about 15 µg g⁻¹) than for the micronutrients iron and manganese (≥ 150–200 µg g⁻¹) (Göransson 1993, 1994). The reason for this is uncertain, but may indicate that plants have specific mechanisms for exclusion or protection against elevated internal amounts of elements when growing with a non-limiting supply of all nutrients (e.g., Godbold et al. 1988). An increase in [Zn] of about 13 µg g⁻¹ DM resulted in a fourfold increase in RG of B. pendula. Such small changes in [Zn], resulting in large changes in plant growth rate, make it difficult to use only internal [Zn] for diagnostic purposes in the field. Information about the quantitative zinc requirement of forest trees is still sparse; but the range of supply rates within which growth limitation occurred in B. pendula is similar to that reported in Pinus radiata D. Don and several Eucalyptus species (Will 1985, Wallace et al. 1986). However, Dell and Wilson (1985) reported that Eucalyptus species differ in their sensitivity to Zn limitation. Evidently there is still a need to establish the growth-limiting [Zn] for various tree species (cf. Brennan et al. 1993).

There were no significant differences in [Zn] of the different leaf fractions at an RG of 0.05 day⁻¹, but at 0.125 or 0.20 day⁻¹, and at FA, the [Zn] was higher in younger leaves than in older leaves (Figure 3). Because small birch plants appear to translocate zinc actively toward the growing meristems at the higher Zn supply rates, it is proposed that the young meristems act as strong sinks for Zn. Although relatively small changes in macronutrient concentrations occurred in the roots at the zinc supply rates tested, higher N, P, Ca, Mg and Mn concentrations were found in leaves and stems (Table 1) at low supply rates of Zn than at high supply rates of Zn (see also Loneragan et al. 1982). Although the physiological basis for such changes is uncertain, it has been suggested that they are a consequence of a loss of control over absorption mechanisms at Zn limitation (Safaya and Gupta 1979, Loneragan et al. 1982). Because zinc acts as a component of enzymes and as a functional, regulatory, or structural cofactor of several enzymes (Marschner 1986), it is possible that all the zinc present in the plant at limited supply rates of zinc is physiologically active. The small treatment differences in Zn concentrations of the plant fractions lend support to this statement (Figure 2, Table 1).

Zinc limitation had little effect on the pattern of dry matter partitioning (Figure 4) among the leaf, stem and root fractions (Figure 4). In contrast, at the equivalent values of N, P or Fe supply (Ingelstad and Lund 1979, Ericsson and Ingelstad 1988, Göransson 1993) larger root mass ratios were recorded for B. pendula, whereas Ericsson and Kähr (1993, 1995) and Göransson (1994, 1998) found smaller root fractions at K, Mg, Mn and Cu limitation. At a zinc limitation of 0.05 day⁻¹, the plants developed a dense root system with short roots. Root growth is one of the most important factors affecting nutrient acquisition by higher plants (Clarkson 1985, Ingelstad and Ägren 1988, Marschner 1993). However, it is not evident how the growth response in terms of dry matter partitioning helped alleviate Zn limitation, because the partitioning of dry matter did not particularly favor root growth. By growing the plants at steady-state nutrition, it was possible to calculate zinc uptake rates with respect to root growth rates. I found that dZn/dW, was smaller at zinc limitation than at higher supply rates and more closely resembled the response found for Fe or K uptake than for N or P uptake (e.g., McDonald et al. 1991, Göransson 1993).

Relative growth rate may be considered in terms of its components, leaf area ratio (LAR) and net assimilation rate (NAR). Although both LAR and NAR were affected at Zn limitation, there was a gradient in the reduction of the LAR that was not apparent in the development of NAR. Specific leaf area was affected by Zn supply in a similar way to that observed in response to nitrogen or phosphorus limitation (e.g., McDonald et al. 1991). Although not measured, this may imply that the plants cannot compensate for a smaller leaf area, consistent with the observation of the “little leaf” syndrome, with increased numbers of leaves. Net assimilation rate, and probably also net photosynthetic rate, was approximately constant until the internal [Zn] was about 10 µg g⁻¹ DM. At lower foliar [Zn], physiological disturbances occurred and net assimilation rate declined rapidly. At RGs higher than 0.05 day⁻¹, changes in processes associated with structural development occurred, and at RGs equal to or lower than 0.05 day⁻¹, changes occur that influence functional development.

Generally, the foliar carbohydrate concentration is either unaffected or elevated by zinc deficiency (e.g., Marschner 1986), although Jeung et al. (1975) found low starch content in zinc-deficient Phaseolus vulgaris L. In the birch plants at zinc limitation, nonstructural carbohydrate concentrations were lower and starch concentrations remained approximately constant compared to values in plants grown with free access to nutrients. McDonald et al. (1986) observed that the starch concentration in small Betula plants tended to be high at low supply rates of nitrogen, whereas Göransson (1993, 1994) found low concentrations of starch (and other carbohydrates) in iron- and manganese-limited Betula plants. Seemingly, there is an equilibrium between carbon input and usage, because both relative root-growth rate and starch reserves were stable. However, it is unclear how such an equilibrium influences current growth because carbohydrate and starch concentrations only reflect the reserves and not the carbon flow to the growing meristems.

It is concluded that low internal [Zn] limits the growth rate of Betula pendula plants and that there is an approximately linear relationship between [Zn] and RG at sub-maximum growth rates. The effect of RG on the uptake rate of zinc per unit root growth rate was large and was 16% at an RG of 0.05 day⁻¹ compared to free access. Plants exhibited persistent and typical zinc-deficiency symptoms at RG values of 0.05 and 0.125 day⁻¹. Low values of RG at zinc limitation are associated with low values of leaf area ratio, specific leaf area and, at severe limitation, low net assimilation rate, and small changes in dry matter partitioning to shoots and roots. Limited supply rates of zinc were associated with constant and relatively low concentrations of nonstructural carbohydrate reserves; and structural development was influenced before functional disturbances occurred.
Acknowledgments

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