Genetic Variation in Nitrogen Uptake and Growth in Mycorrhizal and Nonmycorrhizal *Picea abies* (L.) Karst. Seedlings

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**ABSTRACT.** The purpose of this study was to estimate genetic variation in nitrogen uptake and growth of mycorrhizal and nonmycorrhizal seedlings from 30 open-pollinated families of Norway spruce (*Picea abies*), at two levels of nitrogen (N) supply. Six-week-old seedlings were transplanted into 1.7 L pots filled with pumice, and one-half of them was inoculated with the ectomycorrhizal symbiont *Laccaria bicolor*. A 2 × 2 factorial combination of the presence or absence of the fungus and two nitrogen concentrations (200 mg N/L and 25 mg N/L) was used during 2 wk of establishment. During the following 10 wk, no water or nutrients were supplied. At harvest (week 18) height, shoot and root dry weights, as well as the amount of nitrogen in the shoot and nitrogen acquisition capacity, were determined. There was a strong treatment effect, and most of the traits ranked in increasing order: low N, low N + Mycorrhiza, high N, high N + Mycorrhiza. With low N, plants with mycorrhiza had a shoot dry weight twice that of nonmycorrhizal plants. Significant differences among families were found for most traits in treatments without mycorrhiza, while mycorrhiza strongly reduced the number of significant family differences. The amount of nitrogen in the shoot and all biomass traits showed a significant family × mycorrhiza × nitrogen interaction. Of the two traits related to nitrogen uptake, shoot nitrogen amount showed strong genetic correlations with biomass traits in all treatments (r$_g$ within the range 0.92 to 0.99) whereas nitrogen acquisition capacity did not (r$_g$ within the range −0.53 to +0.23), which implies that the amount of nitrogen in the shoot is a better predictor of growth than nitrogen acquisition capacity. FOR. SCI. 49(2):258–267.

**Key Words:** *Laccaria bicolor*, additive variance, genetic correlations, Norway spruce.
TREE BREEDING has been used to improve yield by selection of superior phenotypes (Cornelius 1994). However, this has generally been done without detailed knowledge of how plant nutrition and mycorrhizal associations influence tree growth. Nitrogen is frequently a growth-limiting factor in Swedish forests (Tamm 1991), but we know little about how nitrogen uptake, translocation, and utilization are influenced by genetics. If they are under genetic control and different sets of genes control these processes, it may be possible to select superior performers with respect to each component. By controlled crosses, it should then be possible to combine these traits to produce trees with superior growth.

Nitrogen metabolism in plants is commonly influenced by mycorrhizal fungi, which are not only involved with nitrogen uptake, but also with water uptake. Ectomycorrhizal fungi increase the absorptive area of host root systems and can access both organic and inorganic nitrogen (Finlay et al. 1992). However, little is known about genetic variation in nitrogen uptake and plant growth in the presence of mycorrhiza (Smith and Read 1997). Marx and Bryan (1971) reported family differences in needle dry matter in Pinus elliottii seedlings inoculated with Thelephora terrestris, and Rosado et al. (1994) showed extremely high heritabilities for root traits and shoot/root ratio in seedlings of the same species inoculated with Pisolithus tinctorius. Lamhamedi et al. (1992) also showed that nutritional status and drought tolerance of Pinus pinaster seedlings varied with the Pisolithus genotype used as mycorrhizal inoculum.

Very little is known about the relationships between genetics, nutrition, and mycorrhiza in Norway spruce (Picea abies [L.] Karst). Norway spruce is one of the most important tree species in boreal and montane forests from central Europe to Asia (Schmidt-Vogt 1977) and is economically important in Sweden. The objectives of our study were to investigate genetic variation in Norway spruce seedlings grown under high and low nitrogen availability in the presence and absence of ectomycorrhizal symbiont Laccaria bicolor (Maire) Orton. Our specific objectives were to determine in growth chamber experiments: (1) plant height, shoot, root, and total dry weight, root/shoot ratios, the amount of nitrogen in shoots, and nitrogen acquisition capacity (NAC) of seedlings from 30 open-pollinated families, (2) interfamily variation and treatment effects, and (3) family by treatment interactions. NAC is defined as the amount of nitrogen in the shoot of an individual under conditions of limited nitrogen availability, relative to the mean nitrogen uptake of that individual’s family when not limited by nitrogen.

Material and Methods

Material

Thirty open-pollinated families of Picea abies from Maglehem seed orchard located in southern Sweden (55°50’N, alt. 60 m asl) were studied in this experiment. The use of this material provides the possibility of future comparisons with data from three field trials established in 1978 with material from the same seed orchard.

The ectomycorrhizal symbiont Laccaria bicolor (Maire) Orton, strain 238, from the laboratory of Prof. J. Trappe, Oregon State University, originally isolated under Tsuga mertensis in Oregon, USA, was used for inoculation. This species was chosen because it grows at Picea abies sites in Sweden and infects roots of Picea abies successfully at relatively high soil nitrogen concentrations (Wallander and Nylund 1992). It has a prolific growth habit in culture and tolerates homogenization, making it suitable for inoculum production for a large-scale genetic experiment.

Experimental Design

The material was studied in a $2 \times 2$ factorial design combining high (200 mg N/L) and low (25 mg N/L) nitrogen treatments in the presence or absence of the ectomycorrhizal fungus. Due to space limitation, the whole experiment was carried out with 4 replications in time to allow sufficient material for statistical analysis. The 30 families were each replicated 5 times per treatment and per replication in time; 30 families × 4 treatments × 20 blocks (4 replications in time × 5 blocks per replication), giving a total of 2,400 plants or 20 plants per family per treatment. In each replication in time, seedlings were arranged in a randomized complete block design in the growth chamber. This design was aimed to extract possible variation due to replication in time or position in the chamber as a block effect.

Our study with only one strain of mycorrhizal fungus required 4 replications in time in a 6.4 m L × 4.5 m B × 2.1 m H growth chamber. A combined study with 30 families of the host plant and several fungi is impossible to conduct, both for reasons of space and the difficulty in starting an investigation at too complex a level.

Cultivation in Growth Chambers

Seeds were sown in a sowing mix made up of fine gravel, sand, perlite, and vermiculite (8:4:3:15). Seedlings were transplanted at an age of 6 wk (see Figure 1) into 1.7 L black plastic pots of 14 cm diameter and 16.5 cm high filled with pumice of 1 mm particle size. Mycorrhizal plants were inoculated by dipping roots in a suspension of fungal mycelium before potting (Kähr and Arveby 1986). Inoculum was prepared from 6-wk-old fungal mycelia cultivated in Pachlewski (Pachlewski and Pachlewski 1974) liquid culture. The mycelia were rinsed to remove residual nutrients, homogenized with the aid of a blender with rotating knives in 1 L deionized water and portioned out in equal volumes to ensure even inoculum concentration.

A study of variation in nitrogen uptake must be carried out such that the amount of nitrogen available in the substrate is

Figure 1. Schematic illustration of one of the four replications in time of the experiment.
known. Therefore, regular watering of the plants until harvest could not be carried out. Instead, we supplied plants with a defined amount of nutrients at the start of the experiment.

During the subsequent 2 wk of establishment, the pots were watered to saturation with balanced nutrient solution containing 200 mg N/L (high N) or 25 mg N/L (low N), depending on the designated treatment. With an average solution volume of 968 mL needed to saturate each pot, the starting amount of nitrogen for the high nitrogen treatment was 194 mg and for the low nitrogen, 24 mg. The nutrient solution contained a mixture of ammonium nitrate and potassium nitrate. Macroelement proportions by weight were 100 N (39 NH₄+−N and 61 NO₃−−N), 84.3 K, 19.6 P, 5.9 Ca, 7.8 Mg, 7.8 S, and microelements according to Ingestad and Lund (1986). The pH of both nutrient solutions was about 4.5.

A 10 wk period without any further fertilization or watering allowed the plants ample opportunity to express their genetic differences with regard to nitrogen uptake, depending on their ability to find and exploit nutrients available in the pot. Drought conditions were avoided by using large pots with a capacity to hold enough wet substrate, covering them with black plastic sheets to avoid evaporation from substrate surface and 90% relative humidity in the chamber. Night length was 3 hr, day and night, temperature was 20°C. A light source with 250 W daylight lamps was used, and light intensity within the spectrum range 400–700 nm was 300 μmol.m⁻².s⁻¹ at plant level.

**Traits Assessed in the Growth Chamber**

Plant height from the cotyledon to the terminal bud was measured 13 and 18 wk after sowing. The length of the elongated hypocotyl was measured and tested as a covariate in the analysis. Shoot dry weight was assessed after drying to constant weight in an oven for 40 hr at 70°C. Root dry weight was measured after 1 wk of drying to constant weight in a freeze drier. The latter method was used to preserve the integrity of ergosterol, which would later be measured to determine the amount of fungal biomass present in the root. The amount of nitrogen in the shoot was determined after grinding the shoot and using an elemental combustion analyzer (NA 1500, Rodano, Italy). Unless otherwise stated, all height, biomass, and nutrient uptake measurements were made at the end of the experiment, wk 18. Nitrogen acquisition capacity for each individual plant was calculated by dividing the amount of nitrogen in the shoot at low N or low N + mycorrhiza by the mean amount of nitrogen in the shoot of that family at high N (free access). NAC for high N with mycorrhiza was calculated in a corresponding way for the sake of comparison.

**Statistical Analyses**

Estimation of variance components and parameters in the model was based on mixed model equations (MME) and the restricted maximum likelihood (REML) method, using the "Mixed" procedure in the SAS software (SAS 1997). MME gives the best linear unbiased predictors (BLUP) of the random effects. To obtain a normal distribution of the residuals and to reduce scaling effects in the joint analysis, the variables were logarithmically transformed prior to the analysis. The following linear models were used for (1) separate analyses of individual treatments, (2) joint analyses of the two low N or high N treatments, and (3) the joint analyses of the four treatments together.

**Model 1. Separate analyses:**

\[ y_{ij} = \mu + ah_{ij} + b_i + f_j + e_{ij} \]

**Model 2. Joint analyses at each of the N-levels:**

\[ y_{ijk} = \mu + ah_{ijk} + b_i + f_j + z_k + fz_{jk} + e_{ijk} \]

**Model 3. Joint analyses:**

\[ y_{ijkl} = \mu + ah_{ijkl} + b_i + f_j + z_k + n_l + fz_{jk} + fn_{jl} + fnz_{jkl} + e_{ijkl} \]

where

- \( y_{ij}, y_{ijk}, y_{ijkl} \) = values of single observation
- \( \mu \) = grand mean
- \( a \) = regression coefficient for elongated hypocotyl length
- \( h_{ij}, h_{ijk}, h_{ijkl} \) = fixed effect of elongated hypocotyl length for each individual seedling \( ij, ijk, ijk \) respectively, used as a covariate.
- \( b_i \) = fixed effect of block \( i \)
- \( n_l \) = fixed effect of nutrient regime \( l \)
- \( z_k \) = fixed effect of mycorrhiza association \( k \)
- \( f_j \) = random effect of family \( j N \sim (0, \sigma^2_f) \)
- \( fn_{jl} \) = random interaction effect of family \( j \) and nutrient regime \( l N \sim (0, \sigma^2_{fn}) \)
- \( fz_{jk} \) = random interaction effect of family \( j \) and mycorrhiza association \( k N \sim (0, \sigma^2_{fz}) \)
- \( fnz_{jkl} \) = random interaction effect of family \( j \), mycorrhiza association \( k \) and nutrient regime \( l N \sim (0, \sigma^2_{fnz}) \)
- \( e_{ij}, e_{ijk}, e_{ijkl} \) = random error terms \( N \sim (0, \sigma^2_e) \)

The families were considered half-sibs, and the additive genetic variance \( (\sigma^2_A) \) was calculated as:

\[ \sigma^2_A = 4\sigma^2_f \]
Additive genetic coefficients of variation were calculated as:

$$CV_A(\%) = \frac{100 \times \sqrt{4\sigma^2_y}}{\bar{x}}$$

where \(\bar{x}\) is trait mean value. The calculation of \(CV_A\) was based on original values.

To estimate the contribution of each of the families to the interaction variances, the ecovalence value (Wricke 1962) for each family was calculated. The ecovalence value is the interaction sum of squares for each family expressed in percent of the total interaction sum of squares. The ecovalence values were calculated on the individual family solutions to the linear models for separate treatments described above. The ecovalence analyses were carried out on shoot dry weight as an example of traits that showed a significant family \(\times\) mycorrhiza \(\times\) nitrogen interaction. Shukla’s stability variance (Shukla 1972) is linearly related to the ecovalence value by Wricke (Kang and Miller 1984). Expressed in percent, the two values will be identical for each genetic entry. The ecovalence values can thus be tested for statistical significance using the method described by Shukla (1972).

Genetic correlations were calculated using ASREML software and based on mixed linear models

$$r_g = \frac{\sigma_{A_{xy}}}{\sqrt{\sigma^2_{A_x} \sigma^2_{A_y}}}$$

where

$$\sigma_{A_{xy}} = \text{covariance between trait } x \text{ and } y$$

$$\sigma^2_{A_x} = \text{additive genetic variance of trait } x$$

$$\sigma^2_{A_y} = \text{additive genetic variance of trait } y$$

Standard errors of the estimates were calculated by the ASREML program from average information matrix using a Taylor series approximation (Gilmour et al. 1998).

**Results**

Roots of plants in all four treatments were examined microscopically for the presence or absence of mycorrhiza at harvest. Most short roots of mycorrhizal plants were surrounded by the characteristic lilac mantle formed by *Laccaria bicolor*. This was absent on the nonmycorrhizal roots. No other fungal type was visible. The substrate from pots containing mycorrhizal plants was characteristically lumpy, reflecting matrices of fungal mycelia holding the grains of the pumice substrate together. This was absent in the substrate of nonmycorrhizal plants.

Plant survival in all treatments was high; only 8 individuals out of the 2,400 did not survive. High N + Mycorrhiza treated plants had more highly branched shoots than plants in the high N treatment. As seen from Table 1, there was a strongly significant effect of mycorrhiza on most traits at each level of nitrogen, but the mycorrhiza effect was stronger at low N than high N. Low N plants were smaller, with reduced needle length and reddish needles, while low N + mycorrhiza plants were characterized by pale-colored needles in their tops. Carbon allocation to the roots was favored under low N conditions, (45% and 48% root of total DW for low N + Mycorrhiza and low N, respectively, as can be calculated from Table 2). The mean shoot nitrogen concentration in the high N plants (25 mg/g dry weight) was slightly reduced by mycorrhiza (23 mg/g dry weight). In the low N plants, the shoot nitrogen concentration was almost twice as high in plants without mycorrhiza (14 mg/g) as in mycorrhizal plants (8 mg/g).

For most traits, the ranking of the treatments was low N, low N + Mycorrhiza, high N, high N + Mycorrhiza. The difference between the two low N treatments was small for NAC (Table 2). A comparison of the mycorrhizal effects on NAC and shoot dry weight shows that there was a limited effect on the NAC, whereas there was a doubling of the dry weight under the low N treatment with mycorrhiza (Table 2). The amount of N incorporated in shoot biomass in the low nitrogen treatment was only slightly higher in the presence of mycorrhiza (2.23 mg) compared to plants grown without this association (2.02 mg) (Table 2).

The variation in NAC within each of the two low N treatments was large, as is evident from Figure 2 and Table 2, in which variances are expressed as a percentage of the total variance. There was a significant family variation in shoot dry weight in all treatments except for the high N + Mycorrhiza (Table 2). The family variation in height at 13 wk, both root

| Table 1. Treatment effect of mycorrhiza—traits, F-ratios and level of significance for low- N and high-N treatments. Level of significance is denoted by: * = 0.05 > P ≥ 0.01, ** = 0.01 > P ≥ 0.001, *** = P < 0.001. Statistical model 2 was used. |
|---------------------------------|----------------|----------------|----------------|
| **Traits**                      | **Low-N and Low-N+mycorrhiza** | **High-N and High-N+mycorrhiza** |
| Height (13 wk), Ht              | 673***         | 5*             |
| Height, (18 wk), Ht             | 768***         | 25***          |
| Shoot dry weight, SDW           | 374***         | 52***          |
| Root dry weight, RDW            | 227**          | 37***          |
| Total dry weight, TDW           | 337***         | 50***          |
| Root-shoot ratio, RSR           | 44***          | 5*             |
| N concentration                | 1165***        | 50***          |
| N amount, NA                   | 14***          | 20***          |
| N acquisition, NAC             | 5*             |                |

* NAC with high-N is not indicated because uptake of nitrogen at high-N was used as the reference.
and shoot dry weights, and NA was much larger in the two nonmycorrhizal treatments than in the two treatments with mycorrhiza (Table 2). The coefficients of additive variance for all traits were relatively large, varying between 8.95 and 27.01% (Table 2).

As is seen from Figures 2–4, there are several rank changes between the two low N treatments. For several traits, poorly performing families showed a more positive response in the corresponding mycorrhizal treatment (e.g., family 23, Figures 2–4).

There was a significant family × mycorrhiza × nitrogen interaction for height at 13 wk, total dry weight, shoot dry weight, root dry weight, and NA (Table 3). For NA this interaction must largely be attributed to the family × mycorrhiza interaction at the low N level as seen from the results of the ANOVAs calculated separately for the low N and high N, respectively (Table 3). Similarly, for NAC, the family × mycorrhiza interaction was significant in the joint ANOVA at the low N level only (Table 3). Only one family contributed significantly to the interaction for shoot dry weight (Figure 2).

Figure 2. The family variation in nitrogen acquisition capacity in the two low N treatments; 25 mg N/L. The families are ranked according to the values in low N treatment without mycorrhiza. Nitrogen acquisition capacity (NAC) is defined as the amount of nitrogen in the shoot of an individual under conditions of limited nitrogen availability, relative to the mean nitrogen uptake of that individual's family under free access to nitrogen.
5). For this trait, we have illustrated the three lowest and the three highest contributing families to the three-way interaction (Figure 6). For each treatment, the family deviation from the mean was standardized by dividing it by the standard deviation in each treatment. The pattern of these six families is supported by their estimated ecovalence values. No family combined stability and good growth. Family 11 was closest to an ideal situation: it maintained a fairly high stable performance across the four treatments.

The genetic correlations among traits that had significant family effects were all strong except for correlations involving NAC (Table 4, bold). Generally the correlations between traits with significant family effects (see Table 4) were stronger than the corresponding correlations involving one or two traits with nonsignificant family effects.

**Discussion**

**Uptake of Nitrogen**

We only know of two studies on the genetic variation in uptake of a nutrient and its impact on growth in a tree species in the presence and absence of mycorrhiza (Marx and Bryan 1971, Rosado et al. 1994). Both studies had a fairly limited number of families, 7 and 15, respectively. Ideally, genetic studies on uptake and its effect on growth should comprise several genetic entries of the host plant and several different fungi.

**Table 3.** Joint ANOVA - traits, variance components for random effects as percent of the total random variation ± standard errors and coefficients of additive variation. $\sigma_{f}^2$, $\sigma_{fz}^2$, $\sigma_{fn}^2$ and $\sigma_{fzn}^2$ are variance components for family, family × mycorrhiza interaction, family × nitrogen interaction and family × mycorrhiza × nitrogen interaction. Level of significance is denoted by: * = 0.05 > P ≥ 0.01 and ** = 0.01 > P ≥ 0.001. Statistical model (3) was used except in cases marked (2) where model 2 was used.

<table>
<thead>
<tr>
<th>Trait</th>
<th>$\sigma_{f}^2$</th>
<th>$\sigma_{fz}^2$</th>
<th>$\sigma_{fn}^2$</th>
<th>$\sigma_{fzn}^2$</th>
<th>CV, %</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (13 wk), ht</td>
<td>1.46 ± 1.39</td>
<td>0</td>
<td>1.96</td>
<td>3.10*</td>
<td>9.47</td>
<td></td>
</tr>
<tr>
<td>Height (18 wk), ht</td>
<td>1.86 ± 1.18</td>
<td>0</td>
<td>1.14</td>
<td>1.20</td>
<td>7.25</td>
<td></td>
</tr>
<tr>
<td>Shoot dry weight, SDW</td>
<td>2.86 ± 1.51</td>
<td>0.85</td>
<td>0</td>
<td>3.58*</td>
<td>7.63</td>
<td></td>
</tr>
<tr>
<td>Root dry weight, RDW</td>
<td>2.09 ± 1.37</td>
<td>0</td>
<td>1.06</td>
<td>2.87*</td>
<td>11.26</td>
<td></td>
</tr>
<tr>
<td>Total dry weight, TDW</td>
<td>3.06 ± 1.54*</td>
<td>1.46</td>
<td>0</td>
<td>4.27*</td>
<td>9.45</td>
<td></td>
</tr>
<tr>
<td>Nitrogen amount, NA</td>
<td>0.83 ± 1.10</td>
<td>0.67</td>
<td>0</td>
<td>3.86*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Nitrogen amount, NA</td>
<td>0</td>
<td>7.12**</td>
<td>—</td>
<td>—</td>
<td>0</td>
<td>Low-N only (2)</td>
</tr>
<tr>
<td>Nitrogen amount, NA</td>
<td>2.73 ± 1.75</td>
<td>1.43</td>
<td>—</td>
<td>—</td>
<td>9.86</td>
<td>High-N only (2)</td>
</tr>
<tr>
<td>N acquisition capacity, NAC</td>
<td>6.20 ± 2.68*</td>
<td>3.52</td>
<td>0</td>
<td>0.34</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>N acquisition capacity, NAC</td>
<td>6.22 ± 3.30</td>
<td>5.87*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Low-N only (2)</td>
</tr>
</tbody>
</table>
The starting average volume of nutrient solution per pot was 968 mL, and this gave an amount of nitrogen of 24 mg for the low N treatment and 194 mg for the high N treatment with concentrations of 25 mg/L and 200 mg/L, respectively. Based on data from a preliminary experiment, approximately 16 and 45% of the initial solution volume in the pots remained at harvest in the high N and low N treatments, respectively. The average amount of nitrogen recovered from the shoot was marginally higher in the mycorrhizal treatments than in the two corresponding nonmycorrhizal treatments (Table 2) and constituted approximately 10% of the nitrogen pool available at the beginning of the period with no watering.

The mean shoot nitrogen concentration in the high N treatment, 23 and 25 mg N/g dry weight in mycorrhizal plants and nonmycorrhizal plants, respectively, was slightly higher than in similar aged *Picea abies* seedlings grown with free access to N in a hydroponic system (Ingestad and Kähr 1985). Thus it may be concluded that the nitrogen regime created in the high N pots was sufficient to meet the nitrogen requirement of our test plants in this treatment (free access conditions). Larger differences might otherwise have been expected between mycorrhizal and nonmycorrhizal plants, but there is some indication that *L. bicolor* is less efficient at transferring assimilated N to its plant host than other ectomycorrhizal species (Gorissen and Kuyper 2000).

NAC is an estimate of how efficient a genetic entry is at taking up nitrogen under limited nitrogen availability, relative to the uptake with free access to nitrogen. The idea behind this estimate is that the uptake at free access might vary among genetic entries, which was the case in our study as seen from Table 2, and that uptake under conditions of limited availability has to be related to the maximum uptake at free access. A problem with the estimation of NAC as a ratio between the uptake at low and high N is that a family with a low uptake of nitrogen in the high N treatment has a higher probability of having a high NAC estimate. This is clearly seen from the negative relationship between NAC in the low N + Mycorrhiza treatment and NA in the high N treatment (\(R^2 = 0.51\), Figure 7). A similar but weaker (\(R^2 = 0.30\)) agreement was obtained for the same relationship in the low N treatment without mycorrhiza.

**Figure 5.** The ecovalence values in percent of individual families for shoot dry weight, the family contributing significantly to the interaction has filled bar.

![Figure 5](https://example.com/figure5.png)

**Figure 6.** Illustration of the G × E interaction for the three most and least contributing families as regards shoot dry weight. For each treatment, the family deviation from the mean was standardized by dividing it by the standard deviation in each treatment. The ecovalence values (%) of the six families are given in parentheses.

![Figure 6](https://example.com/figure6.png)
Another disadvantage with NAC is that it is expected to vary depending on the degree of limitation of a nutrient element, and for that reason, the availability must always be specified for estimates of NAC. Since the same seedling cannot be studied in two different treatments, the denominator in the calculation of NAC is the same for all plants of each family, which is a further disadvantage. As seen from Table 4, NAC was not a good predictor of biomass or height whereas amount of nitrogen, NA, was. Therefore, NA is probably a more useful estimate of uptake since it is a direct assessment of the amount in the shoot. It cannot be ruled out that NAC, estimated for a less severe deficit of nitrogen and for other mycorrhizal fungi, would give more useful estimates.

The family variance components for NAC in the two low N treatments (Table 2) were larger than for most of the other traits, a fact which should be considered before NAC is ruled out as a useful estimator of nitrogen uptake.

### Interfamily Variation and Treatment Effects

In studies of young material, there is always a risk that maternal effects such as seed weight bias the genetic effects. There are conflicting reports on the duration of the maternal effects in conifers ranging from a limited part of the first growth period (Fries 1989) to several years (Perry 1976). The use of seedling weight at the start of the experiment as a covariate in the analyses is one way to overcome maternal effects. However, this was not feasible in our case because of practical difficulties of disentangling roots from substrate and removal of water before weighing. The risk of root injury and drought damage would have been unavoidable. Based on these concerns, we decided to use the length of the elongated hypocotyls as a covariate in the ANOVAs.
As seen from the ANOVAs of individual treatments, there was a large family component both for shoot dry weight and NAC in most treatments (Table 2). This is in agreement with the results of Marx and Bryan (1971) who found significant differences among seven Pinus elliottii families inoculated with Thelephora terrestris. Four of the seven families were inoculated with Pisolithus tinctorius without finding any significant difference among them, although their growth was significantly increased compared to the control. However, with such a low number of families it is hard to prove any differences.

It is striking that the mycorrhiza association reduced the family differences in many traits at both levels of nitrogen (Table 2). This was particularly pronounced for root dry weight since the family variance component in the two treatments with mycorrhiza was below 3%. The coefficients of additive variance were relatively large (Table 2) but somewhat lower than reported by Sonesson and Eriksson (2000) and Sonesson et al. (2002) in their studies of juvenile materials of Pinus sylvestris and Picea abies, respectively.

The positive effects of mycorrhiza on growth are striking both at the low and high level of nitrogen treatment (Table 2). It is evident that this strain of Laccaria bicolor is not particularly efficient at transferring N to its host (cf. Gorissen and Kuyper 2000) and may even have sequestered N within its own mycelium as reported by Colpaert et al. (1996) for the ectomycorrhizal fungus Scleroderma citrinum. However, the mean value of 1.2 for NAC in the high N + Mycorrhiza treatment indicates that mycorrhiza improved nitrogen uptake even under high N conditions.

Although there was only a small difference in average NAC between the two low N treatments there was a doubling of the shoot dry weight (277 versus 137 mg) in the treatment with mycorrhiza. Nor was there any large difference in NA between the means in the two low N treatments (2.02 and 2.23 mg). It could be speculated that the difference in shoot dry weight between the two low N treatments may be attributed to improved water uptake by the mycorrhizal fungus during the 10 wk period without any irrigation. Plants with a good ability to penetrate the substrate, either with the aid of a large root system in combination with mycorrhiza mycelia or just with a large mycelium, would be favored in the presence of a large substrate volume with limited amounts of nutrients and water in the substrate as in our study. The difference in height between the high N + Mycorrhiza and the high N treatment increased from 4.7% to 11.4% during the last 5 wk of the experiment. During this period, availability of water probably became more important and thus lends some support to our speculation that the main contribution from mycorrhiza to improved growth is uptake of water. Our interpretation differs from the general interpretation that mycorrhizal fungi mostly improve the uptake of nutrients in host plants (e.g., Plassard et al. 2000). However, as early as 1935, Cromer suggested that mycorrhizal colonization increased drought tolerance of Pinus radiata seedlings (Cromer 1935). Later, Boyd et al. (1986) and Lamhamedi et al. (1992) supplied direct evidence for increased drought tolerance in mycorrhizal associations.

**Family × Treatment Interaction**

Only one family contributed significantly to the family × mycorrhiza × nitrogen interaction for shoot dry weight (Figure 5). This agrees with observations for the same material in a 2 × 2 factorial study with two temperature levels and two watering regimes (Sonesson et al. 2002). Breeding will be facilitated since the majority of families had low ecovalence values. A culling of the few parents with high ecovalence values would result in a stable breeding population. However, such a culling would in our case result in a loss of parent 23, one of the best performing families in the low N treatment with mycorrhiza (cf. Figure 4). Since nitrogen is one of the most limiting factors for growth of Picea abies in Sweden (Tamm 1991), the results from the joint ANOVAs at the low N treatments for NAC and NA are of particular interest for applied forestry. The strong impact of mycorrhiza on NA and NAC at low N is evident from the significant family × mycorrhiza treatment effects ($P < 0.01$ and $P < 0.05$, Table 3). The corresponding interaction for NA at high N was not significant. It is evident that the mycorrhizal association has a great impact on host plant performance.

**Trait Relationships**

If the predictive power of NA for field growth turns out to be high, the almost perfect correlations between biomass traits and NA (cf. Table 4) would be of great significance for development of low-cost early tests. Any dry weight trait could be used instead for NA in early tests, which would eliminate the extremely costly nitrogen analyses. The correlations between biomass traits and between NA and biomass traits in the two treatments with mycorrhiza were higher ($r_g$ within the range 0.76 to 0.99) than expected from the nonsignificant family effects of some of the traits involved in these correlations. NAC is evidently not a good predictor of any of the growth traits as seen from its low or even negative correlation coefficients in their relationships.

**Conclusions**

1. Larger Picea abies seedling could be produced by inoculation with L. bicolor across families and nitrogen availabilities. However, the response to mycorrhiza was higher with low than high nitrogen availability. The results may be applied in nurseries to produce large seedlings.

2. More poorly growing families in the nonmycorrhizal treatment with low nitrogen availability responded more strongly to mycorrhiza than better growing ones. Probably poorly growing families are better at forming mycorrhiza that in turn improved their water uptake. The strong growth response in lower ranking families caused a reduction in family variance for several growth traits in the mycorrhizal compared to the non-mycorrhizal treatments.

3. The potential exists for selecting families with high nitrogen uptake, one of the components that could be combined with others to obtain superior growth. This is desirable since nitrogen uptake was strongly correlated with several growth traits.
Literature Cited


