Nitrogen Productivity as a Predictor of Growth in *Pinus sylvestris*

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Jonsson, A., Eriksson, G. 2002. Nitrogen Productivity as a Predictor of Growth in *Pinus sylvestris*. *Baltic Forestry*, 8 (1): 8–14

Results from progeny field trials with *Pinus sylvestris* and three growth-chamber experiments using different methods of controling nutrient addition and calculating nitrogen productivity (the amount of biomass produced per unit nitrogen and time) were compared. The aim was to investigate if important nutrient-related traits as nitrogen productivity could be useful in early tests as indicators of future production, if the more sophisticated and expensive methods of estimating nitrogen productivity could be replaced by simplified and cheaper ones and if simulating of field conditions would improve the relationships between juvenile and adult phase. Moderately strong relationships (up to R^2 =0.68) between juvenile traits from the three growth-chamber experiments were obtained in several cases but generally the pattern was inconsistent and therefore difficult to interpret. Nitrogen productivity estimated by approximative methods did not show strong relationship with nitrogen productivity estimated by the Ingestad's method. The relationship between N productivity and five field growth traits improved considerably when the juvenile material was grown with the ectomycorrhizal fungus *Laccaria bicolor* (R^2= 0.36-0.52) compared to the treatment without mycorrhiza (R^2 = 0.03-0.07). This indicates that simulating of field conditions in growth chamber could increase the reliability of early tests. However, none of the relationships with field was significant and the values were too low to be used in breeding.

Key words: Scots pine, *Laccaria bicolor*, mycorrhiza, nitrogen productivity, nitrogen utilization, growth chamber, growth traits/components, early test.

Introduction

Forest tree breeding could be made more efficient if there was a method for screening materials at young age for superior growth at mature age. We want to identify juvenile traits which are good predictors of adult growth capacity. One group of traits which might be tested as such predictors consist of components of plant nutrient response like nitrogen productivity and utilization.

This can be done efficiently by means of retrospective early tests where performance of progenies in growth chamber or nursery tests is related to performance of their siblings in field trials. However, early test studies have sometimes resulted in poor relationships indicating that prediction of future production would not always be reliable (cf. Jonsson 2000).

There are several potential reasons for the weak J-M relationships. One explanation might be the large difference between field and test conditions as regards nutrient availability. Some reports indicate that better agreement could be obtained if field conditions (or at least the limiting factor there) were simulated cf. Li et al. (1991), Dewald et al. (1992). We are mainly interested in the relationship between nitrogen (N) availability and growth as nitrogen is often growth-limiting factor in Swedish forests (Tamm 1991).

Further, nutrient studies have often been carried out by using methods which did not control nutrient supply in an appropriate way. Ingestad (1979) claimed that the relationship between nutrient supply and growth should be studied under steady-state conditions, under which there is an equilibrium between the supplied and used nutrients so the internal concentration of nutrient elements, e. g. nitrogen is held constant. He coined the term nitrogen productivity, which is the rate of growth per unit N taken up by a plant.

The method for studies of nitrogen productivity as developed by nutrient physiologists (i. e. using hydroponic culture in Ingestad's growth units) is not suitable for large-scale genetic experiments, where many families and many individuals per family are included. Therefore, an effort has been made to develop a simplified method for estimation of nitrogen productivity which is based on the principles of Ingestad but modified for genetic studies (Jonsson et al. 1997, Abraitis

et al. 1999). As growth in the 1st growth period (GP) and growth in the consecutive GPs differ in conifers, we usually study plants in at least two growth periods and in a solid substrate. Apart from the way of nutrient supply, those are the main differences from the method by Ingestad.

The first question to be answered was if the results from experiments with different degree of simplification of the original method were in agreement with the results obtained in an experiment carried out according to the original intentions (Ericsson and Kähr, manuscript). The second question was whether nitrogen productivity is related to growth capacity of the young and/or adult material. In other words, can it, or other juvenile characters, be used for prediction of production in field.

Considering the presence of mycorrhizal fungi in field, we were also interested in the influence of mycorrhiza on nitrogen-related traits and growth. Ectomycorrhizal *Pinus sylvestris* seedlings inoculated with *Suillus bovinus* showed similar nitrogen productivity as non-mycorrhizal seedlings according to Ingestad et al. (1986).

The aim of this study was to relate juvenile traits, mainly nitrogen productivity, estimated in Ingestad's growth units for seven families of *Pinus sylvestris* to approximate nitrogen productivity estimated in a simplified way in two previous growth chamber studies (Jonsson et al. 1997, Abraitis et al. 1999). If strong relationship exists between the values compared, it will enable us to use the simplified method for screening materials. Another aim was to relate the juvenile traits to growth traits from field trials and to study if mycorrhiza at the juvenile stage will improve juvenile-mature (J-M) correlations.

Material

Seven open-pollinated families of *Pinus sylvestris* from a seed orchard in central Sweden (Långtora, 30 km south-west of Uppsala) were studied in three experiments (Exp. 1-3) in growth chambers, cf. Abraitis et al. (1999). The families included in the comparison constitute a representative sample as regards their nitrogen productivity assessed in Exp.1 (N $prod_{approx\ 1}$). The mother clones represent plus-trees from southerly Sweden and Finland (latit. 57°50" - 61°25" N). Progenies from those clones were included in progeny tests established in central Sweden in 1981.

Methods

Cultivation and treatment

Exp. 1 and 2.

The seedlings in the two previous studies (Exp. 1 and 2) were grown in a growth chamber for two growth periods under two nutrient treatments (Jonsson et al. 1997, Abraitis et al. 1999). Free access (100 mg N $\cdot l^{-1}$ nutrient solution) and a restricted access to nitrogen calculated to supply about one third of the requirement expected under the given conditions of cultivation were used. It was carried out by watering the substrate with deionized water and subsequent pipetting of 2 ml of balanced nutrient solution to each plant starting after a 6-week period of establishment at free access. N doses given each other day followed a dose curve calculated in advance according to the principles of Ingestad (cf. Jonsson et al. 1997). In Exp.1 and 2, 18 and 21 seedlings respectively were studied per family and treatment.

Exp. 3

In Exp. 3 the original method by Ingestad was used (cf. Ingestad 1979) for studies of variation in N productivity in *Pinus sylvestris* seedlings in presence (m+) and absence (m-) of ectomycorrhizal fungus *Laccaria bicolor* (cf. Kähr & Arveby 1986). Totally, 42 replications with and 42 replications without mycorrhiza fungus were carried out in a growth chamber in Ingestad's units, i. e. in a hydroponic growth system (Ingestad & Lund 1986). Three relative addition rates of nitrogen R_N (Ericsson & Kähr, manuscript) were used - besides the free access with $R_N = 0.06 \cdot day^{-1}$ - suboptimal addition rates 0.04 and 0.03 $\cdot day^{-1}$ were used in order to obtain plants with different, but stable, tissue nitrogen concentration. Light intensity was about 320 $mE \cdot m^{-2} \cdot s^{-1}$ from OSRAM HQI lamps and 6 h night, 20/15°C day/night temperatures and 70% relative air humidity were used. The experiments lasted between two and three months, depending on the nitrogen limitation. For each family 2 growth units ą 60 plants x 3 N treatments x 2 treatments - with resp. without mycorrhiza - were used, that gives in total 720 plants per family. In all, four harvests were performed at which relative growth rate (RGR) was calculated - the first one after lag-phase (at the start of the experiment) followed by three harvests (12-16 plants each) used also for N analyses. N productivity (N prod) was determined according to the rules developed by Ingestad, i. e. as the slope of the regression between RGR of the seedlings and the needle nitrogen concentration.

Juvenile traits assessed in growth chamber

Juvenile traits assessed in growth chamber and evaluated in this study are shown in Table 1.

R/s ratio is root DW/shoot DW.

Nitrogen utilization is defined as plant biomass

produced per unit nitrogen in the needles (Abraitis et al. 1999) and calculated by the equation:

$$\text{N utiliz} = \frac{DW}{DW_{needle} \cdot \text{N conc}}$$

where DW is total plant weight (g), DW_{needle} is needle dry weight (g) and N conc is nitrogen concentration in needles (mg N· g DW $^{-1}$) corrected for the structural N (cf. Ingestad and Kähr 1985).

Nitrogen productivity is defined as plant biomass produced per unit nitrogen in the plant (in our case N in the needles) per unit time (cf. Ingestad 1979). In the two published studies two different approximate methods were developed.

Nitrogen productivity in the three experiments was calculated by means of three methods as described below.

Exp. 1

In the first study nitrogen productivity ($N\ prod_{approx\ 1}$) was calculated for 21 families of *Pinus sylvestris*. In this experiment no harvests of extra plants could be carried out as the volume of the experimental material was already large. $N\ prod_{approx\ 1}$ was expressed per day and based on the period of intensive growth during the second growth period (cf. Jonsson et al. 1997).

Exp. 2

In the second study, except for harvests at the end of growth period 1 and 2, three extra harvests were carried out during the most intensive growth in second growth period and the calculation of nitrogen productivity ($N\ prod_{approx\ 2}$) was based on them - (Abraitis et al. 1999). This was possible because only seven families representative as regards their N $prod_{approx\ 1}$ in Exp. 1 were included.

$N\ prod_{approx\ 2}$ (g DW·mg N^{-1}·day $^{-1}$) for each family was calculated by the equation:

$$N\ prod_{approx\ 2} = \frac{RGR}{\text{N conc}}$$

where:

RGR - relative growth rate between the two harvests in question (g ·gDW^{-1} · day $^{-1}$),

N conc - nitrogen concentration in needles estimated at each harvest (mgN·gDW$_{needles}^{-1}$)

RGR was calculated by the equation:

$$RGR = \frac{\ln \frac{R_{DW}n}{R_{DW}m}}{t}$$

where: $R_{DW}n$ - relative total dry weight at harvest "n" (g), $R_{DW}m$ - relative total dry weight at some earlier harvest "m" (g) and t - number of days between harvests "m" and "n".

The relative dry weight (R_{DW}) at harvest "m" and "n" was calculated by the equation:

$$R_{DW}m \text{ or } n = \frac{DW_{m \text{ or } n}}{DW_{in}}$$

where: $DW_{m\ or\ n}$ - total seedling dry weight at harvest "m" or "n"(g), DW_{in} - initial total dry weight (g). Initial total dry weight was regarded as 20% of the fresh weight at the time of transplanting when the plants were two weeks old (cf. Ingestad & Kähr 1985). $N\ prod_{approx\ 1}$ was calculated in this experiment as well.

Exp. 3

In Exp. 3 the original method by Ingestad was used (cf. Ingestad 1979) for studying and calculating nitrogen productivity (N prod). By plotting ln plant DW at the four harvests against day number, we obtained the slope of the regression line giving the RGR (g·g^{-1}·day^{-1}). For calculation of N productivity the RGR value for each growth unit was plotted against mean needle N concentration from the three harvests and a new regression was calculated for each family, with and without mycorrhiza. The slope of that regression line gives N prod (gDW · gN^{-1} · day^{-1}).

Traits assessed in field

The traits studied were height at 13 and 17 years (H13 and H17), mean yearly height increment (HI 13-17), diameter at breast height (DBH17) and volume (V17) at age 17. Stem volume was calculated according to Brandel (1990) using DBH and tree height at age 17. Mean values of traits across the four existing trials for the three mother and four father clones were used.

Statistical analyses

Strength of relationships between the results from the three growth chamber studies and between the growth chamber studies and field trials was estimated by Pearson family mean correlations.

Results

Exp. 3

The higher R_N was used in Exp. 3, the higher RGR and the lower r/s ratio were obtained. In five of the seven families studied the N concentration in needles was higher in m+ than in m- treatment at R_N=0.06 · day^{-1}. In five families of seven the N productivity was somewhat higher in m- treatment than in m+ treatment.

Correlations between N productivity in Exp. 3 and other traits

We were mainly interested in the relationship between N prod assessed in Exp. 3. and other traits. There were no significant relationships between N prod from Exp. 3 with and without mycorrhiza and the approximative N prod estimates obtained in the two previous experiments.

Correlations with Exp. 1

The relationship between N prod calculated for the treatment without mycorrhiza fungus and traits in Exp.1 was close to zero, while treatment with mycorrhiza resulted in several stronger relationships, as high as R^2=0.43 with N $prod_{approx\ 1}$ in Exp.1.

Correlations with Exp. 2

The pattern was slightly different as regards the correlations calculated with traits from Exp. 2. N prod gave somewhat higher correlations with N $prod_{approx\ 1}$ and plant DW in m- treatment than in m+ treatment. N $prod_{approx\ 2}$ from Exp. 2 did not result in stronger relationships with N prod in Exp. 3 than the N $prod_{approx\ 1}$ estimated in Exp. 1 and 2.

Correlations with field traits

None of the ten correlations within this group was significant. However, the relationships between N prod in Exp. 3 and H13, H17, HI 13-17, DBH17 and V17 were considerably stronger for the m+ treatment (R^2=0.36-0.52) than for the m- treatment (R^2=0.03-0.07). The highest R^2=0.52 was found for the relationship between N prod and H13.

Correlations between RGR, r/s ratio and N concentration in Exp. 3 and other traits

Juvenile traits

For each of those three traits (RGR, r/s ratio and N concentration) in Exp. 3, six correlations were calculated with each other trait, corresponding to the six treatments used in Exp. 3 (m+ and m- x three different R_N).

Correlations with traits in Exp. 1

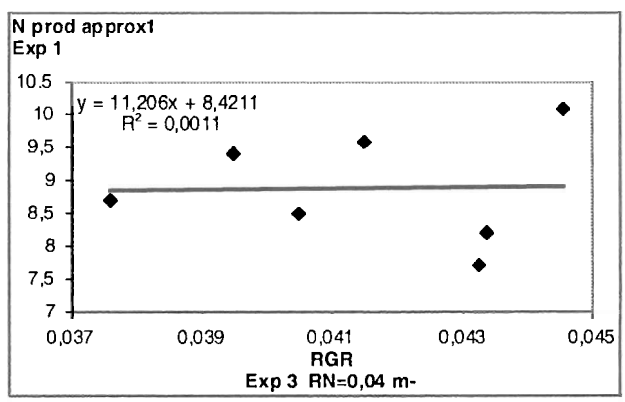
The18 potential relationships (see above) with each of N $prod_{approx\ 1}$, N utilization and plant DW resulted in 54 correlation coefficients in this group. Generally, low values of R^2 were obtained for non-mycorrhizal treatment. In the mycorrhizal treatment the strongest relationships were found in R_N=0.04 · day^{-1} treatment (e. g. the relationship RGR with N $prod_{approx\ 1}$ had R^2 =0.68, Table 2 and Fig. 1). In R_N=0.04 · day^{-1} treatment, the only negative significant correlation was found for relationship between N conc and N $prod_{approx\ 1}$.

Table 1. Juvenile traits from Experiments 1-3 evaluated in this study.

Exp.	plant DW	r/s ratio	RGR	Nconc	Nprod	$Nprod_{approx\ 1}$	N $prod_{approx\ 2}$	N utilization
1	x					x		x
2	x					x	x	x
3		x	x	x	x			

Table 2. Significant relationships between characters in non-mycorrhizal (m-) or mycorrhizal (m+) treatment in Exp. 3 (addition rate R_N = 3-6 % per day) and juvenile characters in Exp. 1 and 2. R^2 and p-values are given, R^2 in bold.

Exp. 3		Exp. 1	Exp. 2	
Treatment	Trait	N $prod_{approx1}$	N $prod_{approx1}$	N utilization
m- R_N = 0.06	r/s			**0.65** 0.028
m+ R_N = 0.03	RGR		**0.64** 0.031	
	N conc		**0.61** 0.038	**0.62** 0.036
m+ R_N = 0.04	RGR	**0.68** 0.022		
	N conc	**0.59** 0.044		



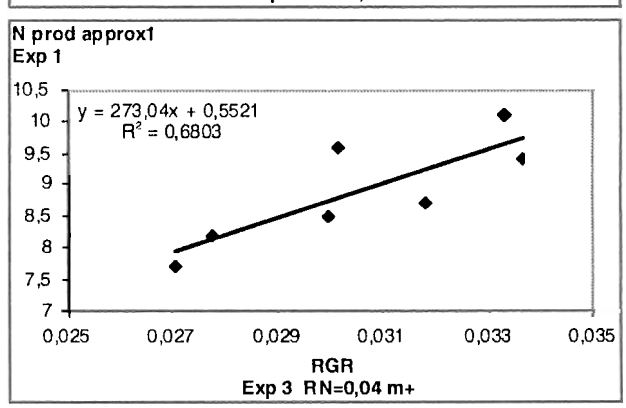


Figure 1. Relationship between N $prod_{approx\ 1}$ in Exp. 1 and relative growth rate (RGR) from non-mycorrhizal (m-) and mycorrhizal (m+) treatment with R_N = 0.04 · day^{-1} in Exp. 3.

Correlations with traits in Exp. 2

In this group, the correlations with N $prod_{approx\ 2}$ are also included, giving totally 72 correlation coefficients. The strongest relationship was found between r/s ratio from R_N=0.06 · day^{-1} m- treatment and N utilization. It was the only significant relationship for m- treatment (Table 2). In m+ treatment, several cases of significant relationships were obtained explaining up to 64 % of variation in juvenile characters in Exp. 2.

To summarize, totally 140 correlations were calculated between juvenile traits from different growth chamber experiments. Only a few of them were significant. A higher number of significant correlations was found between traits assessed in the mycorrhizal treatment in Exp. 3 than in the treatment without mycorrhiza (5 and 1, respectively).

Field traits

Ninety relationships were calculated with field, most of them were weak. The strongest relationships within this group were found between N conc in m+ treatment with R_N=0.04 · day^{-1} and DBH17 (R^2=0.47) or RGR in m- treatment with R_N=0.06 · day^{-1} and HI 13-17 (R^2=0.46).

Discussion

Growth chamber

The special method used in Exp. 3 implying different plant DW due to varying duration of replications in time could cause a variation in absolute values between replications which was not desirable for calculation of family mean correlations. Therefore, only relative traits from this experiment were used.

In a study of nitrogen productivity of *Pinus sylvestris* seedlings inoculated with *Suillus bovinus*, the mycorrhiza did not increase the specific uptake capacity of roots according to Ingestad et al. (1986). Similar results were found in this study - in spite of generally successful inoculation, the mycorrhiza did not increase N prod in Exp. 3, except for family 1.

Further, differences between the seven families studied were small, especially in the treatment with mycorrhiza (cf. slope estimates of N productivity in Ericsson & Kähr, manuscript). This is in agreement with our previous results showing generally less variation in mycorrhiza treatment than in the treatment without mycorrhiza (Mari et al. accepted). In order to have strong relationships broad variation is needed in both treatments/environments.

Generally, we expected higher correlation coefficients in the treatment without mycorrhiza in Exp. 3 as no mycorrhizal fungus was used in the two previous experiments. However, a slightly higher frequence of significant relationships was obtained in mycorrhiza treatment, in spite of a small variation in family means.

The difference between the correlations of N prod from Exp. 3 with traits from Exp. 1 and Exp. 2, respectively, was difficult to explain as the conditions of cultivation were identical in those two experiments and the calculation of nitrogen productivity was the same - N $prod_{approx\ 1}$ (cf. Jonsson et al. 1997).

Non-significant relationship with N $prod_{approx\ 2}$ could be partly explained by the fact that the points of time for harvests in Exp. 2 were planned which had been based on height growth, not on biomass growth. This resulted in a need of extrapolations and lower reliability of the results (cf. Abraitis et al. 1999).

Field trial

Apart from the fact that the families were selected which had been based on their N $prod_{approx\ 1}$, they were representative even for distribution of growth traits assessed in field trial. Thus, the poor correlations cannot be explained by a non-representative material.

As regards correlations with field growth traits, we expected higher correlations in m+ treatment which is most similar to the situation in field. The correlations between N prod and field traits correspond to the expectations being generally higher for m+ treatment than for m- treatment. However, none of the J-M correlations calculated between traits in Exp. 3 and field traits was significant and they were still too weak to be utilized in breeding.

Finally, outside the frame of this study even juvenile traits from Exp. 1 and 2 were related to field traits. No significant family mean correlations were found. A slight tendency to higher values was found for the correlations with DBH and volume in field, an observation in agreement with genetic J-M correlations calculated by Abraitis et al. (1998). According to Abraitis et al. (1998) one of the reasons for weak relationships with field could be limited genetic variation in the progenies in field. Another reason could be that family field performance has been influenced by other factors working in field, e.g. frost-related stresses.

Conclusions

In conclusion, several moderately strong relationships (up to R^2= 0.68) were obtained between the juvenile traits from different experiments but most of them were not significant and did not show a logical pattern.

Considering the N productivity estimation in Exp. 3 as the most relevant, our results indicate that it should not be replaced by time-saving approximate methods.

Stronger relationships between N prod and five field traits were obtained when field conditions were simulated by means of mycorrhiza compared to treatment without mycorrhiza. Use of mycorrhiza in the juvenile material could improve the reliability of early tests for production capacity.

However, the relationships with field trial were too weak to be used in breeding. There are no short cuts - many families have to be studied to get significant correlations.

Acknowledgements

Drs. Monika Kähr and Tom Ericsson are acknowledged for providing results from Exp. 3 and for valuable comments during the preparation of the manuscript. The present study was supported mainly by grants from the European Commission, FAIR5 CT97-3454 project NUTRIGEN. Additional funding was provided by the Swedish Council for Forestry and Agricultural Research.

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Received 10 December 2001

ПРОДУКТИВНОСТЬ АЗОТА КАК ПРЕДСКАЗАТЕЛЬ РОСТА У *PINUS SYLVESTRIS*

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Резюме

В статье представлены результаты испытания потомков сосны в полевых испытаниях и в трех экспериментах в климатической камере, где были использованы различные методы определения продуктивности азота. Целью исследования было (1) определить реакцию генотипов на продуктивность азота как важный признак в молодом возрасте, (2) установить может ли упрощенные методы для установления продуктивности азота быть использованные место сложных и дорогих методов, (3) установить связи "возрост – возрост" между материалом в климатической камере и полевыми опытами. Связи между признаками роста с климатическими испытаниями были крепкими *(R^2=0,68)* , но вобщем заметных закономерностей необнаруженно. Связь между продуктивностью азота, установленной по упрощенному методу и продуктивностью азота, установлуной по сложному методу небыло крепкой. Связь между продуктивностью азота и пятью характерами роста было гораздо крепче, когда материал в климатической камере был зарожен грибом *Laccarija bicolor (R^2=0.36-0.52,* перед заражением *R^2=0.03-0.07).* Это показывает,что симуляция полевых условий в климатической камере может сильно увеличить достоверность селекции в молодом возросте.

Ключевые слова: сосна обыкновенная, *Laccarija bicolor,* микориза, продуктивность азота, климатическая камера, признаки роста.