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Genetic differences in the level of macroelements in spruce (*Picea abies* (L.) Karst.) needles of several clones*

Abstract

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On an evenaged seed orchard of spruce growing in Kórnik 36 grafts were selected representing 6 clones, 6 grafts per clone, and two needle samples were collected from each graft. These were analysed for the concentration of nitrogen, phosphorus, potassium, calcium and magnesium. Statistically significant differences were found between clones for calcium and magnesium and between grafts within clones for all the macroelements. The clonal variance component for calcium concentration was higher than the variance component for grafts within clones. For the other macroelements the opposite was true. This is reflected in highest heritability for calcium percent followed by magnesium, nitrogen and potassium. Clonal height differences are negatively correlated with the macroelement concentration (a dilution effect), however grafts within clones have a positive correlation of height with potassium concentration (absorption ability). Heritabilities for Ca%, Mg% and N% are high.

Additional key words: seed orchards, grafts, foliar analysis, heritability.

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INTRODUCTION

To rationalize mineral fertilization it is very important to know how the fertilized plant manages the mineral nutrients it absorbs. By a choice of genotypes that utilize most efficiently the available mineral elements and at the same time are characterized by a high biomass production we should be able to minimize the expense needed to supply sufficient nutrition.

In numerous experiments performed under uniform cultivation conditions it was found that there is a differentiation in growth and in the utilization of absorbed mineral elements by various provenances of spruce (Kral 1961, Giertych and Fober

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1967, Fober and Giertych 1968, 1970, 1971). Kleinschmit and Sauer (1976), Sauer-Stegman et al. (1978), Kleinschmit et al. (1981) and Kleinschmit (1982) have observed a differentiation of numerous morphological, phenological and physiological traits of spruce both on the provenance and on the individual level, i.e. between clones within the same provenance. As a rule the variability between clones within provenances substancially exceeded that between provenances. These studies were conducted on material propagated vegetatively through rooting of cuttings. Evers (1973, 1979) compared spruces propagated by grafts and by rooting and in both instances he found a considerable individual differentiation in the content of mineral nutrients in the needles.

The aim of the present investigation was to establish the possible differences in the utilization of mineral nutrients between clones of spurce growing in a seed orchard in Kórnik and to see whether these differences are related to the energy of growth of these grafts. The distribution of the grafts and clones in the seed orchard permits the use of a variance analysis separating environmental effects from the genotypic differences between clones. Grafts were treated as replicates within clones.

In view of technical limitations the study was restricted to 6 clones only.

MATERIALS AND METHODS

On an evenaged seed orchard of Norway spruce (*Picea abies* (L.) Karst.) established in 1965 in the Experimental Forest of the Institute of Dendrology of the Polish Academy of Sciences in Kórnik, 36 grafts were chosen representing 6 randomly selected clones with 6 randomly selected grafts per clone. The clone numbers were K-01-16, K-03-34, K-03-35, K-04-22, K-04-25 and K-15-21. In January 1983 the height of the grafts was measured and needle samples were collected for chemical analyses. Needles were collected from the last annual increment from the 3rd whorl of branches from the top, from the outside, southern part of the crown, taking simulaneously two different twigs for analysis. In this way 72 samples of needles were obtained. In each sample nitrogen was measured by the Kjeldahl method (Piper 1957), phosphorus by the modified method of Kuttner and Lichtenstein (Fink 1963) and potassium, magnesium and calcium by the method of flame photometry (Humphries 1956).

The results obtained (Tab. 1) have been analysed statistically by the variance analysis and the new multiple range test. For the estimated mean squares (Tab. 2) it was assumed that the clones were a random sample of Polish plus trees, the grafts a random sample of the studied clones and the twigs a random sample of the within graft variation. Variance components for clones, and grafts within clones were evaluated as well as the heritabilities were estimated (Tab. 3). Relations between the graft height and the concentrations of individual elements in the needles were estimated by correlation analyses, on the clonal level, for all the grafts and for grafts within clones (Tab. 4) by first normalizing the data with respect to clonal means (expressing all values in standard deviation from clonal means).

RESULTS

The mean heights of trees and the concentrations of the studied macroelements in needles of individual clones are presented in Table 1 and in Fig. 1. Clonal differences were significant for tree height and for calcium and magnesium concentrations, while

Table 1

Tree height and concentration of nitrogen, phosphorus, potassium, calcium and magnesium as percent of needle dry weight. Mean values for clones

Clone no.	Tree ht. (m)	N%	P%	K%	Ca%	Mg%
K-01-16	6.97	1.54	0.186	0.916	0.912	0.158
K-03-34	6.42	1.62	0.193	0.967	0.766	0.118
K-03-35	5.28	1.69	0.202	1.072	0.846	0.138
K-04-22	3.87	1.81	0.216	1.040	1.088	0.156
K-04-25	3.10	1.94	0.214	1.034	1.287	0.164
K-15-21	2.50	1.80	0.200	1.173	1.256	0.183

graft differences within clones were significant for all the macroelements (Table 3). The clonal variance component was higher than the graft within clone component for calcium while for the other mineral elements the opposite was true. Heritabilities varied much, highest for calcium percent, then for magnesium, nitrogen and potassium. It was nil for phosphorus percent.

NITROGEN

The mean concentration of this element in the dry weight of needles varied for individual clones from 1.54% to 1.94%. Clone K-04-25 which had the highest concentration of nitrogen is very different from the remainder, which form overlapping groups. The lowest concentration of nitrogen is to be found in clones K-01-16 and K-03-34.

There exists a negative correlation between the mean height of individual clones and the concentration of nitrogen in the needles (Tab. 4). This correlation is also significant over all the grafts and for grafts within clones.

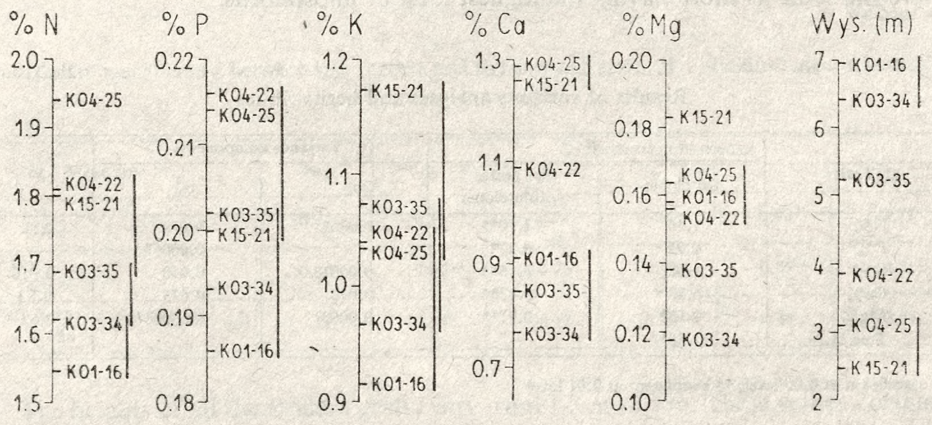

Fig. 1. Range of concentrations of macroelements in needles of Norway spruce for various clones, Clones linked by a common line are not significantly different at 0.05 level in the multiple range test

PHOSPHORUS

The concentration of phosphorus in needles is on the average 0.202%, and for individual clones the values vary from 0.186% to 0.216% (Tab. 1). Compared to nitrogen, the differentiation between clones is less and the multiple range test divides the clones into two overlapping groups (Fig. 1). Thus only the extreme clones differ from each other, clones K-04-22 and K-04-25 from clones K-01-16 and K-03-34.

The correlations of height with phosphorus concentrations in the needles were not significant, however the r values at all levels, clonal, graft, and graft within clone, were negative.

Table 2

Form of variance analysis employed and related formulae

Source of variation	Degrees of freedom	Expected Mean Square	F
Total	$cgr-1=71$		
Clones	$c-1=5$	$A=\sigma_R^2+r\sigma_G^2+gr\sigma_C^2$	A/B
Grafts within Clones	$(g-1)c=30$	$B=\sigma_R^2+r\sigma_G^2$	B/C
Residual	$(r-1)cg=36$	$C=\sigma_R^2$	

c=no. of clones=6; g=no. of grafts per clone=6; r=no. of replicates within graft=2

$$\text{Heritability}=h^2=\frac{\sigma_C^2}{\frac{\sigma_R^2}{gr}+\frac{\sigma_G^2}{g}+\sigma_C^2}=\frac{A-B}{A}$$

POTASSIUM

The mean value of potassium concentration for the whole experiment was 1.034% and for individual clones the values varied from 0.916% to 1.173% (Tab. 1). Similarily as in the case of phosphorus concentration the clones are little differentiated and the multiple range test shows considerable overlap of groups (Fig. 1). Clones with the highest level of potassium in the needles, K-15-21, K-03-35, K-04-22 and K-04-25 were the same as those having the highest level of phosphorus.

Table 3

Results of variance analyses and heritabilities

Trait	F for clones	F for grafts within clones	Variance components σ_C^2	Variance components σ_G^2	h^2
N%	1.46	14.10**	0.0065	0.079	0.32
P%	0.95	5.22**	–	0.00067	–
K%	1.02	8.16**	0.00013	0.040	0.016
Ca%	5.83**	3.42**	0.040	0.035	0.83
Mg%	2.63*	3.91**	0.00031	0.00086	0.62
Tree ht.	12.30**	–			

* significant at 0.05 level, ** significant at 0.01 level

The relation between graft height and potassium concentration shows a significant negative correlation on the clonal level, none on the graft level and a significant positive one on the graft within clone level (Tab. 4).

CALCIUM

The calcium concentration divides the clones into three groups (Fig. 1). The highest calcium concentration, above 1.2% was found in clones K-04-25 and K-15-21. A somewhat lower concentration of about 1% in clone K-04-22 and the remainder, clones K-01-16, K-03-35 and K-03-34 had the lowest, between 0.7% and 0.9%.

There is a significant negative correlation between tree height and calcium concentration on the clonal level and on the graft level but on the graft within clone level it is positive though not significantly so (Tab. 4).

MAGNESIUM

Of the elements studied magnesium appears to differentiate the clones most. The mean value for the whole experiment was 0.153% of magnesium in the needle dry weight and for individual clones the values vary from 0.118% to 0.183% (Tab. 1).

The level of magnesium does not correlate significantly neither at the clonal level nor at the grafts level, though for clones r is rather high and negative as for the other elements. On the graft within clones level the correlation is almost nil (Tab. 4).

TREE HEIGHT

The mean heights of grafts per clone are strongly differentiated (Tab. 1, Fig. 1, Tab. 3). The highest grafts, above 6 m were from clones K-01-16 and K-03-34 and lowest, arround 3 m for clones K-15-21 and K-04-25. The clonal differences in height correlate negatively with the needle content of mineral elements, significantly so for nitrogen, potassium and calcium, but for all the elements the values of r are rather high and the low significance comes from the small number of clones studied (Tab. 4).

Table 4

Correlation coefficients r between tree height and the concentration of individual macroelements in needle dry weight

Mean tree ht.	$r_{0.05}$	Concentration in needles				
		N	P	K	Ca	Mg
per clone $N=6$	0.81	−0.91*	−0.74	−0.83*	−0.90*	−0.71
per graft $N=36$	0.33	−0.50**	−0.26	−0.02	−0.49**	−0.31
In SD from clonal means $N=36$	0.33	−0.36*	−0.13	0.43**	0.20	0.01

The heights of all the studied grafts vary from 1.3 m to 8 m. These values correlate negatively with concentrations of all elements studied except potassium but significantly so for nitrogen and calcium. All the r values at the graft level are lower then on clonal level.

On the graft within clone level, when comparing normalized data arround clonal means, there is negative correlation between graft height and nitrogen and a positive one for potassium. For calcium it is also positive but not significant, for phosphorus negative but not significant and for magnesium nil (Tab. 4).

Discussion

The values of macroelement concentrations in the needles of trees do not contain all the information on the problem of management of mineral nutrients by the plants but to a large extent it indicates the needs of these plants for mineral nutrition. Thus in studies on the mineral nutrition of trees determination of the levels the elements in the photosynthetic tissues constitutes a basic mode of approach (Ingestad 1959, Höhne 1963, Swan 1972 and many others). It needs to be remembered however that the level of mineral nutrients in the needles of a species depends on many factors, such as age of plants, age of the studied needles, distribution within the crown and seasonal changes. The differentiation within the crown is substancial (Wehrman 1957, Strebel 1961, Höhne 1963) but as a rule the variability is unidirectional along gradients (Fober 1976). Thus it was necessary to collect material for analysis from the same position in the crown. In the present study the samples were collected in the winter, during dormancy, when the level of mineral nutrients in the tissues is stabilized by lack of any activity.

In comparison with data obtained by other authors for spruce (Fiedler et al. 1973) the concentrations of individual elements established here were rather typical for old trees. This is particularly indicated by the concentration of calcium, of the order of 0.8-1.3% (Tab. 1). Thus the study material is physiologically old, probably akin to the maternal trees from which the scions were taken for grafting 20 years ago.

As the results have shown the differences between clones are relatively large, which is all the more surprising since only 6 clones were analysed here. The mode of nutrition of individual clones is therefore strongly differentiated. This may be of considerable importance in breeding programs for trees when clones are selected for vegetative propagation. In some countries the cultivation of rooted cuttings of spruce is already being used on a mass scale as for example in West Germany (Kleinschmit et al. 1973, Fröhlich 1982), Finland (Lepistö 1974) and Canada (Rauter 1971, Armson et al. 1980).

The analytico-chemical selection of clones can of course be only of value as an early test, however long term studies of Evers (1979) have shown that clonal differences in the content of mineral elements remain constant as a rule. The value of the results increases when many elements are studied simultaneously. On the basis of studies on clonal variability Evers (1973) concludes that the variability of biotypes may have a profound influence on the diagnosis of the state of nutrition of a stand of trees.

In the experiment described here not only clonal variability was investigated but also the variation between grafts. There arrises therefore the question of the influence of the root stock on the analysed traits. This is particularly important for such a trait as concentration of a mineral nutrient since it has to reach the foliage through the root stock, which is a different clone in each graft. In comparative studies conducted both on grafts and on rooted cuttings (Evers 1979) no influence of the root stock was observed, and that author concluded that the level of element in the foliage is determined by the scion. One can suspect therefore that the results obtained would hold true also for clonal material raised on its own roots. However there is no doubt that the root stock here is part of the environmental component of variation. In the present study it is clearly shown that the variance component for grafts within clones is substancial and for all macroelements except calcium higher than the variance component for clones. While differences between individual clones appear high and significant (in the multiple range test) the clonal variability as such (treating the clones as random representatives of the species) is not very great but heritabilities for the concentrations of some mineral nutrients are large. It is clear that concentrations are much dependent on environmental conditions, including microsite differences, graft union quality and clonal differences between the root stocks. None the less clonal differences for N %, Ca % and Mg % are highly heritable.

On the basis of literature it can be suspected that the growth parameters of trees can correlate with the concentration of macro- and micro-elements in the foliage, however the values of the correlation coefficients are dependent on the site and in particular on the specific situation as regards the extent to which a given element is limiting for growth. Similarily as the concentration of elements in the needles and the values of the correlation coefficient with height alter with needle age, localisation of the needles in the tree crown, the season and the age of the tree etc. Leyton and Armson (1956) have for example demonstrated a significant linear relationship between the energy of growth in young Scots pine trees and the concentration of nitrogen in the needles, provided these were current year needles collected from the upper whorls of the crown. On the other hand in various species from the genus *Larix* the correlation between tree height and concentration of some microelements in the needles were greatest in May and significantly declined in July and September (Mezenceva et al. 1976). Fiedler et al. (1973) believe that the value of such correlations is dependent on the water relations in the plant. When water is a limiting factor for growth, then a minimal production of dry weight leads to an increase in the concentration of nitrogen in the tissues providing a negative correlation between concentration and tree height. According to Giertych (1969) the content of nitrogen, phosphorus and calcium is positively correlated with the phenotypic tree height and negatively with the genotypic as evidenced by mean values for provenances.

For the reasons mentioned above the correlations between heights of trees and the concentration of macroelements in the foliage reported by various authors have to be treated with caution and some authors even question them altogether.

In the conditions of the present experiment it was established that there is a significant negative correlation between clone height and concentiations of nitrogen, potassium and calcium, and the values of *r* for phosphorus and magnesium are also negative and high (Tab. 4). These are genotypic correlations and they indicate that well growing trees make do with less mineral nutrients in their foliage. They are efficient in utilising them for growth. The correlations on the graft level, phenotypic in nature, since they include both the clonal variability and the graft within clone variability, are also negative, but lower than at the clonal level. Only for nitrogen and calcium are they significant and for potassium it is practically nil (Tab. 4). This is hardly surprising since at graft within clone level (on normalized data) which represents the environmental variability (including microsite, graft union quality and root stock variability) for potassium the correlation is significant and positive. The higher concentration of this element was reached by the plants the better they grew. This is efficient absorption but not efficient utilisation. The phenotypic variability had to be intermediate between the clonal and environmental and therefore close to zero. To a lesser extent this is also true for calcium and magnesium, which had a positive *r* value at the environmental level and a clearly intermediate one at the phenotypic level. For nitrogen and phosphorus also the environmental component provided a negative correlation of growth with element level, indicating that where growth conditions were better, dilution of the elements in the foliage resulted. As expected the phenotypic level was also intermediate between the clonal and environmental.

Thus it is primarily for potassium but also for calcium and magnesium that the phenotypic effect is something more than just a dilution of the elements absorbed when growth is high.

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Genetyczna zmienność poziomu makroelementów w iglach świerkowych (Picea abies (L.) Karst.) *różnych klonów*

Streszczenie

Na równowiekowej plantacji nasiennej świerka w Kórniku wybrano 36 szczepów reprezentujących w równej liczbie 6 klonów. Z każdego szczepu pobrano po dwie próbki igieł ostatniego przyrostu, w których oznaczono stężenie azotu, fosforu, potasu, wapnia i magnezu. Klony

różniły się istotnie pod względem stężenia w igłach wapnia i magnezu, natomiast szczepy w obrębie klonów, pod względem stężenia wszystkich badanych makroelementów. Dla stężenia wapnia wariancja klonalna była wyższa od wariancji szczepów w obrębie klonów. Dla innych pierwiastków było odwrotnie. Ma to odzwierciedlenie w obliczonej odziedziczalności, której wartość jest najwyższa dla stężenia wapnia i kolejno się zmniejsza dla magnezu, azotu i potasu.

Międzyklonalne różnice wysokości są negatywnie skorelowane ze stężeniem pierwiastków w igłach (efekt rozcieńczenia), jednakże szczepy w obrębie klonów wykazują pozytywną korelację między wysokością drzew a stężeniem potasu w igłach (zdolność pobierania). Wartości odziedziczalności dla stężenia wapnia, magnezu i azotu są wysokie.

ХЕНРЫК ФОБЕР

Генетическая изменчивость уровня макроэлементов в хвое ели (*Picea abies* (L.) Karst.) *различных клонов*

Резюме

На одновозрастной семенной плантации ели в Курнике выбрали 36 привитых растений ели представляющих 6 клонов. С каждого растения взяли по 2 пробы хвои текущего прироста, в которой исследовали концентрацию азота, фосфора, калия, кальция и магния. Клоны существенно отличались содержанием в хвое кальция и магния, а отдельные подвои в рамках клона концентрацией всех исследуемых макроэлементов. В случае концентрации кальция дисперсия между клонами была большей нежели в рамках клонов. Для других макроэлементов найдена обратная зависимость. Это нашло свое отражение в рассчитанной наследуемости, величина которой является самой высокой для концентрации кальция и уменьшается для магния, азота и калия.

Разницы в высотах внутри клонов отрицательно коррелируют с концентрацией макроэлементов в хвое (эффект разбавления), однако подвои внутри клонов обнаруживают положительную корреляцию между высотой деревьев и концентрацией калия в хвое (способность абсорбции). Величины наследуемости для концентрации кальция, магния и азота высокие.