Variation in Dry Weight and Mineral Nutrient Content of Pinus radiata Progeny

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Introduction

Variations in nutrient content and reaction to nutrient stress have been demonstrated for many agronomic plants. Thus Shea, et al. (1968) have shown for bean crops the advantages gained by selecting strains capable of efficient nutrient utilisation. Similar studies of within species variation in nutrient uptake by trees have been mainly for species with wide natural geographic or edaphic range. For example, significant differences have been observed in foliar nutrient concentrations between provenances of Scots pine (Gerhold, 1959; Steinbeck, 1966), in uptake and distribution of phosphorus between seedlings of Slash pine progeny groups (Walker and Hatcher, 1965), in growth and nutrient uptake for clones of Populus deltoides (Curlin, 1967), and provenances of Jack Pine (Mergen and Worrall, 1965), Norway Spruce (Giertych and Fober, 1967) and *Eucalyptus* cladocalyx (Groves, 1967). Attention has been drawn to the similarity of reaction to contrasting soil types of thc Eucalypt provenances described by Groves and the 'edaphic ecotypes' of Trifolium species described by Snaydon (1962).

Natural stands of Radiata pine (Pinus radiata, D. Don) occur only in California within a limited area of about 7,000 ha and on soils of mainly shallow marine deposits overlying various volcanic and sedimentary country rocks (Roy, 1966). Despite the small range of the species, P. radiata provenances have been recognised. Most plantations in Australia derive only from the further restricted area of the Monterey Peninsula where stands are even less variable (Fielding, 1961; Roy, 1966).

Large differences occur in both the concentrations and total amounts of nutrients in the leaves of neighbouring trees of Radiata pine in Australian plantations. If these differences are genetically based, they have important implications to Australian foresters because soil deficiencies often limit the growth of pine plantations in Australia. There is particular interest in selecting, as parents of future crops, trees of high growth rates and of efficient utilisation of the available nutrients on nutritionally poor soils. However, it must be shown that these characteristics are largely genetically based.

The Study Area

The study area is part of Blue Range plantation at approx. 670 m (2200 ft) above sea level in the foothills of the montains some 25 km (15 miles) west of Canberra. The annual rainfall is about 90 cm (35 in) and occasional light falls of snow occur in most winters (Fielding and Brown, 1961).

In 1961 a block of P. radiata clones had been established as rooted cuttings. Trees of each clone were planted mainly as single rows at right angles to an access road and at about 2 X 2 m spacing. Six clones were selected for detailed study.

Site conditions appeared reasonably uniform over the block, although there was some indication of slight site variation along the rows. The original eucalypt forest was cleared before planting of the pine and afterwards eucalypt regrowth was cut back. A luxuriant grass cover became established, competition from which may have contributed to variable tree development within each clone. The lower branches of many of the larger trees were touching branches of neighbouring trees, but competition between trees did not appear substantial. Since few tree leaves had been shed there had probably been little mutual shading.

In a second clone block about 5 km (3 miles) away, rooted cuttings of three of the clones studied in the 1961 block had also been line planted in 1962. Trees of the 1962 block were examined less intensively than those of the 1961 block but provide some replication and comparison with the main study block.

All trees of the appropriate clones were measured and sampled in late winter (July-August 1967). Of the six clones examined, five had been raised from cuttings taken from young plantation seedling trees selected in a routine search for superior phenotypes of above-average straightness and vigour, and free from crown damage. The ramets of the sixth clone (954) were third generation cuttings propagated from the original parent tree planted in 1934, the second generation trees being 5 years old when cuttings were taken.

Methods of Assessment

The first twenty trees in each of the six rows of the 1961 clone block and three rows of the 1962 block were sampled. In two cases where slightly fewer than twenty trees per line remained all trees were sampled. In most clones some cuttings had failed at planting but as the study was made before intense competition occurred between trees, the slight variations in spacing along the rows were unlikely to be critical.

Tree height and bole diameter at 130 cm (4 ft 3 in) and 60 cm (2 ft) were measured for all trees (Table 1). The diameters of every branch on all sample trees of the 1961 clone block were measured 2.5 cm (1 inch) from the bole. The branches on each tree could then be enumerated by diameter size-classes within whorls. Branch diameter was taken twice at right-angles using vernier calipers and the average calculated. When the tree and branch measurements were complete, two branches were selected randomly and cut from each tree of the six clones of the 1961 block. These branches were ovendried at 85° C within 24 hours after collection, and when dry the leaves were removed and leaves and wood (inclusive of bark) weighed separately.

Representative samples of both leaf and wood tissues of each branch were analysed for phosphorus, calcium, potassium, magnesium and zinc concentrations, using atomic absorption spectrophotometry (Forrest, 1969). The weights of nutrients in the leaves, wood + bark and total for each branch were calculated from the concentrations and dry weights.

In addition, for all trees of the 1961 and 1962 blocks, 1-year-old leaves were collected midway along the most northfacing branch of the lowest whorl initiated during the

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Table 1. — Range of tree sizes and the numbers, size and weights of branches for sample trees of six *P. radiata* clones.

Clone number	546A	579A	582A	602A	605A	954
Tree Sizes						
Av. bole diam. (cm) at 120 cms						
1961 block	6.6	6.4	5.4	6.0	7.8	4.6
Range	3.6—9.7	3.4—9.5	3.3—7.5	3.7—8.7	5.4—10.9	2.6—7.3
1962 block	5.5			5.0	6.2	
Range	2.7—9.2			2.3—7.7	3.4—9.8	
Av. bole height (m)						
1961 block	5.2	4.9	4.1	4.4	5.6	4.0
Range	3.5—6.9	3.2—5.8	2.8—5.4	3.1—6.2	4.1—6.6	2.9—5.5
1962 block	4.5			3.6	4.7	
Range	2.6—6.2			2.0—4.9	3.0—6.1	
Branch Details (1961 block only)						
Average No. of whorls per tree	12.4	10.3	9.9	7.4	10.8	10.5
Range	9—17	6—13	6—13	5—11	9—13	7—14
Average No. of branches per tree	54.0	54.6	44.2	37.1	57.8	48.7
Range	30—77	34—69	25—61	26—55	42—74	27—74
Average branch diameter (mm)	10.6	11.0	10.4	12.3	12.7	7.7
Average size and weight of sampled branches						
Branch diameter (mm)	13.05	12.78	11.07	14.21	15.69	8.63
Wood dry weight (gms)	45.7	54.4	25.7	57.5	74.4	11.47
Leaf dry weight (gms)	78.1	81.1	46.4	86.5	113.9	33.67

past year. The leaf samples were dried within 24 hours after collection, finely ground, and analysed for the previous six nutrients.

Variations in Tree Dimensions

The study trees varied in size, the largest tree of each clone usually being twice as big as the smallest tree in bole diameter and height *(Table 1)*, with no consistent size change along the clone lines. The variation of tree size within clones is greater than between the clone averages. Relative differences between clones in the 1961 block were repeated for clones in the 1962 block, suggesting the differences are genetically rather than environmentally based.

Although all trees in the 1961 block were of the same age i. e. 6 years, the number of whorls per tree varied considerably, even within clones *(Table 1)*. No clone was truly uninodal, although trees of clone 602A had only 1 whorl for most years. Except for clone 605A, the number of whorls per tree was closely related to tree height, the relationships varying between clones.

The number of branches per tree was always linearly related to bole diameter, the relationships varying between clones and being least significant for clone 605A. The number of branches per whorl varied only slightly between trees of each clone, but significantly between clones. Within each clone the average branch diameter per tree was significantly related to tree size, the relationship again varying between clones.

Thus the development of the tree crowns apparently shows consistent genetic influence for the six clones. Trees of clone 602A had fewer whorls and branches per tree, but the average branch diameter per tree was greater with more branches per whorl than other clones. Trees of clones 546A and 579A were similar for most characteristics, with more branches per tree than in most other clones, although 546A had more whorls per tree than other clones. Trees of clone 954 had intermediate numbers of branches and numbers of whorls, but a smaller average branch diameter.

Variations in Branch Weight

Allometric regression relationships for the branches have been calculated for branch wood, leaf and total branch weights against branch diameter in each clone. The equations for each clone are all highly significant (P = 0.01), but between the six 1961 clones, the small variations in slope and intercept were barely significant *(Table 2)*. The

Table 2. — Analysis of variance of regressions of branch weight × branch size and tree canopy weight × bole size for six *P. radiata* clones.

Source of variation	Degrees of freedom	Mean square	F ratio	Significance
Log_e leaf dry weight = a + b Log_e branch diameter				
Between positions	5	0.0422	0.35	N.S.
Non-parallelism	5	0.2336	1.94	P = 0.10
Log_e branch wood dry weight = a + b Log_e branch diameter				
Between positions	5	0.1114	1.86	P = 0.20
Non-parallelism	5	0.0966	1.61	P = 0.20
Log_e total dry weight = a + b Log_e branch diameter				
Between positions	5	0.0514	0.63	N.S.
Non-parallelism	5	0.1589	1.95	P = 0.10
Log_e total crown weight = a + b Log_e bole diameter				
Between positions	5	0.1213	3.26	P = 0.01
Non-parallelism	5	0.0416	1.12	N.S.

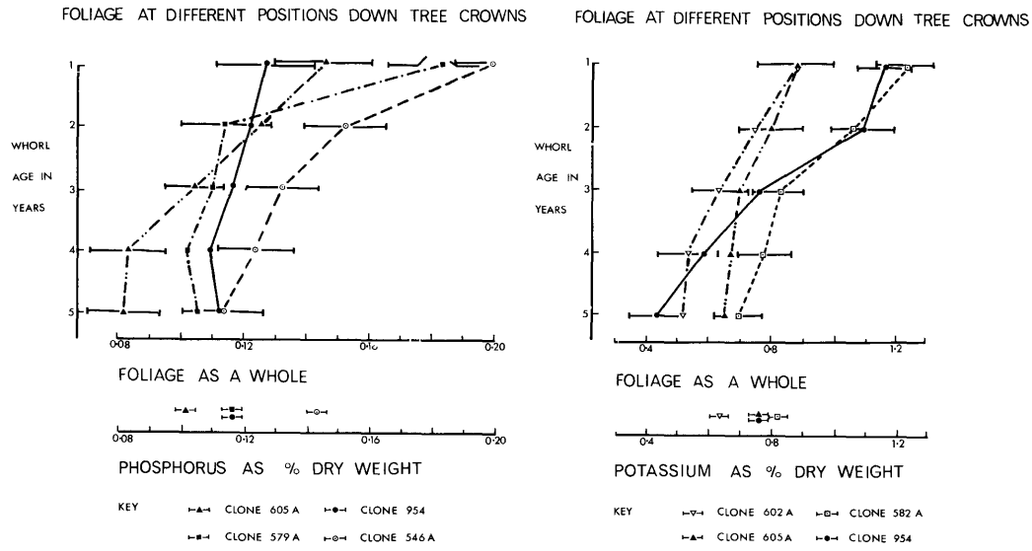

Fig. 1. — Average concentrations of foliar phosphorus and potassium. Twenty trees of *P. radiata* per clone. Horizontal bars indicate standard errors.

branch material of clones 579A was almost equally divided between leaves and wood whilst for clone 954 three quarters of the total branch weight was in the leaves.

Variations in Tree Crown Weights

The crown weights and the weights of branch wood and of leaves for each tree were estimated from the measured branch diameter and the known branch diameter : weight relationship by solving the regression equations for all branches of each tree. The allometric regression relationships between tree bole diameter and the estimated weights of branch wood, leaves and total crown were then calculated for the trees of each clone. The equations for each clone were all highly significant (P = 0.01), with little difference between clones in equation slope, but significant differences in the equation intercepts *(Table 2)*. Thus the crowns of the trees of each clone were characteristically heavy or light resulting from a combination of differences of individual branch weight and number of branches per tree, both of which are apparently controlled by genetic factors.

Variations in Nutrient Concentrations

The concentrations of the various nutrients were determined for the leaves and wood from two branches of each tree, making a total of about 240 branches, i. e. 40 per clone. The average concentration of nutrients in leaves or wood of each whorl, tree or clone could be assessed from the relationships between sample branch diameters and their nutrient contents *(Table 3)*. The patterns of change in nutrient content down the crowns of trees were characteristic within clones, some clones showing large differences through the crown for some nutrients whilst other clones showed relatively little change *(Fig. 1)*.

Difference in nutrient concentrations between clones can be demonstrated also from the concentrations in the standard samples of 1-year old leaves taken from all trees, even though such samples might not represent the average nutrient status of a tree as a whole. For each of the six nutrients determined in the 1-year old leaves from trees in the 1961 clone block, the concentration average differed significantly between the six clones. The range of concentrations was not the same for all nutrients; for example, the average phosphorus concentration for 1-year old leaves of clone 546A was 40% greater than for clone 605A, but for zinc the greatest average was 88% greater than the least *(Table 4)*. The leaves did not consistently contain either a large or small concentration of all nutrients; for example, 1-year old leaves of clone 954 had a large concentration of phosphorus, magnesium and manganese, but

Table 3. — Average nutrient concentration in leaves of all sample branches of the *P. radiata* clones, Blue Range plantations.

Nutrient	546A	579A	582A	602A	605A	954
Phosphorus (% dry weight)	0.133	0.110	0.120	0.110	0.099	0.116
Calcium (% dry weight)	0.475	0.440	0.490	0.460	0.425	0.610
Potassium (% dry weight)	0.79	0.71	0.81	0.62	0.74	0.75
Magnesium (% dry weight)	0.160	0.135	0.155	0.150	0.125	0.130
Manganese (ppm)	285	295	550	340	340	350
Zinc (ppm)	63	54	82	78	69	45

Table 4. — Average nutrient concentrations in 1-year old leaves from clones of *P. radiata* planted in two plantation blocks. Means grouped within a bracket do not differ significantly at P = 0.05.

Phosphorus (% dry weight)			Calcium (% dry weight)		
Clone	1961 block	1962 block	Clone	1961 block	1962 block
579A	0.126		546A	0.179	0.186
605A	0.139	0.151	954	0.199	
602A	0.150	0.148	605A	0.203	0.209
582A	0.165		579A	0.233	
546A	0.167	0.187	602A	0.235	0.214
954	0.175		582A	0.258	
Potassium (% dry weight)			**Magnesium (% dry weight)**		
602A	0.772	0.795	546A	0.091	0.104
954	0.784		605A	0.094	0.096
579A	0.924		579A	0.121	
546A	1.018	1.125	602A	0.139	0.139
605A	1.037	1.058	582A	0.141	
582A	0.176		954	0.153	
Manganese (ppm)			**Zinc (ppm)**		
546A	164	166	954	35	
579A	194		546A	48	38
605A	218	160	605A	47	42
954	255		579A	49	
602A	258	160	582A	57	
582A	298		602A	66	45

a small concentration of calcium, potassium and zinc, the pattern being reversed for clone 579A.

Comparable analyses for the trees in the 1962 clone block gave similar results to the 1961 block *(Table 4)*, even though the two sites were about 5 km apart and differed in aspect, position on slope and degree of slope. However, the soil types were broadly similar.

Variations in Nutrient Content of Sample Branches

The weights of nutrients in the leaves and in the wood of each sample branch were calculated from the nutrient concentrations and dry weight values. The relationships between branch diameter and the weights of nutrients in leaves, wood and total branch were then calculated, the regression equations being of the same allometric form as for dry weight. All regression equations were highly significant.

The regression equations for branches calculated for phosphorus, calcium, potassium and magnesium differed appreciably between clones, with only slight or no differences for manganese and zinc. The large differences reflect differences between clones in branch dry weight and average nutrient concentrations. Thus, branches from trees in the 1961 clone block differed little in nutrient content (or a given branch diameter) within each clone, but substantially between clones.

Variations in Nutrient Contents of Tree Crowns

The amounts of nutrients in the crown of each tree were estimated by solving the appropriate branch nutrient weight × branch diameter regression equations for all branches of the tree. Allometric regression equations as for crown dry weight were then calculated to define the relationships between bole size and the estimated weight of nutrients in the crowns of trees of each clone *(Table 5)*. Since the equations for all nutrients and clones were highly significant (P = 0.01), equations for each nutrient were examined for variation in slope and intercept between clones by analysis of variance *(Table 6)*.

Only for calcium did the slope of the regression vary significantly between clones (P = 0.05), but the intercept differed significantly for all nutrients *(Table 6)*. The nutrient content of the crown of any tree depends on the number, average weight and average nutrient concentration of branches; any or all of these may vary independently between clones.

Table 5. — Regression coefficients and significance of regressions: Log_e nutrients in crown = a + b Log_e bole diameter for six *P. radiata* clones at Blue Range plantation
For significance (P = 0.01, n = 18), T (= $b/S.E._b$) = 2.90

Nutrient and regression values	Clone number (and n)					
	546A 20	579A 20	582A 20	602A 17	605A 18	954 20
Phosphorus						
a	—0.272	—0.169	—0.372	—0.316	—0.749	—0.783
b	1.892	1.817	1.832	1.870	2.218	1.094
T	14.6	8.9	11.7	9.8	12.1	36.1
Calcium						
a	—0.015	0.834	0.586	0.726	—0.383	0.568
b	2.650	2.040	2.337	2.014	3.027	2.328
T	10.8	7.3	9.4	9.6	9.8	33.7
Potassium						
a	1.554	1.712	1.649	1.487	1.339	1.127
b	1.926	1.883	1.789	1.940	2.191	2.130
T	14.2	8.4	11.9	9.8	12.2	35.5
Magnesium						
a	—0.568	0.001	—0.235	—0.052	—1.027	—0.635
b	2.289	1.917	2.100	1.945	2.666	2.109
T	12.2	8.2	10.4	9.8	10.8	36.4
Manganese						
a	—2.434	—1.886	—1.726	—1.739	—2.572	—2.146
b	2.268	1.964	2.239	1.927	2.743	2.040
T	12.1	7.7	4.5	9.8	10.6	40.0
Zinc						
a	—3.647	—3.286	—3.292	—3.048	—4.036	—4.075
b	2.234	1.998	2.204	1.929	2.714	2.182
T	12.4	7.7	9.8	9.7	10.6	34.6

Table 6. — Analysis of variance summary for regression equations: Log_e (weight of nutrients = a + b Log_e bole diameter in tree crown).

Source of variation	Degrees of freedom	Mean square	F ratio	Significance (Equations differ at level shown)
Phosphorus				
Between positions	5	0.09265	3.68	0.01
Non-parallelism	5	0.02453	0.97	N.S.
Error	103	0.02519		
Calcium				
Between position	5	0.15138	2.82	0.05
Non-parallelism	5	0.12817	2.39	0.05
Error	103	0.05359		
Potassium				
Between positions	5	0.10216	3.78	0.01
Non-parallelism	5	0.02465	0.91	N.S.
Error	103	0.02705		
Magnesium				
Between positions	5	0.10986	2.94	0.05
Non-parallelism	5	0.06628	1.77	0.25
Error	103	0.03742		
Manganese				
Between positions	5	0.16629	2.43	0.05
Non-parallelism	5	0.08896	1.30	N.S.
Error	103	0.06844		
Zinc				
Between positions	5	0.12936	3.20	0.01
Non-parallelism	5	0.06009	1.49	0.25
Error	103	0.04041		

Discussion

The six clones studied were propagated by cuttings from trees in commercial plantations, the trees being selected because of phenotypic superiority in characteristics considered desirable in plantation trees, i. e. straight bole, good vigour and healthy crown. Since only about 2 trees per hectare, or 2% were selected the trees in the clone blocks were probably less variable than all trees in the plantation.

The differences between clones might be explained by site or genetic differences. Between-clone variation in dry weight and nutrient content cannot reasonably be attributed to within-site variation because there was relatively little site variation between the rows which were only 2 m apart. Furthermore, the pattern of variation between clones was inconsistent with the position of clones within the block for all characteristics measured. Additionally the relative development of trees and their foliar nutrient

Table 7. — Average weights in crown per unit bole volume (kg per cu m) of organic matter and nutrients for trees of six *P. radiata* clones.

Note: Means grouped within a bracket are not significantly different at P = 0.05.

Foliage dry weight						
Clones:	605A	546A	602A	954	582A	579A
Mean weight:	262.	279.	282.	284.	312.	367.
Total dry weight						
Clones:	954	546A	605A	582A	602A	579A
Mean weight:	380.	426.	474.	481.	545.	595.
Phosphorus						
Clones:	605A	954	582A	602A	546A	579A
Mean weight:	0.39	0.40	0.47	0.49	0.49	0.58
Calcium						
Clones:	546A	605A	602A	954	582A	579A
Mean weight:	1.29	1.42	1.57	1.57	1.78	1.98
Potassium						
Clones:	954	605A	602A	546A	582A	579A
Mean weight:	2.74	3.08	3.16	3.17	3.42	4.07
Magnesium						
Clones:	954	605A	546A	582A	602A	579A
Mean weight:	0.46	0.49	0.53	0.65	0.68	0.76
Manganese						
Clones:	546A	954	605A	579A	602A	582A
Mean weight:	0.08	0.10	0.11	0.12	0.13	0.18
Zinc						
Clones:	954	546A	605A	579A	582A	602A
Mean weight:	0.016	0.023	0.026	0.031	0.033	0.034

concentrations in the 1961 block were virtually identical with the results from the 1962 block for the three clones present in both blocks. Despite obvious experimental limitations, the results seem indicative of genetically induced differences.

Variations between clones in the distribution of organic matter production can be illustrated by the differences in the relative amounts of wood and leaves of branches. The clones also differed in the volume of bole wood produced per unit weight of leaves *(Table 7);* for example, trees of clone 605A had 30% fewer leaves per unit of bole volume than trees of clone 579 A, possibly because their leaves were more efficient photosynthetically.

Differences between clones in nutrient concentrations were estimated for the leaves of the whole canopy *(Table 3)* and for 1-year old leaves *(Table 4).* The nutrient concentrations for 1-year old leaves may not necessarily reflect the average nutrient status of all leaves or the trees as a whole because of changes in average concentration with depth through the crown. For example, the average concentration of potassium in leaves decreased down the crown but the amount of decrease differed markedly between clones *(Fig. 1).*

Clones 954 and 605A differed markedly in potassium concentration of young leaves but had nearly identical average concentrations for the whole crown. Steinbeck (1966) examined the concentration of nutrient in 'one upper lateral branch' of trees in a Scots pine progeny test and clearly, his conclusion that differences in the nutrient concentrations of such samples demonstrated differences between provenances in ability to accumulate nutrients needs cautious acceptance.

The nutrients contained in the crowns of trees at about canopy closure are commonly 70—80% of the total nutrients above ground (Forrest, 1969) and the concentrations of nutrients in leaves are closely related to the concentrations in the bole. The differences between clones in crown nutrient content probably reflect closely the overall effects of genetic composition on the total nutrient content of the trees. Broadly, the trees of clones with larger nutrient content contained twice as much of the particular nutrient as trees from clones with least nutrient amount. Differences in nutrient content between trees of different clones were not necessarily repeated for all nutrients; for example, clone 546A accumulated considerable amounts of phosphorus but little calcium.

Many authors observing differences in nutrient content between provenances, progeny groups or seed lots have discussed the causes of the differences. Differences in root efficiency for absorbing nutrients from the soil or growth medium, the size of the root absorption surface, efficiency of translocation and metabolism, or differential growth responses to other environmental influences, have all been postulated (MERGEN and WORRALL, 1964; WALKER and HATCHER, 1965; STEINBECK, 1966; GROVES, 1967; GIERTYCH and FOBER, 1967).

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Summary

Trees of six clones, whose parents were selected for overall phenotypic superiority, have been examined for crown dry weight and nutrient content characteristics. Variations between clones in branch number and size were similar to those reported previously. Regressions relating branch leaf or wood (inclusive of bark) weight to branch size differ little between clones; but because of varying branch numbers and average size, linear regressions of total crown weight on bole diameter differ significantly between clones, mainly in the value of the intercept.

The concentrations of the six mineral nutrients varied between clones, both in overall average for the tree crowns of each clone and for 1-year old leaves. Evidence is provided that genetic influence in the distribution of nutrients through the crown should be recognised when foliage samples are used to assess nutrient status of trees.

The crowns of trees of similar bole size varied markedly between clones in the amount of each nutrient accumulated. Differences between clones in crown dry weight contributed largely to differences in amounts of nutrients accumulated, but differences in concentration influenced the relationships between clones for each nutrient. In selecting trees for planting, particularly in areas of infertile soil, it could be important to select clones capable of rapid growth with relatively small nutrient uptake.

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Karyotype Analysis of Norway Spruce Picea abies (L.) Karst.

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Introduction

Cytogenetical researches into the chromosome morphology in tree species provide us with the basis allowing to make a series of conclusions in connection of their cytogenetical evolution. It is essential to know what genetical differences result from changes in chromosome morphological constitution and to what extent variation is due to gene mutations.

In addition to the classical work of SAX and SAX (1933) concerning the chromosome morphology of conifers, a series of publications relating to the karyotype analysis of conifer species have appeared in the last years (MEHRA and KHOSHOO, 1956 a, b; AASS, 1957; SANTAMOUR, 1960; NATARAJAN *et al.*, 1961; SAYLOR, 1961; SIMAK, 1962; MORGENSTERN, 1962; CHRISTIANSEN, 1963; YIM, 1963; MERGEN and BURLEY, 1964; PRAVDIN, 1964; SAYLOR, 1964; SIMAK, 1964; BURLEY, 1965; TARNAVSCHI and CIOBANY, 1965; KUMAR *et al.*, 1966; SIMAK, 1966; KRUKLIS, 1967; PEDERICK, 1967; TOYAMA and KUROKI, 1967; SHISHNIASHVILI, 1968; THOMAS and CHING, 1968). Much work still needs to be done in this field.

The previously published papers on cytological investigations of conifers have amply demonstrated the importance of pretreatment methods of the material in making squash preparations (MERGEN and NOVOTNY, 1957; NATARAJAN *et al.*, 1961; SAYLOR, 1961; MERGEN and BURLEY, 1964; WINTON, 1964; BURLEY, 1965; KEDHARNATH and UPADHAYA, 1965; SIMAK and HAPPEL, 1966).

The purpose of the research described in this paper was to determine a more suitable method of making squash preparations from root tips for chromosome morphology studies in Norway spruce and to establish the karyotype of the species.

Materials and Methods

The present investigation was made on seed material collected in Estonia from the Järvselja Forest District. All the trees from which the seed were gathered, were described from a taxonomical point of view, based on differences in the cones and cone scales, as a var. *acuminata* BECK.

Several combinations of pretreatments with 0.002 M aqueous solution of 8-oxyquinoline, with saturated aqueous

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