Summary

In 1955 a total of 224 cuttings of Pinus *radiata,* derived from *18* juvenile clones, was planted on four widely separated sites in New Zealand. The clones were replicated within and between sites. One of the sites was severely deficient in available soil phosphate. In 1967 a final destructive measurements was made. Considerable site effects were evident for growth rate, stem straightness, incidence of malformation, and frequency of branch clusters on the stem. Within sites, clonal repeatabilities were high (mostly between 0.50 and 0.75) for total height, stem straightness, and frequency of branch clusters, but very variable (0.04 to 0.69) for stem diameter. Some drastic clone-site interactions appear to have resulted almost entirely from genetic variation between clones in their ability to tolerate low soil phosphate status. The symptoms of the apparent phosphorus deficiency varied greatly from clone to clone, but in some it masked a tendency towards extreme bad tree form. There were also marked clone-site interactions for branching frequency and stem straightness, which appeared to be largely, but not entirely, the consequence of phosphorus deficiency. These results argue strongly for some regionalisation of seed collection and tree improvement Programmes within New Zealand and indicate that in this connection phosphate status should be a prime consideration.

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Variations in esterase zymogram patterns in needles of Pinus siivestris from provenances in northern Sweden

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Electrophoretic patterns of enzymes (zymograms) show a wide range of variation in natural populations of many organisms, both animals and plants. These patterns have sometimes been found to vary between different stages of development in the same individual and also between individual at the same stage of development. This latter type of variation is genetically determined, and most populations seem to be polymorphic for electrophoretic patterns in at least some enzyme systems.

This paper is a preliminary report from an investigation concerning the enzyme pattern variations in forest tree populations, aiming at a fast and reliable method for evaluation of genetic variation.

The esterase enzyme system was found to show a considerable variation between individual trees. In order to find out if these patterns were genetically determined and stable, or if they were influenced by different environmental factors, the following test was performed. Zymograms were produced from a material in which a collection of mother trees were represented by clonal grafts grown at different localities. The investigators did not know the origin of the samples and it was our task to combine genetically identical samples with guidance of the zymogram patterns, thus testing both the specificity of the pattern and its stability.

Material and methods

The test material consisted of 96 samples. These had been collected by the staff at The institute for forest improvement, northern district, Umeå. 16 mother trees from different provenances were included. Their place of origin is shown in ***Fig.*** 1. Each mother tree was represented by clonal grafts planted at three different plantations. The locations of these, five in all, are also shown in ***Fig.*** 1. Four of them are situated near the cost, whereas the fifth — Sundmo — is in the inland. Of each clone two samples from different grafts were taken at each plantation, giving a total of 16 X 3 X 2 = 96 samples. Each sample had been given a random consecutive number before being delivered to the laboratory.

Needles provided the material for the zymograms, and were all sampled from one year old branches on the south side of the trees. The zymograms were produced with electrophoretic migration on starch gel according to POULIK (1957). Because of the high content of resin, special pretreatments were necessary to obtain a good separation and clearly stained bands.

The pine twigs were collected in October and sent to the laboratory in plastic bags. Needles taken from the twigs were stored, for at a maximum, another seven days in darkness at $\pm 0^0$ C and were exposed to light for 24 hours before the analysis.

2 g of needles and 4 ml of 0.2M Tris-HCl buffer, pH 8.5, supplied with 0.001 M EDTA, 2% polyvinylpyrrolidon (PVP) according to BOUDET (1965) and 0.7% 2-merkaptoetanol according to BHATIA and NILSON (1969), were ground in a high speed laboratory grinder (JANKE, KUNKEL type A 10). The dispersed material was freezed and thawed two times in

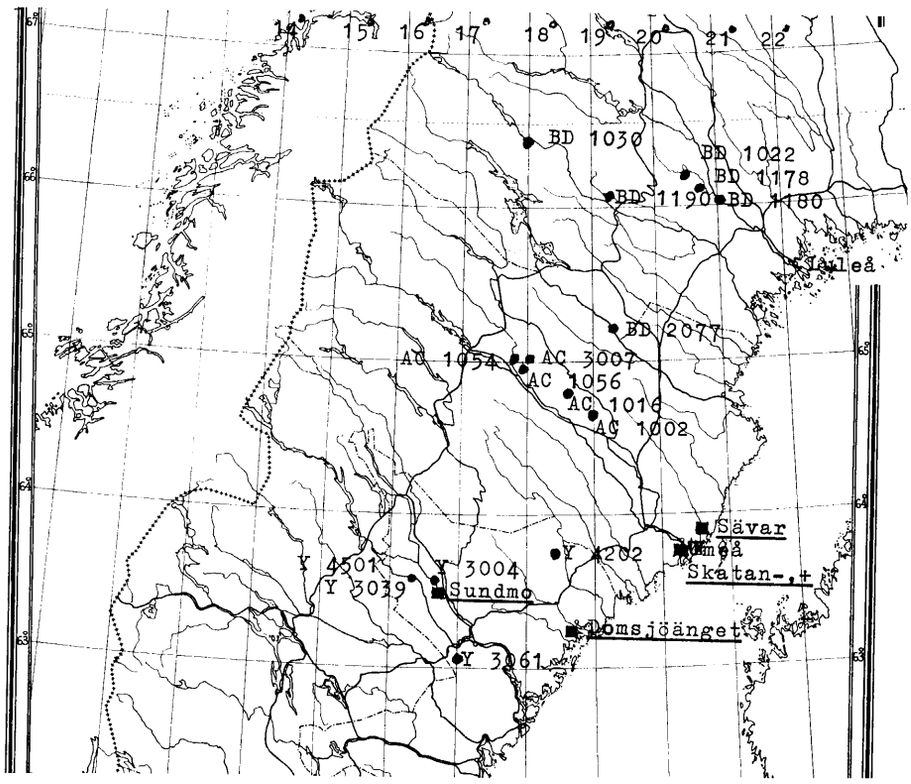

Fig. 1. — Map of northern Sweden showing the place of origin for the 16 mother trees (●) and the sites of the clone plantations (■).

order to burst the cellwalls, after which it was homogenized in an icechilled glass homogenizer for three minutes. The homogenate was centrifuged for 15 min. at 5000 g., and after decantation of the supernatant this was centrifuged another time at 40.000 g for 30 minutes. The top fraction of the supernatant was taken off by pipette and dialyzed for 18 hours at 6° C in a 0.01 M phosphate buffer, pH 7.7, with 0.2% 2-merkaptoetanol added (Bhatia and Nilson, 1969). The enzyme suspension was transferred to pieces of filter paper and allowed to dry in ice box.

The electrophoretic migration took place in starch gel at 300 volt during 2 h 40' at 6° C. The same base buffer has been used in gel and electrode vessel, viz. Tris 10.89 g/l, boric acid 16.69 g/l, EDTA 1.12 g/l, giving a pH of 7.4. For the gel 12 g hydrolyzed starch has been used to 100 ml buffer, diluted 1 : 1 with distilled water. For the vessels undiluted buffer was used. After separation the enzyme pattern has been dyed according to a method by Lawrence, Melnick and Wimer (1960) with some modifications. The gel has been treated with 100 ml phosphate buffer, pH 7.0, for 10 minutes, whereafter 200 mg Fast Red TR salt and 2 ml 1% α-naphtylacetate dissolved in 100 ml distilled water has been added. The enzyme pattern is then readable after 45 minutes at room temperature.

Table 1. — Results of the assignement test for different clones by means of zymogram patterns.

Clone	Stand Skatan + Sävar	Stand Skatan — Domsjö	Sundmo	Numer of samples-assigned to the right clone	
BD 1022	1	1	1	6	
BD 1178	1	1	1	6	
BD 1190	1	1	1	6	
BD 1180	1	0 (1)	1	5 (6)	not distinguishable
BD 2077	1	0 (1)	0 (1)	4 (6)	not distinguishable
AC 1016	1	1	0	5	not distinguishable
AC 1002	1	1	1	6	
AC 1054	1	1	1	6	
AC 1056	1	1	1	6	
AC 3007	1	1	1	6	
Y 3004	1	1	0	5	
Y 3039	1	1	1	6	
Y 4202	1	0	1	5	
Y 4501	1	1	1	6	
Y 3061	1	1	0 (1)	5 (6)	not distinguishable
BD 1030	1	1	0 (1)	5 (6)	not distinguishable
Total:	16	13	11 = 40 (45)	88 (94)	

Results

When zymograms had been obtained from all samples these were combined in groups according to their pattern. The result of this analysis is shown in *Table 1.*

Eleven groups of six zymograms were easily recognizable, each representing one mother tree. One group of 18 samples showed much the same pattern, but because of differences in the staining intensity of the bands, they could be separated into three clones, BD 1180, BD 2077, AC 1016, with only a few misclassifications. There was also a confusion between the clones Y 3061 and BD 1030, which showed great similarities. One real misclassification was made between Y 3004 and the 18 sample group mentioned above. This zymograms had an unclear pattern, probably because of low vitality of the sampled twig. Two samples from Y 4501 originating from Sävar showed a pattern somewhat diverging from the rest of the clone, which may be due to the fact that these grafts had only grown in the plantation for one breeding season.

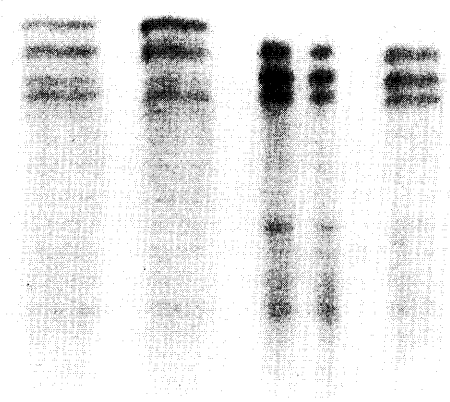

Fig. 2. — Zymograms from two clones, each represented by samples from two different locations. From left: BD 1190 grown at Skatan +; BD 1190 grown at Sundmo; BD 1180 grown at Skatan +; BD 1180 grown at Sundmo.

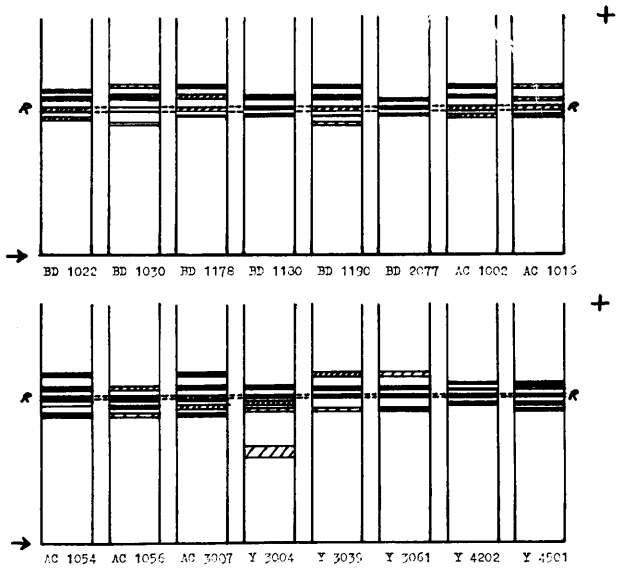

Fig. 3. — Esterase zymogram patterns from all clones included in the assignment test.

Further, one sample showed a marked divergence from all the others, but could by elimination be correctly assigned to clone Y 4202. It is at present under investigation if this graft could have been erroneonsly registered at the plantation.

In *Table 1* a correct assignment is represented by 1, a mis-classification by 0. Since there were no obvious differences between grafts from different plantations, the most relevant interpretation of the test result is given in the last column, thus 88 correct assignments out of 96, an average of 0.92. If clones BD 1180, BD 2077, AC 1016 are combined in one group and Y 3061 and BD 1030 in another, 94 correct assignments are reached, an average of 0.98.

After having obtained information about the local origin of the samples, these could be paired within locations. The result of this is also shown in the table, giving an average of correct pairing of 0.83, and, if the grouping used above was considered, 0.94.

Some examples of enzyme pattern variations within and between clones are given in *Fig. 2. Fig. 3* shows zymogram patterns from all clones included in this test.

Discussion

The results from the assignment test show that the influences of graft and location are insignificant, but that there is a considerable individual variation, which is presumably genetically determined. This is an important qualification for future genetic investigations of forest trees by this method.

It is not to be expected that all enzyme systems should be as stable against environmental influences as the esterases are. However, there ought to be several valuable enzyme systems, and it is our intention to survey the possibilities and if possible increase the number of known systems with individual variation and environmental stability.

The next step is to evaluate the mechanism of inheritance for the polymorphic systems by studying samples of offspring with known descent. It may then be possible to find an association between these marker loci and economically important characters, for instance branch angles and hardiness. The method may also be of value in investigations of enzyme systems governing the intensity of flowering and the resistance against different calamities in forest trees.

Finally, if a number of genetic polymorphic systems of this kind becomes known, they can give information about the origin and degree of inbreeding for special trees and about genetic differentiation between and within populations of forest trees.

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