GENETIC VARIATION IN NITROGEN CONCENTRATION, ACCUMULATION AND UTILIZATION EFFICIENCY IN 20 *LARIX LARICINA* FAMILIES

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ABSTRACT. -- Twenty half-sib familes of Larix *laricina* from 5 stands in Nova Scotia were grown under 3 nitrogen levels (12.5, 50, 200 ppm) in a greenhouse for 20 weeks. Tissue nitrogen concentrations were determined by using an automatic analyser. The results showed that the effect of nitrogen on tissue nitrogen concentrations, accumulations and utilization efficiency was stronger than the genetic effect. The family effect was significantly different for total plant nitrogen concentration, accumulation and utilization efficiency. The family x fertilizer interaction was significantly different at the 1% level only for total plant nitrogen accumulation. Narrow-sense heritability estimates were 0.43, 0.49 and 0.32 for total plant nitrogen concentration, accumulation and utilization efficiency, respectively. Families that were capable of accumulating more nitrogen and utilizing it more efficiently also grew best.

INTRODUCTION

Increased use of fertilizers in forestry has stimulated interest in selecting and breeding for genetic response to fertilization. Because wood production can be increased by both environmental and genetic manipulation, gains should result from combining the best cultural treatments with optimum genetic responses to those treatments (Zobel and Roberds 1970, Kitzmiller 1972). Since the early 1960's, several investigations have been carried out to determine the magnitude of genetic variation in nutrient response among and within tree species. Results have indicated that within some tree species, there are inherent differences in nutrient response (Jahromi et al. 1976, Roberds et al. 1976, Bell et al. 1979). There are "specialists" that perform well only at high or Tw nutrient regimes, and "generalists" that do well at all nutrient regimes. The principal reason for these differences is that some genotypes are capable of absorbing and utilizing nutrients more efficiently than others (Walker and Hatcher 1965, Woessner et al. 1975, Clark 1983), and these genotypes also grow well.

Eastern larch or tamarack (Larix *laricina* [Du Roi] K. Koch) is one of the most widely distributed North American conifers and grows under extremely varied climatic and soil conditions. Thus it should be expected to possess considerable genetic variation (Wright 1976). Because tamarack was not of great economic importance in the past, genetic information on it is limited. Results from provenance trials have indicated that there are significant differences in survival, height and diameter growth (Rehfeldt 1970, Sajdak 1970, Jeffers 1975, Cech et al. 1977, Riemenschneider and Jeffers 1980). Park and Fowler (1982T examined the genetic structure in natural stands using material

from controlled pollination. Self-pollination resulted in an increase in the proportion of empty seeds and in reduced germination, survival, and growth.

In recent years it has been shown that height growth of young tamarack exceeds that of all of its common coniferous associates on both upland and lowland sites (Mead 1978), and it is now regarded as a favorable reforestation species in North America. In addition to selection for improved stem form and growth, fertilization is an option to increase yields even more. However, forest managers will wish to plant genotypes that utilize fertilizers most efficiently. Since variation in response to fertilizers has never been explored, it was made the objective of this study. This paper reports on tissue nitrogen concentrations, accumulations, and utilization of 20 tamarack families grown at 3 nitrogen levels in the greenhouse.

MATERIALS AND METHODS

Plant Materials

Seeds used in this study were collected from 4 parent trees in each of 5 stands in Nova Scotia (Table 1). The seeds were stratified by soaking in cold water and then stored moist at 2 °C in the refrigerator for 5 days.

Table 1. Location of stands sampled in the Province of Nova Scotia.

Stand No.	Location	Latitude N	Longitude W	Elevation m
1	Garden of Eden	45°22'	62°15'	152
2	Chignecto Game Sanctuary	45°34'	64°26'	107
3	Stanley	45°07'	63°53'	30
4	Scotsburn	45°44'	62°53'	15
5	Georgefi el d	45°13'	63°35'	107

Greenhouse Procedure

On 10 January 1983, 3 seeds were directly sown into 330 cm^3 styroblocks filled with a 2:1 peat-vermiculite mixture. The experi ment was located in a greenhouse at the University. A factorial design with 3 replicates x 3 nitrogen levels x 20 families was used. After thinning 28 days later to 1 tree per container, there were 5 trees per plot. The photoperiod was held at 18h. and the temperature at a mean of 22 °C.

Three nitrogen levels (12.5, 50 and 200 ppm) were maintained for a period of 14 weeks. Initially macronutrient solutions for each nitrogen treatment were made of 100 x strength as well as separate micronutrient solutions. Basal nutrient solutions (Table 2) were freshly prepared from these concentrated stock solutions weekly and applied to the seedlings. Sufficient quantities were applied to thoroughly wet the growing medium so that surplus solution drained at the bottom and accumulation of nutrients was avoided.

Measurements

Seedling heights were measured at 2-week intervals starting the 6th week after sowing. At the age of 20 weeks the seedlings were harvested. Total heights and root collar diameters were recorded and the seedlings divided into roots and shoots. After oven-drying to constant weight at 70 °C, they were weighed to the nearest mg. The roots of the 5 trees in a family plot were pooled, as were the shoots, and then ground in a Wiley mill and passed through a 20-mesh screen. Tissue nitrogen concentrations expressed as N% of dry weight were determined by means of the Kjeldahl method using the automated Büchi system. Tissue N% of dry weight and tissue dry weight were used to calculate tissue N accumulations.

Two methods were used here to determine differences in N utilization efficiency (NUE) among the families. In the first, NUE was expressed as mg of dry weight produced per mg of N absorbed following the lead of some plant breeders (Loneragan and Asher 1967, O'Sullivan et al. 1974, Whiteaker et al. 1976, Makmur et al. 1978, Giordano et 77⁻1982), although it has seen criticized by others (Siddiqi and Glass T⁻P1). The second method is simply based on dry-matter production and this has been considered more applicable since in most circumstances (particularly in forestry) high nutrient concentrations are the exception rather than the rule. Thus the method is said to be more reliable in the discovery of genotypes that thrive under less-than-optimum nutrient levels (Fox 1978, Chichester 1981).

Statistical Analysis

The analysis was based on the model

$$Y_{ijkl} = \mu + A_k + P_i + F_{ij} + AP_{ik} + AF_{ijk} + E_{ijkl}$$

where Y_{ijkl} is the mean of a particular character of the l-th progeny of the j-th family within the i-th population grown under the k-th N level;

μ is the experimental mean;

A_k is the effect of the k-th N treatment level;

P_i is the effect of the i-th population;

F_{ij} is the effect of the j-th family within the i-th population;

AP_{ik} is the effect of the interaction between the k-th N level and the i-th population;

AF_{ijk} is the effect of the interaction between the k-th N level and the j-th family within the i-th population;

E_{ijkl} is the random error

Table 2. Target nutrient concentrations and sources used to prepare stock solutions.

Element	Target Concentration (ppm)	Source
Macro-Nutrient Solutions		
N	N1 - 12.5 N2 - 50 N3 - 200	NH_4NO_3
P	60	H_3PO_4(88%)
K	151	K_2SO_4
Ca	17	irrigation water
Mg	8	3 ppm in irrigation water, remainder as $MgSO_4 \cdot 7H_2O$
S	68.7	62 ppm as K_2SO_4, remainder as $MgSO_4.7H_2O$
Trace Element Solution		
Fe	4	Fe chelate 9%
Cl	4	KCl
B	0.5	H_3BO_4
Mn	0.5	$MnSO_4.4H_2O$
Zn	0.05	$ZnSO_4$
Cu	0.02	$CuSO_4.5H_2O$
Mo	0.01	$(NH_4)_6Mo_7O_{24}.4H_2O$

For this analysis the fertilizer effect was considered fixed while population and family effects were assumed to be random, giving rise to a mixed model (Steel and Torrie 1980). The format of the analysis of variance is shown in Table 3. Narrow-sense heritability was estimated as follows. It was assumed that members of individual families were half-sibs. Individual tree heritability then is

$$h_F^2 = \frac{4\,\sigma_F^2}{\sigma_E^2 + \sigma_P^2 + \sigma_F^2 + \sigma_{AP}^2 + \sigma_{AF}^2}$$

Genetic and phenotypic correlations between growth characteristics and total plant N concentration and accumulation were estimated (Becker 1975).

Table 3. Format of the analysis of variance.

Source of variation	Degrees of Freedom		Expected Mean Squares 1/
Fertilizers (A)	(a-1)	2	$\sigma_E^2 + n\sigma_{AF}^2 + nf\sigma_{AP}^2 + npf\sigma_A^2$
Populations (P)	(p-1)	4	$\sigma_E^2 + na\sigma_F^2 + naf\sigma_P^2$
Families in Populations (F)	p(f-1)	15	$\sigma_E^2 + na\sigma_F^2$
Fertilizers x Populations (AP)	(a-1)(p-1)	8	$\sigma_E^2 + n\sigma_{AF}^2 + nf\sigma_{AP}^2$
Fertilizers x Fam. i.P. (AF)	p(a-1)(f-1)	30	$\sigma_E^2 + n\sigma_{AF}^2$
Pooled Error (E)	apf(n-1)	120	σ_E^2

1/ ϕ_A^2 is variance due to the fixed effect of A.

RESULTS

General Responses

A summary of treatment effects (Table 4) shows that at higher levels of N, the concentration of this element and its accumulation increased, but the utilization efficiency shows the opposite trend.

Correlations between several of the characters including seed weight (Table 5) were significant for concentration and utilization efficiency (negatively correlated), accumulation and dry weight, and dry weight and utilization efficiency.

Table 4. Mean effects of nitrogen treatments on different characters.

Characteristics	N level 12.5	50	200
N concentration in tops (%)	0.707	1.258	2.048
N concentration in roots (%)	0.854	1.224	2.137
N concentration in total plant (%)	0.780	1.241	2.092
N accumulation in tops (mg)	4.470	25.000	47.610
N accumulation in roots (mg)	2.687	6.310	8.907
N accumulation in total plant (mg)	7.157	31.310	56.517
Total plant N utilization efficiency	133	81	48
Total plant dry weight (g)	0.953	2.511	2.742

Table 5. Correlation coefficients of several characters based on means over all treatments[1/].

		X1	X2	X3	X4	X5
X1	Seed weight	1.00	-0.00	0.30	0.31	0.30
X2	Total plant N concentration		1.00	0.02	-0.28	-0.64**
X3	Total plant N accumulation			1.00	0.87**	0.11
X4	Total plant dry weight				1.00	0.51**
X5	N utilization efficiency					1.00

[1]/Significance level: **, 1% (18 d.f.)

Analysis of Variance

The analysis of variance (Table 6) indicates significant fertilizer effects for concentration, accumulation, dry weight and utilization efficiency. Populations exerted no significant influence and this is not surprising since the stands sampled were not separated by large distances. However, there were significant differences among families for all four characters, and the family x fertilizer interaction was also significant for accumulation and dry weight. This interaction signifies that the ranking of families at different N levels changed.

If one examines individual families for nutrient utilization efficiency, it is apparent that they may be segregated into four groups according to a method described by Fox (1978) and illustrated by Figures 1 and 2. The responses of the families in the upper right hand quadrant indicate high efficiency in both nutrient uptake and dry-matter production, while those in the lower right hand quadrant are less efficient. That the groups for uptake and production consist largely of the same families is also shown by the significant correlation coefficient between accumulation and dry weight (Table 5). The remaining two quadrants contain groups that either performed well only at low nutrient level (families 1 and 20) or poorly at both high and low levels.

Table 6. Mean squares and significance levels (*, 5%; **, 1%).

Source of Variation	Total Plant			
	N concentration	N accumulation	N utilization efficiency	Dry weight
Fertilizers (A)	26.5837**	36546.10**	109277.50**	56.825**
Populations (P)	0.0017	10.86	24.81	0.036
Families i.P. (F)	0.0076**	32.32**	63.77*	0.103**
A x P	0.0032	15.48	34.18	0.058
A x F	0.0048	27.97**	36.81	0.082**
Error	0.0034	11.24	35.30	0.049

Variance Components and Heritability

The variance components and heritabilities are given in Table 7. Although the error variance components contributed from 59 to 90% of total variance, the family components are also substantial and range from 8.2 to 12.2%. As a result, the individual-tree heritabilities for N concentration, accumulation and utilization efficiency are 0.43, 0.49, and 0.32, respectively.

Table 7. Variance components (V.C.), heritabilities (h^2) and standard errors (S.E.) for nitrogen concentration, accumulation and utilization efficiency.

Source of variation	Total Plant Nitrogen					
	Concentration		Accumulation		Utilization efficiency	
	V.C.	V.C.%	V.C.	V.C.%	V.C.	V.C.%
Populations (P) - σ^2_P	-0.00013	0	-0.596	0	-1.082	0
Families i.P.(F) - σ^2_F	0.00047	10.8	2.342	12.2	3.163	8.2
Fertilizers x P - σ^2_{AP}	-0.00011	0	-1.041	0	-0.219	0
Fertilizers x F - σ^2_{AF}	0.00047	10.8	5.574	29.1	0.501	1.3
Error - σ^2_E	0.00340	78.3	11.244	58.7	35.305	90.5
Total variance	0.00434	100.00	19.160	100.0	38.969	100.0
h^2	0.433		0.489		0.325	
S.E. h^2	$\pm$0.269		$\pm$0.259		$\pm$0.255	
S.E. σ^2_F	$\pm$0.00029		$\pm$1.241		$\pm$2.484	

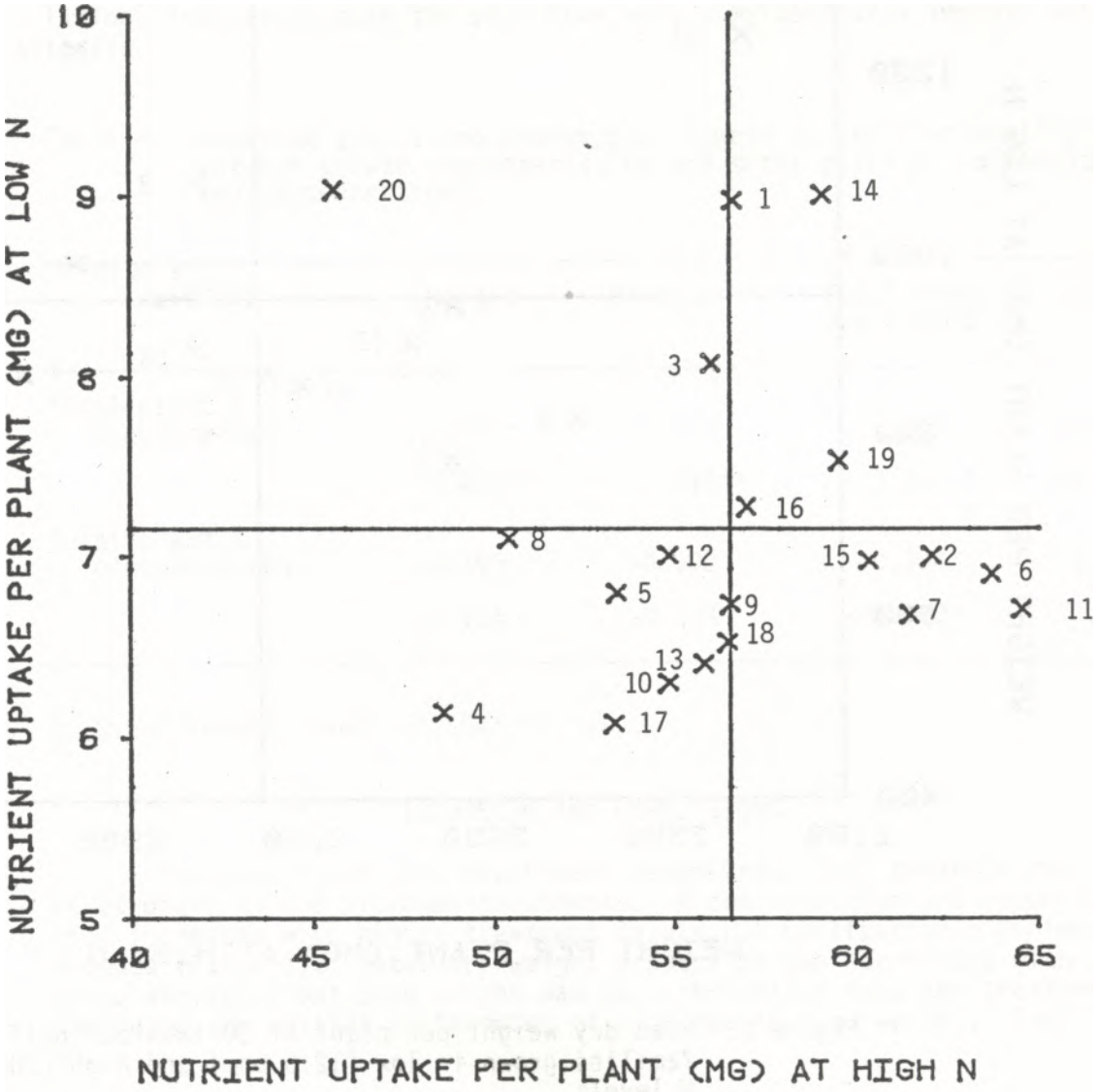

Figure 1. Mean N uptake per plant of 20 tamarack half-sib families grown in low (12.5 ppm) and high (200 ppm) N levels.

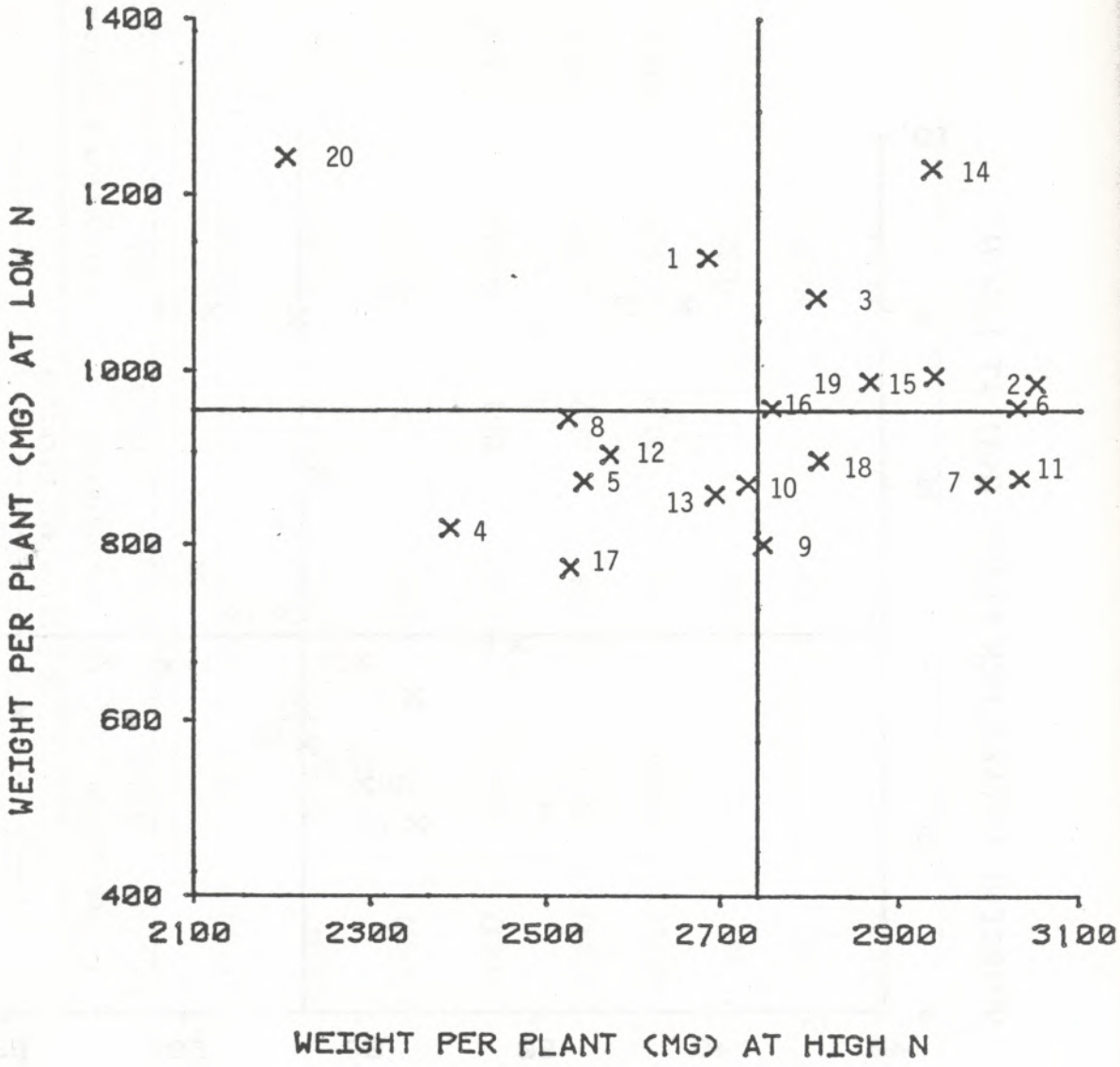

Figure 2. Mean dry weight per plant of 20 tamarack half-sib families grown in low (12.5 ppm) and high (200 ppm) N levels.

Genetic and Phenotypic Correlations

There were low negative genetic and phenotypic correlations between growth characteristics (except for shoot/root ratio) and total plant concentration (Table 8). Thus selecting for total N concentration would result in nonsignificant response in growth characteristics. In contrast there were positive genetic and phenotypic correlations between growth characteristics (except for shoot/root ratio) and total N accumulation; indicating that the selection will simultaneously improve both traits.

Table 8. Genetic (upper) and phenotypic (lower) correlation coefficients between growth ch aracteristics and total plant N accumulation and concentration

Trait	Height	Diameter	Total Plant Dry Weight	Shoot/ Root
Total plant N accumulation	0.345	0.430	1.001	-0.606
	0.428**	0.516**	0.890**	-0.355**
Total plant N concentration	-0.395	-0.002	-0.304	0.457
	-0.104	-0.133	-0.200*	0.052

[1]/ Significance level: *, 5 %; **, 1 %.

DISCUSSION AND CONCLUSIONS

The results of this experiment demonstrate that tamarack responded strongly to the nitrogen treatments. N concentration and accumulation increased with higher treatment levels but utilization efficiency dropped (Table 4). Total dry weight production was correlated with these variables but seed weight was not, indicating that the treatments overruled any initial differences of size due to seed weights (Table 5).

The most important result was that the responses of individual families differed significantly for every measured variable and that a significant family x treatment interaction existed for N accumulation and dry weight (Table 6). Because the family component was substantial, and half-sib families have a certain genetic relationship, there were sizable heritabilities for N concentration, accumulation and utilization efficiency. The standard errors for family variances and heritabilities were much smaller than the estimates themselves, therefore the estimates are relatively reliable.

The interactions revealed that the responses of individual families to different N levels were not simple or uniform. There are "efficient responders" (Lyness 1936), namely those that grew well at both high and low N levels (upper right hand quadrant, Figs. 1 and 2), and "efficient non-responders", i.e. those that grew well only at low levels (upper left quadrant). The third category is made up of those that performed poorly at both high and low levels (lower left quadrant), or only at a high level (lower right hand). Thus the possibility of selection of different groups exists.

Although these results are encouraging, they were conditioned by the environments in which the plants were grown. These environments were very different from those in which selection would be carried out and realistic genetic parameters estimated (Dudley and Moll 1969). However, abundant evidence from field experiments of other species would seem to suggest that considerable variation in nutrient utilization also exists when forest trees are tested in field environments (Zobel and Roberds 1970, Roberds et al. 1976). Such experiments are needed for tamarack to verify this point.

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LITERATURE CITED

Becker, W.A.
1975. MANUAL OF QUANTITATIVE GENETICS. 3rd ed. Washington State Univ. Press. 170 pp.

Bell, H.E., R.E. Stettler and R.W. Stonecypher.
1979. FAMILY X FERTILIZER INTERACTION IN ONE-YEAR-OLD DOUGLAS FIR. Silvae Genet. 28:1-5.

Cech, F.C., R.N. Keys and D.H. Weingartner.
1977. SEVENTH-YEAR RESULTS OF A TAMARACK PROVENANCE STUDY. Proc. 24th Northeastern For. Tree Improv. Conf., pp. 55-59.

Chichester, F.W.
1981. SELECTING FOR NUTRIENT USE EFFICIENCY WITHIN FORAGE GRASS SPECIES. I. DEVELOPMENT OF SCREENING SYSTEM. Jour. Plant Nutrition. 4:231-246.

Clark, R.B.
1983. PLANT GENOTYPE DIFFERENCES IN THE UPTAKE, TRANSLOCATION, ACCUMULATION AND USE OF MINERAL ELEMENTS REQUIRED FOR PLANT GROWTH. Plant and Soil. 72:175-196.

Dudley, J.W. and R.H. Moll.
1969. INTERPRETATION AND USE OF ESTIMATES OF HERITABILITY AND GENETIC VARIANCES IN PLANT BREEDING. Crop Sci. 9:257-262.

Fox, R.H.
1978. SELECTION FOR PHOSPHATE EFFICIENCY IN CORN. Comm. Soil Sci. Plant Anal. 9:13-37.

Giordano, L.B., W.H. Gabelman and G.C. Gerloff.
1982. INHERITANCE OF DIFFERENCES IN CALCIUM UTILIZATION BY TOMATOES UNDER LOW-CALCIUM STRESS. J. Amer. Soc. Hort. Sci. 107:664-669.

Jahromi, S.T., R.E. Goddard and W.H. Smith.
1976. GENOTYPE X FERTILIZER INTERACTION IN SLASH PINE. Growth and nutrient relations. For. Sci. 22:211-219.

Jeffers, R.M.
1975. SURVIVAL AND HEIGHT GROWTH OF TAMARACK PLANTED IN NORTHERN WISCONSIN. USDA For. Serv. Res. Note NC-190. 3 pp.

Kitzmiller, J.H.
1972. RESPONSE OF SYCAMORE (PLATANUS OCCIDENTALIS (L.) FAMILIES TO NITROGEN FERTILIZATION. Ph.D. Thesis, N.C. State Univ., Raleigh, N.C. 126 pp.

Loneragan, J.F., and C.J. Asher.
1967. RESPONSE OF PLANTS TO PHOSPHATE CONCENTRATION IN SOLUTION CULTURE: RATE OF PHOSPHATE ABSORPTION AND ITS RELATION TO GROWTH. Soil Sci. 103:311-317.

Lyness, A.S.
1936. VARIETAL DIFFERENCES IN THE PHOSPHORUS FEEDING CAPACITY OF PLANTS. Plant Physiol. 11:665-688.

Makmur, A., G.C. Gerhoff and W.H. Gabelman.
1978. PHYSIOLOGY AND INHERITANCE OF EFFICIENCY IN POTASSIUM UTILIZATION IN TOMATOES GROWN UNDER POTASSIUM STRESS. J. Amer. Soc. Hort. Sci. 103:545-549.

Mead, D.A.
1978. COMPARATIVE HEIGHT GROWTH OF EASTERN LARCH AND BLACK SPRUCE IN NORTHERN ONTAIRO. For. Chron. 54:296-297.

O'Sullivan, J., W.H. Gabelman and G.C. Gerloff.
1974. VARIATION IN EFFICIENCY OF NITROGEN UTILIZATION IN TOMATOES (LYCOPERSICON ESCULENTUM MILL.) GROWN UNDER NITROGEN STRESS. J. Amer. Soc. Hort. Sci. 99:543-547.

Park, Y.S. and D.P. Fowler.
1982. EFFECTS OF INBREEDING AND GENETIC VARIANCES IN A NATURAL POPULATION OF TAMARACK (LARIX LARICINA [DU ROI] K. KOCH) IN EASTERN CANADA. Silvae Genet. 31:21-26.

Rehfeldt, G.E.
1970. GENECOLOGY OF LARIX LARICINA (DU ROI) K. KOCH. IN WISCONSIN. I. PATTERNS OF NATURAL VARIA[I0g. Silvae Genet. 19:9-16.

Riemenschneider, D.E. and R.M. Jeffers.
1980. HEIGHT AND DIAMETER OF TAMARACK SEED SOURCES IN NORTHERN WISCONSIN. USDA For. Serv. Res. Pap. NC-190. 6 pp.

Roberds, J.H., G. Namkoong and C.B. Davey.
1976. FAMILY VARIATION IN GROWTH RESPONSE OF LOBLOLLY PINE TO FERTILIZING WITH UREA. For. Sci. 22:291-299.

Sajdak, R.L.
1970. PROVENANCE TESTING AT MICHIGAN TECHNOLOGICAL UNIVERSITY. In USDA For. Serv. Res. Pap. NC-47. p. 4-5.

Siddiqi, Y.M. and A.D.M. Glass.
1981. UTILIZATION INDEX. A MODIFIED APPROACH TO ESTIMATION AND COMPARISON OF NUTRIENT UTILIZATION EFFICIENCY IN PLANTS. J. Plant Nutrition. 4:289-302.

Steel, R.G.D. and T.H. Torrie.
1980. PRINCIPLES AND PROCEDURES OF STATISTICS. 2nd ed. McGraw-Hill, New York. 633 pp.

Walker, L.C. and R.D. Hatcher.
1965. VARIATION IN ABILITY OF SLASH PINE PROGENY GROUPS TO ABSORB NUTRIENTS. Soil Sci. Soc. Amer. Proc. 29:616-621.

Whiteaker, G., G.C. Gerloff, W.H. Gabelman and D. Lindgren.
1976. INTRASPECIFIC DIFFERENCES IN GROWTH OF BEANS AT STRESS LEVEL OF PHOSPHORUS. J. Amer. Soc. Hort. Sci. 101:472-475.

Woessner, R.A., C.B. Davey, B.E. Crabtree and J.D. Gregory.
1975. NUTRIENT CONTENT OF THE ABOVE TISSUE OF 12-WEEK-OLD LOBLOLLY PINE IN INTRAPROVENANCE CROSSES. Can. J. For. Res. 5:592-598

Wright, J.W.
1976. INTRODUCTION TO FOREST GENETICS. Academic Press, New York. 463 pp.

Zobel, B. and J.H. Roberds.
1970. DIFFERENTIAL GENETIC RESPONSE TO FERTILIZERS WITHIN TREE SPECIES. In First North Amer. For. Biol. Workshop. Michigan State Univ., East Lansing, Mich. Abstract, 2 pp.