Family x fertilizer interaction in one-year-old Douglas-fir[1])

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Summary

During the summer of 1973 a greenhouse study was conducted to evaluate the response of 39 full-sib families of Douglas-fir to three different levels of nitrogen fertilization. Approximately 60 seedlings from each of 39 families were involved in a factorial treatment design. Height and diameter of each tree were measured after ten weeks; nitrogen concentration were measured after 14 weeks of height, diameter, dry weight, shoot/root ratio, and foliar growth.

Data were analyzed using a split-plot analysis of variance. Germination results and seed-size effects were evaluated; estimates of variance components, and of genetic correlations were made. Results may be summarized as follows:

1. Family X nitrogen interaction was observed for all parameters measured after 14 weeks except foliar nitrogen concentration. Some families performed well at one particular nitrogen level while others performed well at all levels. The interaction of height and diameter growth with nitrogen indicate that selection for growth based upon the ability to efficiently use nitrogen fertilizer might be a successful undertaking in Douglas-fir.
2. Significant family effects were found for all parameters measured. Height, diameter, dry weight, shoot/root ratio, and foliar nitrogen concentration were under substantial genetic control, making selection and breeding for general combining ability potentially successful.
3. Height, dry weight, and shoot/root ratio were positively correlated with each other, both phenotypically and genotypically. From this one can predict that artificial selection for one trait would result in a positive response of the others.
4. Foliar nitrogen concentration was negatively correlated with dry weight whereas total foliar nitrogen was positively correlated. This indicates that the faster growing families had less nitrogen per unit of foliar dry weight but more nitrogen per tree than slower growing families.
5. Correlation analyses of seedling height and weight on seed weight indicated no apparent seed-size effect.

The results from this limited study indicate that in certain Douglas-fir material family X nitrogen fertilizer interactions can be identified after the first growing season. How reliable an indicator this is of subsequent field performance under different nitrogen regimes only long-term studies will tell.

Key words: Pseudotsuga menziesii, genotypic correlations, genotype X environment interaction, phenotypic correlations.

Zusammenfassung

Sämlingsnachkommenschaften aus 39 Kreuzungen von zufällig ausgewählten Einzelbäumen eines Douglasienbestandes in Oregon wurden in Töpfen angezogen und dabei wöchentlich gedüngt, wobei drei verschiedene Stickstoffdünger mit unterschiedlicher Konzentration zur Anwendung kamen. 10 und 14 Wochen nach dem mittleren Keimdatum jeder Familie wurden Höhe, Durchmesser und Trokkengewicht festgestellt. Als Ergebnis konnte beobachtet werden, daß eine Interaktion Familie X Stickstoffverwertbarkeit besteht, wobei manche Familien mehr auf bestimmte Stickstoffkonzentrationen reagieren als andere.

Introduction

As forest management in the Pacific Northwest is becoming more intensive, cultural practices such as site preparation, browsing protection, fertilization, and thinning are becoming an integral part of woodland management programs. This is particularly true for the emerging plantation silviculture involving select genetic stock. It raises the question as to how progenies from good performers in natural stands will perform under these manipulated conditions.

A case in point is the increasing application of nitrogen fertilizer both in plantations and established stands. Nitrogen seems to be a limiting factor of tree growth on many sites and, therefore, much effort is made in developing appropriate schedules to supply this nutrient artificially (Gessel et al. 1965, Miller and Pienaar 1973, Miller and Young 1976). While the economics of this cultural practice is undergoing some changes in light of the rapid price increase of commercial fertilizer, the overall interest is not diminishing and the attention is shifting to alternative sources such as sewage sludge or biological nitrogen fixation. Regardless of the diverse opportunities for nitrogen manipulation, it seems safe to say that increasing attention will be paid to the nitrogen status in the planning of future plantations. Accordingly, forest managers may want to know whether there are genetic differences among families affecting performance at different nitrogen levels; whether there is merit in identifying some "specialist" strains for specific regimes on the one hand, and "generalist" strains on the other; and, if so, how one best goes about identifying them.

This article presents the results of a study designed to test family X nitrogen fertilizer interaction in Douglas-fir, *Pseudotsuga* rnenziesii (Mirb.) Franco. The overall plan involved a first phase in the greenhouse, to be followed by a subsequent phase of congruent design in the field. The first phase of intensive monitoring during the seedlings' first growing season was conducted in 1973 and is reported here. For a variety of reasons, Phase II was not carried out although some of the plant material still exists in a field planting.

Both the response of Douglas-fir to fertilization and the interaction between forest tree genotypes and their environments have been well documented (Gessel et al. 1951, Heilman and Gessel 1963, Steinbeck 1966). However, studies showing an obvious genotype X fertilizer interaction are rare. Such an interaction was shown for Pinus sylvestris by Brown (1970), for Platanus occidentalis by Kitzmiller (1972), and for Pinus *elliottii* by Jahromi et al. 1976). For Douglas-fir, two studies (Rediske et al. 1968, Carlson 1974) indicated this interaction. The former involved a complete fertilizer (N, P, K) application, thereby confounding the nitrogen response with that of other nutrient. The latter reported a significant family X nitrogen interaction for both total and rate of height growth which supports the findings of this study.

Materials and Methods

Plant material. The seed used in this study originated from 39 biparental crosses of selected parent trees located

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on a 100,000-acre tree farm in Columbia County, Oregon. Parent trees were reasonably well distributed over the tree farm. Some parent trees were selected for form and growth rate while others were randomly selected. Crosses among parent trees were random with each parent being used only in one cross.

Hypotheses and experimental design. Two main hypotheses were formulated for this study. The first was that there would be significant differences between families in their performances at given nitrogen levels. The second hypothesis was that there would be a significant family × nitrogen interaction. To test these hypotheses, a split-plot experimental design was used (SNEDECOR and COCHRAN 1967, STEELE and TORRIE 1960) with two adjacent replications located in the same greenhouse compartment. A 3×39 factorial experiment with nitrogen confounded in blocks of 39 families was used. This approach not only avoided treatment errors by grouping trees by fertilizer application, but also shifted statistical resolution from the main effects of fertilizer to the interaction effect of family by fertilizer. Plots of 20 trees per family were used with border rows around the outside edge of blocks.

Cultural techniques. Seedlings were grown in 8-cubic inch styroblocks (MATTHEWS 1971) in a mixture of half peat and half vermiculite. This has proven to be a satisfactory mixture for growing Douglas-fir seedlings (PIESCH and STETTLER 1971). Three levels of fertilization were applied weekly. The low and high levels were Hoagland's nitrogen deficient solution supplemented with nitrogen in the form of urea (HOAGLAND and ARNON 1938). The intermediate treatment was a standard HOAGLAND's solution. After 9 applications these treatments were equivalent to 25, 100, and 175 pounds of nitrogen per acre (or 28, 112, and 196 kg/ha) for the low, intermediate, and high levels, respectively. The corresponding equivalents after 13 applications were 36, 100, and 252 pounds per acre (or 40, 112, and 282 kg/ha). Uniform distribution of water was accomplished by using a fan-type spray nozzle.

Measurement of parameters. Height and diameter measurements were taken 10 and 14 weeks after mean (family) germination date. Additionally, dry-weight measurement and foliage analysis, using the micro-Kjeldahl method (BROADSTREET 1965, JACKSON 1958), were performed. It was expected that the effect of seed size on seedling growth would be of particular importance in this study. In order to account for this, 10 randomly selected seeds from each family were weighed and their seedlings identified throughout the study. After final measurements had been taken, correlations were calculated between seed weight, seedling height, and dry weight.

Statistical analysis. A split-plot analysis of variance (STEEL and TORRIE 1960, SNEDECOR and COCHRAN 1967) was carried out using plot means *(Table 1).* On traits where

Table 1. — Form of split-plot analysis of variance used to analyze data from 2 blocks of 39 families grown at 3 levels of fertilization. (Individual data.)[1]

Source	DF	EMS (A fixed, C random)[2]
A	(a-1)	$\sigma^2 - n\sigma^2_p + nc\sigma^2_{AB} + \frac{nab}{a-1}\sigma^2_{AC} + \frac{nbc}{a-1}\Sigma A^2$
B	(b-1)	$\sigma^2 + n\sigma^2_p + nc\sigma^2_{AB} + nac\sigma^2_B$
E	(a-1)(b-1)	$\sigma^2 + n\sigma^2_p + nc\sigma^2_{AB}$
C	(c-1)	$\sigma^2 + n\sigma^2_p + nab\sigma^2_C$
AC	(a-1)(c-1)	$\sigma^2 + n\sigma^2_p + \frac{nab}{a-1}\sigma^2_{AC}$
e	a(b-1)(c-1)	$\sigma^2 + n\sigma^2_p$
w	abc(n-1)	σ^2

[1]) For mean data $\sigma^2 = \frac{\sigma^2_w}{K} + \sigma^2_p$ except for source w where $\sigma^2 = \sigma^2_w$.

[2]) σ^2_w = variance due to differences among trees as estimated from a random sample of 90 to 100 plots.

σ^2 = variance due to differences among trees.

σ^2_p = variance due to differences among plots.

σ^2_{AC} = variance due to interaction between families and nitrogen treatments.

σ^2_c = variance due to differences among families.

σ^2_{AB} = variance due to interaction between blocks and nitrogen treatments.

σ^2_B = variance due to differences among blocks.

ΣA^2 = variance due to differences among nitrogen treatments.

A = nitrogen main effect.

B = block effect.

E = main-plot error.

C = family effect.

AC = family x nitrogen interaction.

e = sub-plot error.

w = variance within sub-plots.

a = number of levels of A = 3.

b = number of blocks = 2.

c = number of families = 39.

n = number of individuals within a cell = 3.

k = the harmonic mean of the numbers of individuals in each sub-plot used to estimate (σ^2_w). For height and diameter measurements k = 9.48. For foliar nitrogen concentration k = 2.95.

MS = mean square.

DF = degrees of freedom.

Table 2. — Analysis of variance of response to treatments.

	GROWTH PERIOD						
	10-WEEK		14-WEEK				
SOURCE OF EFFECTS	DIA.	HT.	DIA.	HT.	DRY WT.	S/R[1]	F.N.[2]
NITROGEN	NS	NS	NS	NS	NS	NS	**
FAMILY	**	**	**	**	**	**	*
NITROGEN X FAMILY	NS	NS	*	*	**	**	NS

NS = Not significant.
* = Significant at the 5 percent level.
** = Significant at the 1 percent level.
[1]Shoot/root ratio.
[2]Foliar nitrogen concentration.

individual variation estimates were desired, analyses were carried out using a random sample of trees from each plot. Expected mean squares and cross products were used to estimate components of variance and co-variance. All analyses were done on a Honeywell 635 computer made available by Weyerhaeuser Company.

Results and Discussion

Seed size effects. Correlations between seed weight and seedling height, and between seed weight and seedling dry weight (R^2 values of 0.002 and 0.004, respectively) indicated no significant relationships. These are similar findings as those of LAVENDER (1968) for Douglas-fir, but contrary to the findings of other researchers working with pine or spruce (MITCHELL 1934, SPURR 1944, RIGHTER 1945, CALLAHAM and HASEL 1960, BURGAR 1964).

Response to treatments. The results of analyses of nitrogen, family, and nitrogen × family effects are presented in *Table 2.* In addition, analyses of shoot/root ratios and foliar nitrogen concentration are provided. Examination of *Table 2* reveals that, except for foliar nitrogen concentration, there were no significant differences among the nitrogen treatments. This may be the result of the experimental design, the length of the growing period, or a combination of the two. The split-plot experimental design places less precision on the main-plot factor (nitrogen treatments) and more on the sub-plot (family) and interaction (nitrogen × family) factors (FEDERER 1955). The relatively small degrees of freedom provided a weak test for the nitrogen treatments. WALKER and HATCHER (1965) suggested this interpretation in their work with slash pine *(Pinus elliottii).* The analysis of foliar nitrogen concentration showed that after 14 weeks foliar nitrogen concentration was proportional to the amount of nitrogen supplied. A longer growth period, or an experiment over several growing seasons, might permit seedlings to respond significantly to the nitrogen treatments.

Table 2 shows that for all traits measured the family (genetic) effect was significant, suggesting that the traits measured were under some genetic control.

A goal of this study was to determine whether family × nitrogen interactions existed in these seedlings for the parameters measured. Indeed, this turned out to be the case for all measurements taken at 14 weeks except foliar nitrogen concentration. The lack of any interaction for the 10-week height and diameter measurements suggests that 10 weeks were not sufficient for certain families to express their inherited ability to use nitrogen more efficiently than others. The lack of a significant interaction for foliar nitrogen concentration suggests that those families that are efficient in taking up nitrogen at low concentrations are also efficient in taking up nitrogen at higher concentrations. However, a consideration of total foliar nitrogen content rather than of the foliar nitrogen concentration might provide a different result.

One may view this family × nitrogen interaction more easily by considering the relative ranking consistency of particular families. The two typical situations that might result from this interaction are a change in ranking or a differential magnitude of response *(Figure 1).*

Of particular interest to both forest managers and forest tree breeders is the identification of families which perform best with regard to nitrogen regimes. Examination of *Ta-*

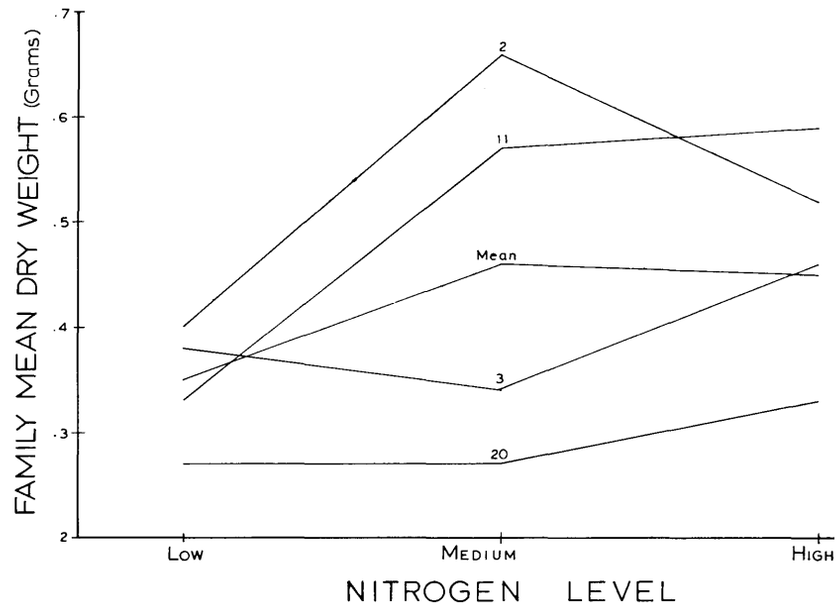

Figure 1. — Family dry weight means at 14 weeks plotted over nitrogen level, showing two kinds of genotype × fertilizer interaction: Families 3 and 11 show a change in ranking; families 2 and 20 show a differential magnitude of response. The mean is for all families in the experiment.

ble 3 reveals three types of families detected in this study: (1) "specialists" that perform well only at high nitrogen regimes (families 11 and 27), (2) "specialists" that perform well only at low nitrogen regimes (families 10 and 19), and (3) "generalists" that do well at all nitrogen regimes (families 2 and 21).

Phenotypic and genotypic correlations. In order to calculate phenotypic and genotypic correlations, co-variance analyses were conducted on each pair of traits.[3]) Correlation coefficients based upon these analyses are presented in *Table 4*. All phenotypic correlation coefficients were positive except those involving foliar nitrogen concentration.

Since the former traits are all measurements of growth response, this result was expected; however, the negative correlations between the growth responses and the foliar nitrogen concentration were not expected. Apparently those families that performed best in terms of growth also had a lower foliar nitrogen concentration. This phenomenon could be explained by a "dilution effect" on nitrogen concentration resulting from the increased biomass production (R. B. WALKER, personal communication). While foliar nitrogen concentration may be lower in faster growing families ,the total nitrogen content should be higher. To test whether this was indeed the case, five families were randomly selected and their mean dry weight and mean total foliar nitrogen content compared *(Table 5)*. It can be seen that there was a strong positive relationship between these two parameters ($R^2 = 0.997$), suggesting that the faster growing families had taken up and used more nitrogen than the slower growing families. JAHROMI *et al.* (1976) reported similar findings regarding both nitrogen concentration and total nitrogen in slash pine.

Table 3. — Family ranking according to means of 14 week measurements of height and dry weight.

	14 HEIGHT			14 D.W.		
FAMILY	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
1	25	18	32	14	28	28
2	7 *	8	1	8	1	7
3	21	35	21	11	37	16
4	6	16	2	30	38	29
5	28	23	24	19	12	15
6	5	22	7	20	15	6
7	23	36	28	23	32	9
8	35	21	39	31	31	38
9	9	20	17	5	21	3
10	3	10	11	4	7	11
11	27	2	9	26	3	2
12	24	31	33	15	8	39
13	11	24	25	18	4	14
14	20	29	20	33	18	20
15	34	37	34	17	34	30
16	33	34	14	10	26	27
17	22	6	13	7	19	13
18	32	32	36	22	35	25
19	8	19	27	6	14	17
20	38	33	38	38	39	37
21	2	9	6	1	10	1
22	1	7	3	13	30	5
23	19	27	37	2	23	21
24	16	30	35	9	11	19
25	14	26	18	35	36	34
26	36	25	12	21	27	10
27	13	15	5	27	17	4
28	12	3	4	28	25	23
29	26	17	31	32	20	22
30	15	12	15	3	5	8
31	29	13	30	36	22	26
32	10	1	8	16	13	24
33	37	5	16	25	9	31
34	17	14	10	12	6	12
35	30	39	19	24	24	18
36	18	11	22	34	2	33
37	4	4	26	29	16	32
38	39	38	23	37	29	35
39	31	28	29	39	33	36

*Underscored figures relate to specific families discussed in text.

[3]) Correlations between diameter and dry weight, diameter and shoot/root ratio, and diameter and foliar nitrogen concentrations were not determined because diameter measurements were not taken on the same trees selected for measurement of these other traits.

Table 4. — Genotypic and phenotypic correlations.[1]

Trait	14-wk ht	Dry wt	Shoot/ root	Foliar N. Con.
10-wk ht	0.83[2]	0.68	0.18	-0.05
	0.84	0.64	0.33	-0.14
14-wk ht	-	0.55	0.51	-0.05
	-	0.56	0.79	-0.21
Dry wt	-	-	0.10	-0.27
			0.37	-0.52
Shoot/root	-	-	-	-0.13
	-	-	-	-0.27

[1]Since diameter variance was very small, accurate correlations with other traits could not be determined.
[2]The upper numbers are the phenotypic correlation coefficients, the lower numbers the genotypic correlation coefficients.

Table 5. — Comparison of mean foliar dry weight and mean total foliar nitrogen content for five randomly selected families.

Family	14-week Dry weight (g)	14-week Total nitrogen content (mg)
12	0.229	5.12
1	0.243	5.83
22	0.282	6.49
17	0.283	6.23
10	0.314	6.01

Table 4 also shows that the genetic correlations between height, dry weight, and shoot/root ratios were all positive, suggesting that selection for one trait would result in a positive response in the others. However, the correlations involving foliar nitrogen concentration were either very small or negative, suggesting that selection for foliar nitrogen concentration would result in a nonsignificant response in the other traits.

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New Methods to Prepare Squashes to Study Microsporogenesis in Pinus resinosa Ait. II. The Effects of Boiling Tissue Pieces in Various Fluids

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Abstract

Squashes of microsporophylls of *Pinus resinosa* were prepared as follows: The material was fixed in ethanol-acetic acid (3 : 1 v/v) (EA), and stored in ethanol-glycerin-water (1 : 1 : 1 v/v) (EGW). The best treatment for leptotene to diakinesis was heating in glycerin-water-dimethyl sulfoxide (2 : 1 : 7 v/v) with sodium bicarbonate 0.05 g/100 ml (GWDS) at 170° C for 30 min, followed by heating in glycerin-water (9 : 1 v/v) with sodium bicarbonate 0.25 g/100 ml (GWS) at 260° C for 2—8 min. This resulted in untangling of prophase chromosome masses, and thus in an unobstructed view of chromosome details. The best treatment for metaphase to tetrads was heating in GWS at 260° C for 2—8 min without prior heating in GWDS.

Key words: Pinus resinosa, microsporogenesis, cytology, squashes, meiosis.

Zusammenfassung

Es wurde eine neue Methode zur Herstellung von Quetschpräparaten von Mikrosporophyllen bei *Pinus resinosa* AIT. entwickelt, durch welche eine gute Entwirrung der Prophase Chromosomenmassen und damit ein ungehinderter Einblick in die Feinstruktur der Chromosomen erreicht wird.

Introduction

During earlier experiments to develop new squash techniques for cytological studies of conifers (BONGA, 1978), it was observed that prolonged boiling of small pieces of male cone of *Pinus resinosa* in ethanol-glycerin-water (1 : 1 : 1 v/v) resulted in well-separated sporophyte cells and meiocytes with darkly stained, slightly swollen chromosomes and a well-cleared cytoplasm. This suggested that boiling of specimens could play an important role in the development of new squash techniques for conifers. The objective of the present study was to determine if the boiling technique could be improved so that less swelling of the chromosomes would occur, while maintaining the high degree of cell separation and clearing of the cytoplasm.

Material and Methods

Male reproductive shoots of *Pinus resinosa* AIT. were fixed in ethanol-acetic acid (3 : 1 v/v) (EA) for 24 h and then stored in ethanol-glycerin-water (1 : 1 : 1 v/v) (EGW) (GERLACH, 1969) at room temperature.

To obtain rapid and controlled heating of the material, the heating unit shown in *Figure 1* was constructed. It consisted of a clay disc (14 × 4 cm) with four vertical holes for the specimen containers and one small hole in the center for a thermometer. This disc was placed on top of a thermostatically controlled hotplate located inside a fume hood. Specimens were boiled in aluminium cups made by folding a piece of aluminium foil over the end of a glass tube. Aluminium foil cups were preferred to glass cups because aluminium foil transmits heat faster than glass. The mass of clay of the disc, having a large capacity to store heat, served to reduce temperature fluctuations.

Small pieces of specimen (about 2 × 1 × 1 mm) were transferred to the aluminium foil cups and a specific amount (usually 8 drops) of the fluid used for boiling the tissues (referred to as the boiling fluid), was added. The