A Diallel Cross in Black Spruce, Picea mariana (Mill.) B.S.P.

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Introduction

The development on an effective breeding program foi a given species depends upon information on the inheritance mechanisms of its economically important characters, particularly the relative importance of additive, dominance and epistatic genetic variances (BELLMANN and AHRENS 1965) These variances can only be obtained from experiments based on controlled crosses with specific mating designs (COMSTOCK and ROBINSON 1952). One of these designs, the diallel cross, is particularly useful for this purpose when a complete evaluation of variance components is desired including those due to selfing, maternal, and reciprocal effects (COCKERHAM 1963), and when the number of parents can be limited as in theoretical studies, as contrasted to a program of crossing and progeny testing resulting from established seed orchards (HINKELMANN and STERN 1960). In a complete diallel, a set of p parents is crossed in all combinations, leading to p^2 progenies, but the selfs and reciprocals may be omitted (GRIFFING 1956). In a reduced cross of this kind efficient estimates of heritability and degree of dominance can be obtained from 8—10 parents (PEDERSON 1971).

This paper reports results from a complete 7 X 7 diallel in black spruce, one of the most common species in the boreal forests of North America, which is utilized and regenerated on a large scale. Variance components are given for all sources of variation, phenotypic and genetic correlations and heritabilities are calculated, and breeding implications discussed.

Materials and Methods

In May 1970 seven 16-year-old trees in a Petawawa plantation from the Lake Simcoe-Rideau Region in Ontario (HILLS 1959) were crossed in all combinations using standard methods. Little insect damage occurred and all cones were harvested in September 1970. Seed set was low in the selfings but some full seed was obtained from all 49 combinations; the average was 13 seeds per cone and the range 1—44 seeds.

Following stratification of the seed for three weeks, a greenhouse experiment was established in March 1971. The randomized block design consisted generally of 10 "Nippon" paper pots per plot, 6 replications, and 2 environments; about one-tenth of the plots in the last replications of each environment were not sown because of insufficient seed. The greenhouses were illuminated for 16 or 18 hours, respectively, and kept at temperatures ranging from 18—30° C. Forty-eight of the 49 seedlots germinated and mortality was light. After three months the seedlings were established in a nursery experiment with 12-tree plots in 2 rows, 5 replications in randomized blocks, and 2 environments with only minor differences in soil. This experiment was terminated in the fall of 1972.

In the greenhouse experiment, the cumulative number of germinated seedlings was counted every second day from the 8th to the 18th and again on the 21st day after sowing and expressed as the germination rate index (BARTLETT 1937). The germination percentage calculated from the number of seeds sown and germinated until the 21st day was transformed by arcsin (STEEL and TORRIE 1960). The number of cotyledons of the first five seedlings in each plot was counted when germination was complete. The height of all seedlings in each plot was measured at the end of their greenhouse growth period. In the nursery experiment, height and survival of the seedlings were recorded in September 1972.

Plot means of the six measured characters were then analysed using the least-squares method by means of the computer program prepared by SCHAFFER and USANIS (1969). Sources of variation and degrees of freedom are given in *Table* 1. Because of insufficient seed and mortality, 56 plots were missing in the greenhouse experiment and 44 in the nursery experiment. The computer program provided for automatic calculation of missing-plot vaiues and subtraction of degrees of freedom.

***Table** 1.* — Sources of variation and degrees of freedom in the analyses of variance1)

Source Description	Abbreviation	Derivation2)	Degrees of freedom1) Greenhouse expt.	 Nursery expt.
Environment	ENV	e — 1	1	1
Replication	REP	e(r — 1)	10	8
General combining ability	GCA	p — 1	6	6
Selfing	SELF	p	7	6
Specific combining ability	SCA	p(p — 3)/2	14	14
Maternal effect	MAT	p — 1	6	6
Reciprocal effect	REC	(p — 1) (p — 2)/2	14	13
Gen. comb. ab. X Env.	GE	(e — 1) (p — 1)	6	6
Error	ERR		467	375
Total			531	435

1) Degrees of freedom for SELF, REC and ERR reduced by missing plots and complete absence of certain families.
2) e, r, and p refer to number of environments, replications and parents, respectively.

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1) Contribution in honor of the late Professor KLAUS STERN, Forstliche Fakultät, Universität Göttingen, Germany.

The analysis was based upon the random model (model II), assuming that we are dealing with random samples from the parent population, so that inferences are made about parameters in this population. Approximate F tests were then made by dividing the mean squares of general combining ability by those of specific combining ability, and the remaining mean squares by the error mean squares (GRIFFING 1956, p. 471). Since the mean squares and variance components for the selfing were exceedingly large and dominated some of the analyses, they were excluded from most subsequent computations to obtain results comparable to those of other workers who avoided this complication by omitting the selfs in their crossing scheme.

It was assumed that epistatic components of genetic variance are negligible in the population examined. The variance component for general combining ability (σ_{gca}^2) then estimates one-fourth of the additive genetic variance (σ_A^2), and the component for specific combining ability (σ_{sca}^2) one-fourth of the dominance variance (σ_D^2). Genetic correlations were directly calculated and printed by the computer from the GCA covariance component of two characters in the numerator and the square root of the product of their GCA variance components in the denominator (KRIEBEL *et al.* 1972). This was done separately for the greenhouse and nursery experiment. Phenotypic correlations were derived for both experiments at the same time from mean values of each family obtained from sums over all replications and environments. The variance components of the analysis were not suitable for this purpose because the number of families and replications differed in the two experiments and the results could not all be analysed by the computer at the same time. These phenotypic correlations are the ordinary product-moment correlations which can be tested for significance in the usual manner, but no such test is available for the genetic correlations (pers. comm. from Prof. K. HINKELMANN).

Results

The major results of the analyses are reflected in the following statistics:

1) Variance components *(Table 2)* indicate the great influence of selfing, particularly for germination percent, survival percent, and second-year height. The error variance component for these characters is also very large. Environment and replication effects are notable for germination rate, first-year and second-year height.

2) The significance of the approximate F values and the percentage contributions to total variance of the major sources of variation (selfing excluded) *(Table 3)* indicate that they may be ranked in this order: general combining ability (significant for 5 characters, largest percentage contributions), maternal effects (significant for 5), reciprocal effects (3), and specific combining ability (2). Thus GCA is most important and SCA least.

Table 2. – Variance components, standard errors, and heritabilities on family basis of the six characters.

Source	Germination rate	Germination percent	Cotyledon number	First-year height	Survival percent	Second-year height
ENV (σ_{env}^2)	0.2380 ± 0.3049	0.7778 ± 1.5031	0.0457 ± 1.1393	1.5242 ± 1.3525	-0.4496 ± 0.7338	0.4600 ± 0.8906
REP (σ_{rep}^2)	0.7773 ± 0.3296	3.2211 ± 2.0360	0.0423 ± 0.1211	0.7094 ± 0.2954	-1.4323 ± 0.8300	2.9058 ± 1.3890
GCA (σ_{gca}^2)	0.2445 ± 0.1293	4.7024 ± 6.2573	1.3575 ± 0.7363	0.0704 ± 0.0488	0.5280 ± 3.3592	2.0212 ± 1.7574
SELF (σ_{self}^2)	0.1855 ± 0.1922	170.9125 ±95.1583	0.5565 ± 0.8969	0.5633 ± 0.3090	172.7674 ±98.8049	66.6599 ±34.0397
SCA (σ_{sca}^2)	0.0506 ± 0.0435	25.9675 ±11.3693	-0.1990 ± 0.1308	0.0118 ± 0.0143	5.1744 ± 4.5007	0.2909 ± 0.2696
MAT (σ_{mat}^2)	0.0117 ± 0.0261	15.8071 ± 8.9118	0.3974 ± 0.2243	0.0065 ± 0.0060	0.7502 ± 0.5353	-0.0121 ± 0.0749
REC (σ_{rec}^2)	0.1621 ± 0.0789	9.2509 ± 4.5090	-0.1654 ± 0.1271	0.0045 ± 0.0119	-5.3492 ± 0.9169	0.3868 ± 0.2963
GE (σ_{ge}^2)	0.0095 ± 0.0040	0.2466 ± 0.6239	0.0909 ± 0.1189	0.0179 ± 0.0130	0.1675 ± 1.2405	-0.0783 ± 0.0277
ERR (σ_{error}^2)	1.3016 ± 0.0850	74.6352 ± 4.8738	10.9707 ± 0.7164	0.6184 ± 0.0403	136.2932 ±10.0726	7.8476 ± 0.5735
σ^2 (family mean)[1]	0.5820	62.0708	2.3501	0.1535	15.0164	3.4351
h^2 (family)[2]	0.42	0.08	0.58	0.46	0.04	0.59

[1] $\sigma^2 \text{(family mean)} = \sigma_{gca}^2 + \sigma_{sca}^2 + \sigma_{mat}^2 + \sigma_{rec}^2 + \sigma_{ge}^2/e + \sigma_{error}^2/er.$

[2] $h^2 \text{(family)} = \sigma_{gca}^2/\sigma^2 \text{(family mean)}.$

Table 3. – F values of the analyses of variance [1] and percentages of the major variance components (VC %)[2].

Component	Germination rate		Germination percent		Cotyledon number		First-year height		Survival percent		Second-year height	
	F	VC%	F	VC%	F	VC%	F	VC%	F	VC%	F	VC%
GCA	14.9***	52	2.6	8	28.8***	77	16.2***	75	2.9*	8	29.7***	75
SCA	2.0*	11	9.3***	47	0.7	0	1.4	13	1.6	80	1.7	11
MAT	5.2***	2	34.6***	28	5.9***	23	2.7*	7	0.9	12	1.8*	1
REC	3.7***	35	3.7***	17	0.7	0	1.2	5	0.3	0	1.9*	14

[1] Significance levels: *, 5%; **, 1%; ***, 0.1%.

[2] Negative components = 0

3) Narrow-sense heritabilities on a family basis *(Table 2)* are low for germination percent (0.08) and survival percent (0.04); and high for germination rate (0.42), cotyledon number (0.58), first-year height (0.46), and second-year height (0.59). The standard errors for GCA were smaller than the components themselves in four of the six characters, thus making the heritability estimates fairly reliable.

4) There were significant (5% or 1% level) phenotypic correlations *(Table 4)* between: germination rate and cotyledon number, germination rate and first-year height; germination percent and survival percent; first-year height and survival percent, first-year height and second-year height; and survival percent and second-year height.

5) Substantial negative genetic correlations were found *(Table 4)* between: germination rate and germination percent, germination rate and cotyledon number; germination percent and cotyledon number, germination percent and first-year height. Noteworthy positive genetic correlations included: germination rate and first-year height; survival percent and second-year height. Phenotypic and genetic correlations showed the same trend by having the same sign in six of the seven pairs of values.

Table 4. — Phenotypic[1] and genetic correlations (underlined).

	Characters	X_2	X_3	X_4	X_5	X_6
X_1	Germination rate	—0.04 —0.56	—0.30* —0.60	+0.50** +0.48	+0.01 —	+0.21 —
X_2	Germination percent		—0.26 —0.89	+0.10 —1.85	+0.37** —	+0.12 —
X_3	Cotyledon number			+0.07 +0.12	—0.01 —	+0.03 —
X_4	First-year height				+0.34* —	+0.46** —
X_5	Survival percent					+0.65** +1.09
X_6	Second-year height					— —

[1] Significance levels of the phenotypic correlations: *, 5%; **, 1%.

Discussion

The results of this first diallel cross in black spruce are consistent in several respects.

A sizable proportion of additive variance is present. High GCA variance components and heritabilities for germination rate, cotyledon number and first and second-year height indicate this. These are all characters of growth and size not directly related to reproductive fitness, namely the capacity to leave progeny (Lerner 1958, Falconer 1960).

Although the overall importance of dominance variance is smaller, it is present typically in fitness or survival characters. A large proportion of variance contributed by SCA for germination percent and survival percent reflects this. These characters also showed much inbreeding depression (indicated by a large variance component for selfing) and their heritability is low *(Table 2)*. Therefore this study agrees with the general theory that the closer a character is connected to fitness, the more inbreeding depression it will show, the lower will be its additive variance and its heritability (Falconer 1960, Tigerstedt 1967).

The components for maternal and reciprocal effects are more difficult to interpret. Falconer (1960) classifies maternal variance as environmental and indicates that maternal influences are one generation out of phase with other effects. The largest maternal components in this study were associated with germination rate, germination percent and cotyledon number *(Table 3)*, all characters that are strongly correlated with seed weights in this species (Morgenstern 1969). Probably reciprocal effects are very similar and both may reflect the health and vigor of a parent independent of its genetic contribution.

A large additive variance in characters of growth and size as determined here is in agreement with the genetic system of black spruce as a pioneer species. Although black spruce may occupy swamp habitats in the temperate part of its range thus giving the impression of a climax species, these habitats are extreme and do not permit normal species succession. More typical is its behaviour in its boreal range where it is most frequent and regenerates rapidly on open sites, for example, after fire. Levins (1964) and Rehfeldt and Lester (1969) have discussed the systems of adaptation of pioneer and successionally advanced species. In the former, additive variance prevails, heterozygosity is important and a high individual tolerance of non-optimal environments exists, while the rate of speciation is low. In the latter, epistatic and dominance variances are more important, genetic variances within populations more limited, isolation more prevalent, and the rate of speciation greater. Black spruce is a species that fits into the first category with its predominantly clinal variation pattern and the small degree of differentiation between local populations (Morgenstern 1969). — The heritability estimate for height growth based on additive variance obtained here, if expressed on an individual-tree basis, is also in close agreement with an earlier estimate obtained from open-pollinated families (Morgenstern 1973).

Among the genetic correlations, those between germination rate and height growth are of special interest because they indicate a possibility for early selection. However, this possibility needs to be confirmed from long-term juvenile-adult correlations, and correlations with additional characters of economic value.

The large amount of additive variance has implications for the development of a breeding program. For characters related to growth and yield, in particular, selection is promising. Since yield is much subject to environmental modification, progeny testing is needed. The progenies of open pollinated mothers are easy to obtain, and tests based on such families of half-sibs should be effective. To increase the selection differential, the best families should then be screened for promising individuals, possibly one individual only per family, to minimize inbreeding in this new generation of seed producers. In this way a breeding program can be started rapidly, without the necessity of establishing seed orchards and making controlled crosses, both of which are costly and time consuming procedures, and require more skills and facilities than may be available in the early phases of a breeding program.

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Summary

A 7 × 7 diallel cross was made in a plantation of black spruce to evaluate the relative role of additive and dominance genetic variance. Seed from all 49 families was sown and progeny grown for one season in a greenhouse and a second season in a nursery experiment. Six characters related to germination, survival and height were measured. A general least-squares analysis was made.

The results indicated that for characters related to reproductive fitness such as germination percent and survival percent, variance components were large for selfing and moderate to large for specific combining ability, general combining ability, maternal and reciprocal effects. Narrow-sense heritabilities of these characters were low. For characters not directly related to reproductive fitness (germination rate, cotyledon number, first- and second-year height), components for selfing and specific combining ability were smaller but for general combining ability larger. The heritability of these characters was high. A strong genetic correlation between germination rate and first-year height was found. Therefore general combining ability, i. e. additive genetic variance, is most important.

The relative role of additive and dominance variance in controlling character inheritance in black spruce, a pioneer species, is in good agreement with the fitness theory of R. Levins. Since all characters related to yield are largely controlled by additive variance, breeding by selection is promising, and testing of open-pollinated families a possibility.

Key words: Combining abilities, heritability, genetic system, breeding program.

Résumé

L'auteur fit un croisement diallèle 7 × 7 dans une plantation d'Épinettes noires afin d'évaluer le rôle relatif de la variance additive et de la variance de dominance génétique. Des graines de toutes les 49 familles furent semées et la progéniture fut produite durant une saison en serre, et une seconde saison en pépinière. L'auteur mesura six caractères ayant trait à la germination, la survie et la hauteur. Puis il effectua une analyse générale par moindres carrés.

Les résultats indiquent qu'en ce qui concerne les caractères reliés à la capacité de reproduction, tels le pourcentage de germination et le pourcentage de survie, les composantes de la variance étaient élevées pour les croisements intraspécifiques, et modérées à élevées pour la capacité spécifique de combiner, la capacité générale de combiner, et pour les effets maternels et réciproques. Les héritabilités, prises dans un sens étroit, de ces caractères étaient faibles. Chez les caractères non directement reliés à la capacité de reproduction (taux de germination, nombre de cotylédons, hauteur après la première et la deuxième années), les composantes pour les croisements intraspécifiques et pour la capacité spécifique de combiner étaient plus faibles, mais pour la capacité générale de combiner, elles étaient plus élevées. L'héritabilité de ces caractères était élevée. L'auteur trouva une forte corrélation génétique entre le taux de germination et la hauteur après un an. Par conséquent, la capacité générale de combiner, i. e. la variance génétique additive, s'avère très importante.

Le rôle relatif de la variance additive et de la dominance pour contrôler l'héritage des caractères chez l'Épinette noire, espèce pionnière, va bien de pair avec la théorie d'aptitude de R. Levins. Vu que tous les caractères reliés au rendement sont largement contrôlés par la variance additive, les croisements par sélection s'avèrent prometteur et il y a aussi la possibilité de tester les familles fécondées à ciel ouvert.

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