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Analysis of Genetic Variation in a Pinus strobus x P. griffithii F_1 Hybrid Population

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Summary

28 *P. strobus* x *P. griffithii* full-sib and 2 half-sib (parents) families were arranged (during artificial inoculation with *Cronartium ribicola*) in a rondomized complete block design. Each family was represented by a 14-seedling plot in each of three blocks. At age 6, the seedlings were transplanted in the field by using the same experimental design as in the inoculation tent. 13 traits were measured when the seedlings were 9 years old. The main results were, as follows: (1) Significant differences were found among hybrids for 11 out of 13 tested traits; (2) Differences among male and female trees were significant for the most traits, including blister rust resistance, diameter, basal area, volume and stem straightness; (3) Blister rust resistance, diameter, basal area and volume growth of the hybrids were 105%, 33%, 58% and 63%, respectively, higher than *P. strobus* and 33%, 25%, 114% and 400%, respectively, higher than *P. griffithii;* (4) The ratios GCA: SCA variance were 2:1 for basal area, 3:1 for number of stems, 1:1 for stem straightness and 1:0 for all the other traits; (5) Both positive and negative GCA effects which differed significantly from zero were found; (6) The narrow-sense heritabilities ranged from 0.383 for stem straightness to 0.853 for diameter; (7) If the best 2, 8, 14, and 20 out of 28 tested families were selected, a genetic gain of 14.1%, 9.0%, 6.1%, and 3.6%, respectively, in blister rust resistance and 25.2%, 16.1%, 10.9%, and 6.4%, respectively, in volume growth rate could be achieved.

Key words: Pinus strobus, P. griffithii, hybrid, *Cronartium ribicola,* resistance, general combining ability, genetic correlation, additive variance, heritability, genetic gain.

Introduction

Hybridization and backcrossing in advanced generations can be used as mean of combining desirable traits from two species into a new strain which can then be used to advantage in a tree breeding programme. Interspecific hybridization between eastern white pine (*Pinus strobus* L.) and blue pine (*P. griffithii* MC.CLELL) was performed by some workers, mainly for improving blister rust (*Cronartium ribicola* FISCH. ex RABENH.) resistance (PATTON, 1964; HEIMBURGER, 1964; BLADA, 1987) and growth traits (ZSUFFA, 1979a; KRIEBEL, 1982; LEANDRU, 1982). To date, the main results obtained from *P. strobus* x *P. griffithii* hybridization have been:

— Hybrid progenies contained a higher percentage of resistant seedlings than intraspecific *P. strobus* crosses (HEIMBURGER, 1962; PATTON, 1964; ZSUFFA, 1979b; BLADA, 1987);

— One year old hybrid progenies showed considerable variability in height, crown size and needle length (PATTON, 1964);

— Juvenile F_1 hybrids outgrew in height the *P. strobus* controls (ZSUFFA, 1979a; LEANDRU, 1982; BLADA, 1987);

— Differences were found among *P. griffithii* male parents in their ability to transmit resistance to the hybrids with *P. strobus* (PATTON, 1964; BLADA, 1987);

— The best families were from 22% to 44% superior in volume to the best *P. strobus* families in three 17 to 22 years old progeny tests (KRIEBEL, 1982);

— According to KRIEBEL (1982), some workers suggest that non-additive variance was most important in interspecific hybridization of white pines whereas in a *P. strobus* x *P. griffithii* F_1 population the additive genetic variance was predominant (BLADA, 1987);

— Blister rust resistance and height growth in 5-year-old hybrids were shown to be under polygenic control and heritable; narrow-sense heritabilities, at family level, were 0.64 and 0.65 for blister rust and height growth, respectively; the expected gains were 11% in resistance and 3% in height growth if the best 8 families were selected (BLADA, 1987).

This paper reports the results of factorial analyses of some traits in a 9 year old *P. strobus* x *P. griffithii* F_1 hybrid population.

Materials and Methods

Initial materials and mating design

The mating design and genetic model followed those of KOMSTOCK and ROBINSON's (1952) Experiment II adapted to this case. Both populations and parents were taken at

Table 1. — Measured traits.

	Traits	Units	Symbol
1	Blister-rust resistance	Index 1...10	BR_1
2	Trees free of blister rust	%	BR_2
3	Trees survivers (free+cankered)	%	BR_3
4	Annual height growth in 1989	dm	Ha
5	Total height growth	dm	Ht
6	Diameter at 1/2 of height	cm	D
7	Basal area at 1/2 of height	dm	BA
8	Stem volume	dm	V
9	Number of stems	Index 1...3	NS
10	Stem straightness	Index 1...3	SS
11	Branch thickness	Index 1...3	BT
12	Branches/whorl	N♀	NBW
13	Crown widh	dm	CW

random without regard to blister rust resistance and to any other trait except female flower production.

The 7 eastern white pine females and the 4 blue pine males of unknown origin were factorially crossed in 1979. Before crosses, the parents were not tested for any trait. The seeds were stratified according to KRIEBEL's (1973) methodology and then sown (spring 1981) in individual polyethylene pots (22 cm x 18 cm x 18 cm) in a potting mixture consisting of 70% spruce humus and 30% sand.

The seedlings grew in pots throughout the first 6 years; because of the "pot stress" the height and diameter growth of the hybrids was about half that of the control hybrids that were outplanted at age 4 (BLADA, unpublished data).

Inoculation, experimental design and testing

The seedlings were artificially inoculated in 1982, 1983 and 1984, between 20 August and 30 August, when they were 2, 3 and 4 years old. During each inoculation, the pots with seedlings were placed in a polyethylene tent and arranged in a randomized complete block design. Each family was represented by a 14-seedling plot in each of the 3 blocks. Two half sib progenies, representing the mean of the open-pollinated parents, were included as control.

Inoculum material consisted of heavily infected leaves of *Ribes nigrum* L. harvested from a single population. Other details concerning inoculation and inoculation tent were more or less similar to those described by BINGHAM (1972).

At age 6 the seedlings were transplanted in the field by using the same experimental design as in the inoculation tent. Therefore, the nursery test took place between 1981 and 1986 and the field test between 1987 and 1989.

Measurements

The 13 traits listed in *table 1* were measured in the autumn of 1989 at age 9.

As stated in *table 1*, the blister rust resistance was measured by three indices. The first index (BR_1) reflects the economic and biological impact as well as the incidence of disease; this index takes into consideration both the number and severity of the lesions. Its numerical values were assigned, as follows: 1 = trees dead or total susceptibility; 2 = 4 or more serious stem lesions; 3 = 3 severe stem lesions; 4 = 3 more or less severe stem lesions; 5 = 2 severe stem lesions; 6 = 2 more or less stem lesions; 7 = 1 severe stem lesion; 8 = 1 more or less severe stem lesion; 9 = branch or very light stem lesions; 10 = no lesions or total resistance.

The second and the third indices (BR_2 and BR_3) were calculated based on the BR_1 index data; i.e. all trees with the score 10 were considered "trees free of blister rust (BR_2)" and trees with a score greater than 1 were considered "trees surviving (BR_3)" Before statistical analysis, the percentages were transformed to the arc $\sin\sqrt{\%}$ values.

A subjective 1 to 3 index was used for the assesment of the following 3 traits: (a) "Number of stems (NS)", where: 1 = 3 or more stems; 2 = 2 stems; 3 = 1 stem; (b) "Stem straightness (SS)", where: 1 = sinuous; 2 = middle straight; 3 = straight; (c) "Branch thickness (BT)", where: 1 = very thick; 2 = middle thick; 3 = thin.

The other traits do not require additional explanation. Family means were basic data for statistical analysis.

Statistical analysis

The statistical model assumes that the replicates were fixed and that the males and females were random samples from basal population. The formula for this model is:

$$X_{ijkh} = m + M_i + F_j + (MF)_{ij} + B_k + (MFB)_{ijk} + e_{ijkh} \quad (1)$$

where: X_{ijkh} = the observation of the h-th full-sib family from the cross of the i-th male and j-th female in the k-th block; m = the general mean; M_i = the effect of the i-th male (i = 1, 2, . . . I); F_j = the effect of the j-th female (j = 1, 2, . . . J); $(MF)_{ij}$ = the effect of the interaction of the i-th male and j-th female; B_k = the effect of the

Table 2. — Model for analysis of variance according to COMSTOCK and ROBINSON Experiment II.

Source of variation	DF	MS	E (MS)
Blocks (B)	K-1	MS_B	
Males (M)	I-1	MS_M	$\sigma_e^2 + K\sigma_{MF}^2 + KJ\sigma_M^2$
Females(F)	J-1	MS_M	$\sigma_e^2 + K\sigma_{MF}^2 + KI\sigma_F^2$
Males x Females (MF)	(I-1)(J-1)	MS_{MF}	$\sigma_e^2 + K\sigma_{MF}^2$
Pooled errors	(IJ-1)(K-1)	MS	σ_e^2
$\sigma_M^2=(MS_M-MS_{MF})/KJ$; $\sigma_F^2=(MS_F-MS_{MF})/KI$; $\sigma_{MF}^2=(MS_{MF}-MS_E)/K$; $\sigma_e^2=MS_E$			

k-th block (k = 1, 2, . . . K); $(MFB)_{ijk}$ = the effect of the interactions of the i-th male j-th female and k-th block; e_{ijkh} = the random error.

The analysis of variance with the expectations of mean squares and formulas for estimating the variance components are given in *table 2.*

Standard errors (SE) of variance components were computed by the following formula (ANDERSON and BANCROFT, 1952):

$$SE = \sqrt{\frac{2}{K_1^2} \sum_g \frac{MS_g^2}{f_g + 2}} \qquad (2)$$

where: K_1 = coefficient of the variance component; MS^2_g = the g-th mean square used to estimate the variance component; f = the degrees of freedom of the g-th mean square.

The narrow-sense (h^2_A) and the broad-sense (H^2) heritability formulas, at family level, according to GRAFIUS and WIEBE (1959), were:

$$h^2_A = \frac{\sigma^2_M + \sigma^2_F}{\sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_e/K} \qquad (3)$$

$$H^2 = \frac{\sigma^2_M + \sigma^2_F + \sigma^2_{MF}}{\sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_e/K} \qquad (4)$$

There were also calculated heritability estimates due to male (h^2_M) and female (h^2_F) variances.

Two kinds of genetic gain were calculated, as follows:
(1) As compared to the hybrid population mean, and
(2) As compared to each parent mean by using the formulas (5) and (6), respectively:

$\Delta G = ih^2\sigma_P$ (FALCONER, 1960) (5)

$\Delta G = h^2S$ (ROBINSON, COMSTOCK and HARVEY, 1949) (6)

where: i= intensity of selection; σ_P= phenotypic standard deviation; S = selection differential.

Estimation of general combining abilities (GCA) followed GRIFFING's (1956) Method 4 and formula adapted to a factorial design.

Genetic correlation among traits at the additive level were calculated by using the "corelation key" of the Texas Instruments-51 III computer (see Owner's Manual, 1978, p 36); the basic data were the general combining ability effects, that is the additive genetic effects.

The index of selection (I_s) was calculated for each family taking into consideration only blister rust resistance (BR_1) and stem diameter as economically important traits. The calculation formula was (BLADA, unpublished data):

$$I_s = X_1 . h^2_{A_1} + X_2 . h^2_{A_2} \qquad (7)$$

where: X_1 and X_2 are the means of blister rust resistance (BR_1) and stem diameter, respectively and h^2_{A1} and h^2_{A2} are the heritabilities of the two traits.

The heterosis (H) was calculated by comparing the hybrid population mean to the best parent mean, by using the formula:

$$H_{(\%)} = (d/P) . 100 \qquad (8)$$

where: d= difference between the hybrid population mean and the best parent mean (P) for the trait taken into consideration. This is in accordance with the term "hybrid vigor" or heterosis that refers to size superiority over both parents but it is essential to understand that the term may be properly used for things other than size (ZOBEL and TALBERT, 1984).

Results

Genetic variation

Significant differences ($p < 0.05$; $p < 0.01$; $p < 0.001$) were found among hybrid families for 11 of 13 tested traits (*Table 3*, row 2). Therefore selection within hybrid population is possible for the most economically important traits.

Differences among male trees were significant ($p < 0.05$; $p < 0.01$; $p < 0.001$) for all traits except blister rust resistance (BR_1), height growth, branch thickness and crown widh (*Table 3*, row 3).

There were significant differences ($p < 0.05$; $p < 0.01$; $p < 0.001$) among female trees for all traits (*Table 3*, row 4);

Male x female interactions were nonsignificant for all traits except stem straightness (*Table 3*, row 5). These and the above-mentioned data suggest that non-additive action of the genes was absent whereas the additive effect was significant for most traits.

Substantial variation was demonstrated at the family level for most traits (*Table 4*). The best family measured 7.50 points in blister rust resistance (BR_1), while the

Table 3. — Analysis of variance of the tested traits in *P. strobus* × *P. griffithii* hybrid population.

Source of variation	D. f.	Mean squares of the following traits												
		BR1	BR2	BR3	Ha	Ht	D	BA*)	V	NS	SS	NBW	BT	CW
Blocks	2	0.495	223.1	632.4	0.308	2.038	0.093	0.0001	0.0551	0.059	0.038	1.282	0.490	5.780
Hybrids	27	1.688^{++}	146.4^{+}	155.0	0.696^{+++}	1.735^{++}	0.038^{+++}	0.0037^{++}	0.0114^{+++}	0.051^{+++}	0.081^{+++}	0.200^{++}	0.113	0.596^{+++}
– Males (M)	(3)	1.881	399.8^{++}	341.4^{+}	1.061^{++}	0.080	0.070^{++}	0.0033^{+}	0.0100^{+}	0.226^{+++}	0.287^{+++}	0.299^{+}	0.111	0.080
– Females (F)	(6)	4.837^{+++}	270.8^{++}	411.9^{+++}	2.357^{+++}	7.020^{+++}	0.128^{++}	0.0150^{++}	0.0430^{+++}	0.038^{+}	0.055^{+}	0.582^{+++}	0.353^{+++}	2.202^{+++}
– M×F	(18)	0.606	62.7	38.3	0.081	0.249	0.004	0.0021	0.0011	0.026	0.054^{+++}	0.057	0.034	0.147
Pooled error	54	0.769	68.5	90.1	0.242	0.662	0.007	0.0007	0.0029	0.014	0.018	0.084	0.075	0.143

*) The values for BA were multiplied by 100.

Table 4. — Distribution of the hybrids into homogeneous groups for some traits according to Duncan test.

BR_1			D			Ht			V			I.s	
Family	Mean (Index)	Duncan test $p<0.05$	Family	Mean (cm)	Duncan test $p<0.05$	Family	Mean (dm)	Duncan test $p<0.05$	Family	Mean (dm^3)	Duncan test $p<0.05$	Family	Values
512-C	7.50		522-C	2.23		633-B	14.5		516	0.53		512-C	6.94
512-B	7.40		516	2.17		630-B	14.5		516-B	0.53		512-B	6.39
630-B	7.30		633-B	2.13		633	14.4		512-C	0.48		633-B	6.19
630	7.20		512-B	2.13		630	14.4		512-B	0.48		630-B	6.16
628-B	7.20		516-B	2.07		516	14.2		633-B	0.48		628-B	6.13
526	7.13		633	2.03		516-B	14.1		633	0.48		526	6.09
628	7.13		634	2.03		631	14.0		634	0.45		633	6.04
633-B	7.07		631	2.00		631-B	13.9		634-B	0.45		630	6.01
526-B	7.03		526	1.97		634	13.8		631	0.41		628	6.00
633	6.97		522-C	1.97		615	13.7		631-B	0.41		526-B	5.93
613-B	6.93		629	1.97		634-B	13.7		615-B	0.41		613-B	5.91
613	6.83		628-B	1.97		613-B	13.6		615	0.41		613	5.75
631	6.53		630-B	1.93		615-B	13.6		629	0.39		631	5.75
629	6.43		634-B	1.93		512-C	13.6		526	0.39		634	5.69
631-B	6.43		622	1.93		613	13.5		526-B	0.39		629	5.65
634	6.40		615	1.93		512-B	13.5		629-B	0.39		516	5.62
629-B	6.33		613-B	1.90		526	12.7		630-B	0.38		631-B	5.60
634-B	6.30		621-B	1.90		629	12.7		630	0.38		634-B	5.54
615	6.20		631-B	1.90		622	12.7		622	0.37		516-B	5.53
615-B	6.10		522-B	1.87		628-B	12.7		622-B	0.37		615	5.48
516	6.10		526-B	1.87		526-B	12.6		628-B	0.35		629-B	5.47
522-C	6.03		628	1.87		629-B	12.6		628	0.35		522-C	5.41
516-B	6.00		629-B	1.87		622-B	12.6		522-C	0.34		615-B	5.33
522-B	5.93		615-B	1.83		628	12.6		522-B	0.34		522-B	5.25
621-B	5.23		630	1.83		621-B	12.3		613-B	0.33		621-B	4.86
621	5.17		622-B	1.80		522-C	12.3		613	0.33		621	4.72
622	4.93		613	1.80		621	12.2		621-B	0.32		622	4.70
622-B	4.83		621	1.80		522-B	12.2		621-B	0.31		622-B	4.51

poorest one measured only 4.83, i.e. 36% more susceptible. Also, the fastest growing family measuring 14.5 dm in height was 55% taller than the poorest one and produced 71% more in volume. Other traits also showed substantial variation.

Hybrid vigor or heterosis

It was of interest to know whether or not the *P. strobus* x *P. griffithii* hybrid does have hybrid vigor under the test environment. According to the data (*Table 5*, row 4) it does. The hybrid is outperforming both parents in the following traits: blister rust resistance (BR_1, BR_2, BR_3), annual growth, diameter, basal area, volume growth, number of branches per whorl and crown widh. Quantitative values of the heterosis for these traits are listed in the *table 5*.

Variance components

Variance components, standard errors and heritabilities are shown in *table 6*.

The analyses indicated that GCA variance was the major source of variation in most traits studied in this experiment.

Whereas GCA variance showed preponderance, the SCA variance was almost absent for all traits except number of stems and stem straightness. Consequently, most traits could be improved by using additive genetic variance.

The relative importance of additive and non-additive genetic variance is indicated by their proportions. The ratios GCA : SCA variance were 2:1 for basal aret, 3:1 for number of stems, 1:1 for stem straightness and 1:0 for all

Table 5. — The mean performance of the hybids (compared to the parents) and the heterosis effect.

	BR_1 (Index)	BR_2 (%)	BR_3 (%)	Ha (dm)	Ht (dm)	D (cm)	BA (dm^2)	V (dm^3)	NS (Index)	SS (Index)	NBW (Index)	BT (Index)	CW (dm)
P strobus (♀)	3.15	14.0	24.1	3.7	13.2	1.5	0.019	0.246	2.9	2.6	3.2	1.0	6.3
Hybrids (♀ + ♂)	6.45	23.7	87.4	3.9	13.3	2.0	0.030	0.400	2.9	2.6	3.4	2.0	7.4
P griffithii (♂)	4.85	16.1	38.7	1.4	6.3	1.6	0.014	0.080	2.2	1.7	3.3	2.0	4.0
Heterosis effct *) (%))	+ 33	+47	+126	+ 5	+ 1	+25	+58	+ 63	0	0	+ 3	0	+17

*) Compared to the best parent

Table 6. — Variance component estimates, standard errors[1]) and heritabilities on family basis of the traits.

Components	BR_1	BR_2	BR_3	Ha	Ht	D	BA	V	NS	SS	BT	NBW	CW
σ^2_{GCA-F}	0.353 ±0.202	17.34 ±11.40	31.13 ±17.19	0.190 ± 0.098	0.564 ± 0.293	0.103 ± 0.005	0.0107 ±0.0001	3.500 ± 0.002	0.100 ± 0.003	0.008 ±0.015	2.658 ± 0.024	4.375 ± 0.092	0.171 ± 0.092
σ^2_{GCA-M}	0.061 ± 0.057	16.05 ±12.08	14.43 ± 10.30	0.047 ± 0.032	-0.008 ± 0.004	0.031 ± 0.002	0.0005 ± 0.0001	0.424 ± 0.001	0.952 ± 0.007	1.110 ±0.009	0.367 ± 0.003	1.152 ±0.009	- 0.003 ± 0.003
Total σ^2_{GCA}	0.414	33.39	45.56	0.237	0.564	0.134	0.0112	3.924	1.052	1.118	3.025	5.527	0.171
σ^2_{SCA-MF}	-0.054 ± 0.080	-1.93 ± 0.79	-17.27 ± 6.96	- 0.054 ± 0.017	-0.138 ± 0.049	- 0.010 ± 0.001	0.0046 ± 0.0001	- 0.600 ± 0.001	0.400 ± 0.003	1.200 ± 0.006	- 4.100 ± 0.006	- 0.900 ± 0.008	0.001 ± 0.018
Total σ^2_G	0.414	33.39	45.56	0.237	0.564	0.134	0.0158	3.924	1.452	2.318	3.025	5.527	0.172
σ^2_e	0.256 ±0.059	22.83 ± 4.31	30.03 ± 5.67	0.081 ± 0.015	0.221 ± 0.042	0.023 ± 0.004	0.0023 ±0.0004	0.967 ± 0.180	0.467 ±0.088	0.600 ± 0.113	2.500 ± 0.470	2.800 ± 0.500	0.048 ± 0.009
σ^2_p	0.67	56.22	75.59	0.318	0.785	0.157	0.0181	4.891	1.919	2.918	5.525	8.327	0.220
σ_p	0.818	7.498	8.694	0.564	0.886	0.125	0.0043	0.070	0.139	0.171	0.235	0.289	0.469
h^2_F	0.527	0.308	0.412	0.597	0.718	0.656	0.591	0.715	0.052	0.003	0.481	0.525	0.777
h^2_M	0.091	0.286	0.191	0.148	—	0.197	0.028	0.087	0.496	0.380	0.066	0.139	—
h^2_A	0.618	0.594	0.603	0.745	0.718	0.853	0.619	0.802	0.548	0.383	0.547	0.664	0.777
H^2	0.618	0.594	0.603	0.745	0.718	0.853	0.873	0.802	0.757	0.794	0.547	0.664	0.784
$\sigma^2_{GCA-F} : \sigma^2_{GCA-M}$	6:1	1:1	2:1	4:1	1:0	3:1	21:1	8:1	1:9	0:1	7:1	4:1	1:0
$\sigma^2_{GCA} : \sigma^2_{SCA}$	1:0	1:0	1:0	1:0	1:0	1:0	2:1	1:0	3:1	1:1	1:0	1:0	1:0

[1]) Variance components and standard errors of some traits were multiplied as follows: D by 10; BA and V by 1000; SS, BT and NBW by 100.

Legend:

$\sigma^2_{GCA—F}$ and σ^2_{GCA-M} = the general combining ability variance due to females and males, respectively;
σ^2_{SCA-MF} = the specific combining ability variance due to male × female interactions;
σ^2_G, σ^2_e, σ^2_p = the genetic, error and phenotypic variance, respectively;
h^2_M h^2_F h^2_A = the male, female and narrow-sense heritabilities, respectively;

the other traits. This suggests that a selective breeding strategy utilizing additive variation would be efficient.

The ratios GCA-F : GCA-M variance showed a preponderance of the former variance, present in *P. strobus*, for all traits except number of stems and stem straightness (*Table 6*, row 13). For blister rust resistance (BR_1), diameter, basal area and volume, the GCA-F variance averaged about 6, 3, 21, and 8 times larger, respectively, than SCA-M variance. Therefore, the most important amount of variation to be used in an improvement programme can be found in the *P. strobus;* according to this study, there was no usuable variation in *P. griffithii,* but the sample (only 4 males) was small and further confirmation is needed.

This test indicates that, since the preponderance of genetic variation was due to GCA effects, the estimation of parental breeding value could be accomplished by one of more economical progeny testing methods such as interspecific controled polycrossing. If SCA is low, as in this case, any male tester is suitable if its effect is reasonably uniform.

Combining ability

The estimates of general combining ability effects are listed in *table 7.*

Positive and negative GCA effects, which differed significantly from zero, were found mainly in the female parents.

Parents 65 and 315 have the largest GCA effects for growth traits and even acceptably blister rust resistant. Parent 62 exhibited the largest positive GCA effects for blister rust resistance whereas parent 215 had the largest negative GCA effect for eight traits including blister rust resistance. Consequently, the breeding strategy would be

Table 7. — Estimates of general combining ability (GCA) effects.

Parents	Traits / effects												
	BR_1	BR_2	BR_3	Ha	Ht	D	BA	V	NS	SS	NBW	BT	CW
G. C. A. - Females													
2	0.064	-1.964	3.464	0.169	0.245	-0.086$^+$	-0.003$^+$	-0.032	-0.009	0.076	-0.131	-0.076	-0.171
62	0.414	-1.214	6.464	0.636$^+$	0.845$^+$	-0.036	-0.001	-0.004	0.074	0.043	0.119	-0.243$^+$	0.312$^+$
65	0.231	3.702	-0.202	0.519$^+$	0.778$^+$	0.081$^+$	0.003$^+$	0.061$^+$	0.024	0.043	0.286$^+$	0.274$^+$	0.612$^+$
215	-1.411	-9.464$^+$	-2.119	-0.448$^+$	-0.855$^+$	-0.086$^+$	-0.003$^+$	-0.056$^+$	0.057	0.043	-0.114	-0.110	-0.588$^+$
315	0.298	3.036	0.714	-0.198	-0.495	0.198$^+$	0.007$^+$	0.104$^+$	-0.060	-0.057	0.269$^+$	-0.009	0.295
326	0.081	2.702	-0.452	-0.398	-0.838$^+$	-0.036	-0.001	-0.039	-0.076	-0.107	-0.198	0.007	-0.405$^+$
68	0.323	3.203	2.131	-0.281	-0.671$^+$	-0.035	-0.001	-0.034	-0.010	-0.040	-0.231	0.157	-0.055
G. C. A. - Males													
21	0.210	3.250	2.988	0.138	-0.069	-0.048	-0.002$^+$	-0.019	0.019	-0.014	-0.140	-0.012	-0.026
26	-0.205	-3.321	-2.726	-0.138	0.069	0.048	0.002$^+$	0.019	-0.005	0.138$^+$	+0.140	0.012	0.021
21 - B	0.300	4.250	3.893	0.238	0.031	0.052	-0.001	-0.018	0.019	-0.381$^+$	-0.040	0.088	0.074
26 - B	-0.305	-4.179	-4.155	-0.238	-0.031	-0.052	0.001	0.018	-0.033	+0.257$^+$	+0.040	-0.088	-0.069

based on the parents with the largest GCA. This would be particularly important when the trait to be improved is quantitatively inherited.

Genetic control

The histograms in *figure 1* show the frequency distribution of blister rust resistance (A) and height growth (B) values in the *P. strobus* x *P. griffithii* hybrid population. According to genetic theory (LERNER, 1958; MATHER and JINKS, 1977) polygenes are responsible for the expression of continuously distributed characters. Since the frequencies in *figure 1* are normally distributed, it is assumed that blister rust resistance and height growth of the 9-year-old hybrids are polygenically controlled; similar results were obtained on the same biological material at age 5 (BLADA, 1987).

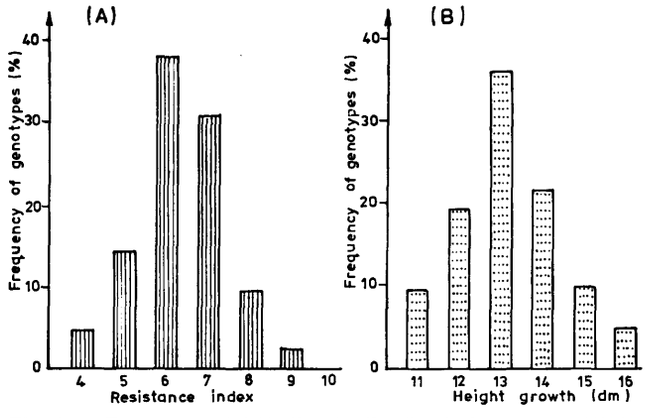

Fig. 1. — Frequency distribution in the *P. strobus* x *P. griffithii* hybrid population evaluated for blister rust resistance (A) and height growth (B).

Table 8. — Correlation coefficients among traits for GCA genetic effects (D.f = 9).

Traits	BR_1	BR_2	BR_3	Ha	Ht	D	BA	V	NS	SS	NBW	BT	CW
BR_1		0.864^{++}	0.624$^+$	0.552	0.431	0.394	0.296	0.354	-0.218	-0.349	0.185	0.292	0.671$^+$
BR_2			0.462	0.337	0.131	0.486	0.309	0.310	-0.323	-0.600	0.067	0.600	0.537
BR_3				0.656$^+$	0.389	-0.050	-0.294	-0.172	0.392	-0.505	-0.142	-0.155	0.304
Ha					0.927^{++}	0.150	0.002	0.227	0.561	-0.110	0.429	0.035	0.737$^+$
Ht						0.100	0.095	0.321	0.489	0.199	0.554	-0.020	0.736$^+$
D							0.910^{++}	0.870^{++}	-0.308	-0.290	0.747^{++}	0.415	0.654$^+$
BA								0.959^{++}	-0.428	0.087	0.813^{++}	0.306	0.631$^+$
V									-0.279	0.151	0.894^{++}	0.257	0.761$^+$
NS										0.017	0.074	-0.226	0.148
SS											0.220	-0.314	0.148
NBW												0.111	0.779^{++}
BT													0.364
CW													—

Table 9. — Expected genetic gain (ΔG%) for some traits.

Traits	ΔG compared to hybrid mean if selected the best .... families				ΔG compared to P.strobus mean if selected the best .... families				ΔG compared to P. griffithii mean if selected the best .... families			
	2	4	8	20	2	4	8	20	2	4	8	20
BR_1	14	9	6	4	29	19	13	7	19	12	8	5
BR_2	34	22	15	9	57	37	25	15	50	32	22	13
BR_3	11	7	5	3	39	25	17	10	24	16	11	6
Ht	9	6	4	2	9	6	4	2	18	12	8	5
D	10	6	4	3	13	8	6	3	12	8	5	3
BA	16	10	7	4	25	16	11	6	34	21	14	9
V	25	16	11	6	41	26	18	11	126	81	54	33
SS	5	3	2	1	5	3	2	1	7	5	3	2

Heritability

The high levels of additive variance were reflected in higher narrow-sense heritabilities that ranged from 0.383 for stem straightness to 0.853 for diameter. The maternal heritability (h^2_F) estimates were consistently higher than paternal (h^2_M) ones, except for number of stems and stem straightness. The obvious explanation is that the maternal heritabilities were biased upward by maternal variances. All heritability estimates are presented in *table 6.*

Genetic correlations

Significant ($p < 0.05$) and highly significant ($p < 0.01$; $p < 0.001$) positive genetic correlations were obtained among some traits, as follows (*Table 8):*

(1) Among diameter, basal area, volume, number of branches per whorl and crown widh;

(2) Among the three types of blister rust resistance;

The correlations indicate that selection for one trait will cause a simultaneous improvement for the others.

Genetic gain

If the best 2, 8, 14, and 20 out of 28 hybrid families would be planted in suitable sites, a genetic gain in blister rust resistance and volume growth of about 14%, 9%, 6%, 4% and 25%, 16%, 11%, 6%, respectively, could be expected in comparison with the hybrid population mean (*Table* 9, rows 1 and 7).

In comparison with the *P. strobus* parent population mean, a genetic gain in the same 2 traits and at the same intensity of selection of 29%, 19%, 13%, 7% and 41%, 26%, 18%, 11%, respectively, could be expected (*Table 9,* rows 1 and 7). These results and others listed in *table 9* suggest that plantations with *P. strobus* x *P. griffithii* F_1 hybrids, could be economically profitable.

Conclusions

The mating between fast growing *P. strobus* and moderate to high blister rust resistant *P. griffithii* resulted in a hybrid that demonstrated heterosis in most traits, including blister rust resistance and volume growth rate.

Enough genetic variation was demonstrated to encourage selective breeding for most economically important traits.

The breeding strategy would have to use the additive genetic variance.

The magnitude of variation in GCA effects revealed that it may be possible to select parents with superior breeding value for some important traits.

Positive correlations for GCA effects suggest that correlated responses for some traits will be obtained if selection is done on only one.

Due to the moderate to high value of narrow-sense heritability estimates, a similar genetic gain could be expected in most of the tested traits including blister rust resistance and growth traits.

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Allozyme Variation and Inheritance in Leaves of Populus deltoides, P. nigra, P. maximowiczii and P. x canadensis in Comparison to Those in Root Tips

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Abstract

Methods for extraction and electrophoresis of leaf tissue enzymes in *Populus deltoides* MARSH., *P. nigra* L., *P. maximowiczii* HENRY, and *P.* x *canadensis* MOENCH are described. Eleven enzymes were assayed in 54 individual clones of these poplars, and in progeny and parents of seven controlled crosses of *P. deltoides* with *P. deltoides*, *P. nigra*, and *P. maximowiczii*. 10 of these enzymes were analysed earlier in root tips of the same individuals. In addition, colorimetric esterase was analysed. A total of 33 genes coding for 11 enzyme systems were observed. 87% to 91% of the genes expressed in leaf tissue of the 3 *Populus* species and one interspecific hybrid were also expressed in root tips. Allozymes of 26 loci expressed identically in leaves and root tips. Four loci, *Per-1*, *Per-2*, *Per-3*, and *Sdh-2* that expressed in root tips of the *Populus* species, however were not detected in leaf tissue. In addition, 2 peroxidase loci *Per-L1*, and *Per-L2*, were detected only in leaves. Single-gene control was observed for isozyme variants of each of the 4 enzyme zones (*CE-1*, *CE-2*, *PER-L1*, *PER-L2*) investigated or detected only in leaves. Allozymes in one species were allelic to allozymes in other species at 16 loci. *Populus deltoides*, *P. nigra*, and *P. maximowiczii* could be differentiated by isozymes of *Ce-1* in leaves.

Key words: Poplars, enzyme electrophoresis, clones, clone genotypes, gene expression, hybrids.

Introduction

Populus deltoides MARSH. (section *Aigeiros* DUBY), *P. nigra* L. (section *Aigeiros*), *P. maximowiczii* HENRY (section *Tacamahaca* SPACH.) and their interspecific hybrids are important for poplar breeding and intensive plantation programs (ZSUFFA, 1976; Anonymous, 1979; DICKMANN and STUART, 1983). *Populus deltoides* x *P. nigra* hybrids are named as *P.* x *canadensis* MOENCH syn. *P.* x *euramericana* (DODE) GUINIER. Allozymes and their multilocus genotypes are useful for various genetic, breeding and phylogenetic studies in these *Populus* species (RAJORA, 1986, 1988, 1989a and b, 1990a; RAJORA and ZSUFFA, 1986, 1989, 1990).

Methods of enzyme electrophoresis, allozyme genotypes of clones, inheritance and linkage of allozymes, and diagnostic (species-specific) allozyme genes and alleles in root tips of *P. deltoides*, *P. nigra*, *P. maximowiczii*, and *P.* x *canadensis* have been described previously (RAJORA, 1988, 1989a and b, 1990a and b; RAJORA and ZSUFFA, 1989). Leaves are the most abundant and easily available tissues for electrophoretic analysis, thus, it is highly desirable to develop methods of enzyme extraction and electrophoresis in leaf tissue. In poplars, such methods have been developed for leaf tissues of *P. tremuloides* MICHX. (CHELIAK and PITEL, 1984), and P. balsamifera L. (FARMER et al., 1988).

We undertook the present investigation to develop methods of enzyme extraction, electrophoresis and detection in leaf tissue of *P. deltoides*, *P. nigra*, *P. maximowiczii*, and *P.* x *canadensis*. We examined (i) enzyme banding patterns and allozyme coding genes, (ii) allozyme genotypes of the clones, (iii) diagnostic loci and alleles for species and their interspecific hybrids, and (iv) isozyme inheritance. The results of these investigations are compared with the results earlier obtained for root tips.

Materials and Methods

Populus Species and Clones

54 individuals of *P. deltoides*, *P. nigra*, *P.* x *canadensis*, and *P. maximowiczii* were studied: 16 individuals of *P. deltoides* representing var. *deltoides* and var. *occidentalis* (RAJORA, 1989a); 13 individuals of *P. nigra* representing var. *nigra*, var. *italica*, var. *plantierensis*, cv. Vereecken, and cv. Ichenheim (RAJORA, 1989b); 17 individuals of *P.* x. *canadensis* each representing a different cultivar (RAJORA and ZSUFFA, 1989); and 8 individuals of *P. maximowiczii* (RAJORA, 1988). Except for P. x *canadensis* cv. I-214, all of these individuals were picked from among the individuals whose allozyme genotypes and phenotypes were determined earlier in root tips (RAJORA, 1988, 1989a and b; RAJORA and ZSUFFA, 1989).

Controlled Crosses

10 progeny of each of the 2 intraspecific *P. deltoides* crosses, 3 *P. deltoides* x *P. nigra* crosses, and 2 *P. deltoides* x *P. maximowiczii* crosses were studied. These crosses were made in 1983 (RAJORA, 1986, 1990b), and the sampled progenies were established in a field-progeny test in Ontario. Root-tip allozyme genotypes of these offspring were obtained previously. Inheritance and linkage of allozymes were studied earlier in root tips of much larger numbers of progeny (40 to 104 per cross, with a total of 807 for 12 crosses) of these and 5 other controlled crosses (RAJORA, 1990b).

Tissue Preparation and Enzyme Extraction

Tissues of very young expanding leaves from the sprouts of rooted cuttings were used for enzyme electrophoresis.

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