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GENETIC VARIATION IN BLISTER RUST RESISTANCE AND GROWTH TRAITS IN *PINUS STROBUS* H *P. PEUCE* HYBRID AT AGE 17 (EXPERIMENT 1)

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ABSTRACT

A 7 × 4 factorial crossing was performed between *Pinus strobus* (female) and *P. peuce* (male) to combine the rapid growth of *P. strobus* with high resistance of *P. peuce* to blister-rust. The resulting 28 families were artificially inoculated at age two and planted in the field at age 6. Nine traits were measured at age 17. The results indicated that: (1) the effects of female parents were highly significant for growth traits and blister-rust resistance, whereas the male parents had highly significant effects only on growth traits; (2) the hybrid performance was intermediate between the two parent species, in all traits; (3) highly significant positive correlations were found among growth traits, but no significant correlations were found between any growth trait and blister-rust resistance; (4) good general combiners, not only for growth but for blister-rust resistance, too, were found among eastern white pine parents as three of 11 parents had positive significant *gca* effects for blister-rust resistance and five for volume growth; and (5) a genetic gain of 9.5% in blister-rust resistance and 18.3% in volume growth could be achieved.

Key words: *Pinus strobus, P. peuce*, factorial cross, combining ability, heterosis, breeding value.

INTRODUCTION

Eastern white pine (*Pinus strobus* L.) is a fast growing species but susceptible to blister-rust caused by *Cronartium ribicola* Fisch. ex Rabenh. In North America, the inefficiency of conventional control methods for blister-rust has stimulated improvement in resistance through breeding (RIKER *et al.* 1943; BINGHAM *et al.* 1953; RIKER & PATTON 1954; HEIMBURGER 1956, 1962, 1972a, 1972b).

The existence of resistance to blister-rust within the eastern white pine was demonstrated, and consequently, emphasis was placed on an intraspecific approach to determine how such resistance was inherited and how it could be employed in a reforestation programme (RIKER & PATTON 1954). On the contrary, according to his experiments, HEIMBURGER (1972a) stated that there is a lack of resistance genes in eastern white pine and, therefore, the introduction of such genes from related species might be the only way to obtain a reasonably realistic resistance. The eastern white pine improvement in North America was based both on intraspecific (RIKER *et al.* 1943; HEIMBURGER 1972A, ZSUFFA 1981) and interspecific crosses (HEIMBURGER 1962, 1972b; PATTON 1966; ZSUFFA 1979). Unfortunately, many early experiments with eastern white pine were abandoned. However, in Idaho and Montana, U.S.A., the breeding for blister-rust resistance in western -white pine (*Pinus monticola* Dougl), has been continuing, with good results, for about half a century.

Balkan pine (*P. peuce* Griseb.) was recommended as a potential source of resistance genes since performance of *P. strobus* × *P. peuce* hybrids has demonstrated the ability of Balkan pine to transmit its blister-rust resistance to offspring (PATTON 1966; HEIMBURGER 1972a; SOEGAARD 1972).

Eastern white pine was of great interest in Romania for many decades, due to its good adaptability, rapid growth and desirable wood properties (RADU 1974). Taking into account the potential value of hybrids for growth, BENEA (1960), BENEA & TOMESCU (1966), LEANDRU (1982) launched a comprehensive hybrid-breeding programme. They demonstrated that *P. strobus* × *P. peuce* hybrids exhibited rapid growth and an opportunity for producing F_2 generations.

Since 1973, severe blister-rust attacks have occurred in Romania in *P. strobus* planted stands (BLADA 1982; 1990). For this reason, plantings with this species were completely stopped.

However, because of the potential importance of eastern white pine and the danger the pathogen represents, a genetic resistance improvement programme was started in 1977, and included both intraspecific and interspecific hybridization approaches. An objective of this programme was to establish hybrid seed orchards composed of selections with high general combining ability parents for resistance (BLADA 1982).

Previous reports (BLADA 1987; 1989) on *P. strobus*

× *P. peuce* F_1 hybrids revealed that:

- the high wood production of eastern white pine could be combined with blister-rust resistance of Balkan pine, using intermediate hybrids;
- there was sufficient genetic variation among eastern white pine parent genotypes;
- there was enough additive variance in parent populations to warrant breeding for blister-rust resistance as well as growth traits ;
- a significant genetic gain can be achieved if the best hybrid families are planted on large and suitable areas.

F_1 hybrid population from reciprocal crosses between eastern white pine and Balkan pine supported evidence that extranuclear genes controlling blister-rust resistance and growth traits could be found within eastern white pine (BLADA 1992).

The objective of this paper was to provide information about genetic variation and genetic gain from selection within a 17-year old *P. strobus* × *P. peuce* F_1 hybrid population.

MATERIALS AND METHODS

Initial material and mating design

Seven *P. strobus* female and four *P. peuce* male parents were selected in planted populations of unknown origin. The selection was made without regard to any trait except for flower production, assuming that both categories of parents were random samples from presumed random mating populations. However, the question as to whether or not the parent populations were random mating populations, can not be answered.

In 1979, a 7 × 4 factorial mating was performed. The seed samples were stratified according to KRIEBEL's (1973) recommendations and then sown (spring 1981) in individual polyethylene pots (22 × 18 cm) in a potting mixture consisting of 70 % spruce humus and 30 % sand. The seedlings grew in pots during the first six years.

So far, the literature appears to contain no published data concerning full factorial mating design between *P. strobus* and *P. peuce*

Inoculation and experimental design

The seedlings were artificially inoculated in 1982 1983 and 1984 between 20 and 30 August, when they were two, three and four years old, respectively. During each inoculation the potted seedlings were placed in a polyethylene tent and arranged in a randomized complete block design. Each family was represented by a 10-seedling row-plot in each of the three blocks. Two open pollinated progenies of eastern white pine and Balkan pine, representing the mean of the parents, were included as control.

Inoculum consisted of heavily infected leaves of *Ribes nigrum* L. collected from a single mixed-clone plantation. Other details concerning inoculation and inoculation tent were similar to those described by BINGHAM (1972).

The first results of this Experiment 1 were published after six years of nursery testing (BLADA 1989).

At age six, the hybrids were planted out at 3 × 3 m spacing, in the Caransebes-Valisor Forest District (at about 45° 27' N latitude, 22° 07' E longitude, and 310 m altitude) by using a randomized complete block design similar to that from inoculation tent. The infected trees, but still living, were planted, too. No thinning was carried out by age 17.

Measurements

Nine traits were assessed when the hybrids were 17 years old (Table 1). The blister-rust resistance (BRR) was scored using an index that took into account both the number and severity of the lesions: 1 = dead tree or total susceptibility (all trees killed by rust in previous years were included in this cumulative category); 2 = four or more serious stem lesions; 3 = three severe stem lesions; 4 = three more or less severe stem lesions; 5 = two severe stem lesions; 6 = two more or less severe lesions; 7 = one severe stem lesion; 8 = one more or less severe lesion; 9 = branch or very light stem lesions; and 10 = free of rust or no rust attack.

Percentages of the "Trees free of blister-rust" (TFBR) and "Trees survived" (TS) were calculated based on BRR index data, *i.e.*, all trees with a score 10 were TFBR and all trees with a score 2 to 10 were considered tree survivors. All percentages were transformed to the arc sin square root of percents for analysis of variance.

Stem straightness was assessed using a 5 point visual index, where 1 = crooked and 5 = very straight.

Branch thickness was measured at the 6 major branches within each of the sixth and seventh whorls; branch thickness represents the average thickness from these two whorls. To avoid the swelling of the trunk the branch diameter was taken with a calliper, at 3 cm away from the stem.

The other traits do not require supplementary explanations.

Plot-mean and individual tree data were subjected to randomized block and factorial analysis of variance.

Table 1. Measured traits.

Traits	Units	Symbol
1. Blister rust resistance	Index 1,....,10	*BRR*
2. Trees free of blister-rust	%	*TFBR*
3. Trees survivors	%	*TS*
4. Trees height	dm	*H*
5. Diameter at 1.30 m	cm	*D*
6. Basal area	dm^2	*BA*
7. Stem volume	dm^3	*V*
8. Stem straightness	Index 1, ..., 5	*SS*
9. Branch thickness	cm	*BT*

Statistical analyses

In order to estimate the genetic components of variance the following statistical model, applied to plot means, was assumed:

$$x_{ijkh} = m + M_i + F_j + (MF)_{ij} + B_k + e_{ijkh} \qquad [1]$$

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 = 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 k-th block (k = 1, 2,..., K); e_{ijkh} = the random error. It should be noted that M × B, $F \times B$ and $M \times F \times B$ interactions were pooled for the error term.

When parents were random samples from random mating population and when the families were planted in a complete randomized block design a random model for statistical analysis could be used (Comstock & Robinson 1952). But, in this case a fixed model was chosen because, as previously stated, the question as to whether or not the parent populations were random mating populations, could not be answered.

The generalized form of the ANOVA used for the factorial mating design, according to Halauer & Miranda (1981, p.66), is shown in table 2. The within plot mean square was calculated by separate analysis. The mid-parent heterosis (*MPH*) was calculated according to Halauer & Miranda (1981, p. 347) formula. The high-parent heterosis (*HPH*) was calculated by the same formula where *HP* replaced *MP*.

$$MPH = [(F_1 - MP) / MP] \times 100 \qquad [2]$$

$$HPH = [(F_1 - HP) / HP] \times 100 \qquad [3]$$

where: F_1, *MP* and *HP* are the F_1 hybrid mean, the mid-parent mean and the high-parent value, respectively. As shown above, two estimates of heterosis were computed, one compared to the best parent (*HP*) and the other compared to the mean of the parents, or mid-parent mean (*MP*) from open pollinated controls. According to the broad, modern concept, there exists positive or negative heterosis, luxuriant, adaptive, selective or reproductive heterosis and labile or fixed (Mac Key 1976). Only positive and negative heterosis was estimated in this experiment.

The general combining ability effects (*gca*) of each parental tree and the specific combining ability effects (*sca*) of each male-female cross were calculated, using Griffing's (1956) Method 4, adapted to a factorial design. The statistical model was:

$$x_{ij.} = X... + g_i + g_j + s_{ij} + e_{ijk} \qquad [4]$$

where: $x_{ij.}$ is the mean of the i-th female tree crossed to the j-th male tree over k replicates; $X...$ is the general mean; g_i is the *gca* effect associated with the i-th female tree; g_j is the *gca* effect associated with the j-th male tree; s_{ij} is the *sca* effect associated with the cross between the i-th female tree and j-th male tree; e_{ijk} is the residual effect.

Table 2. Analysis of variance of the design II mating design in one environment (Hallauer & Miranda 1981).

Source of variation	d.f.	MS	E(MS)
Blocks (*B*)	$r - 1$		
Males (*M*)	$m - 1$	MS_5	$\sigma^2 + r\sigma^2_{mf} + rf\sigma^2_m$
Females (*F*)	$f - 1$	MS_4	$\sigma^2 + r\sigma^2_{mf} + rm\sigma^2_f$
$M \times F$	$(m - 1)(f - 1)$	MS_3	$\sigma^2 + r\sigma^2_{mf}$
Pooled errors	$(r - 1)(mf - 1)$	MS_2	σ^2
Total	$(rmf - 1)$		
Within plot[2]	$rmf\,(k - 1)$	MS_1[1]	

[1] M_1 is the within plot mean square and includes the within-plot genetic variance and environmental variance; k = the number of plants measured in each plot.

The computational formulae were as follows:

$$gca_i = x_{i.} - X... \qquad [5]$$

$$gca_j = x_{.j} - X... \qquad [6]$$

Genetic gain was calculated as twice the average of *gca*'s or the average of the breeding values of the three and five parents respectively, selected for the next breeding works (Table 8).

The observed (OGV_{ij}) and predicted (PGV_{ij}) genetic values of the full-sib family produced by mating the *i*-th female tree and *j*-th male tree were calculated, such as:

$$OGV_{ij} = x_{ij} - X... \qquad [7]$$

$$PGV_{ij} = gca_{i\cdot} - gca_{.j} \qquad [8]$$

where: x_{ij} is the least-square mean of a particular full-sib family; *X...* is the general mean of the hybrid population; $gca_{i.}$ and $gca_{.j}$ = female and male parent trees general combining ability effects.

Comparisons of rankings of full-sib families based on observed and predicted genetic values have been used to illustrate some of the practical implications of relative magnitudes of additive and non-additive genetic effects in breeding.

The specific combining ability (sca_{ij}) of the *i*-th female tree mated to the *j*-th male tree was calculated, such as:

$$sca_{ij} = OCV_{ij} - PGV_{ij} \qquad [9]$$

RESULTS AND DISCUSSIONS

Genetic variation

Analyses of variance indicated highly significant difference ($p < 0.001$) among hybrid families for all traits except stem straightness (Table 3, row 2). Hence, selection at family level within hybrid population could be carried out for the most economically important traits.

There was a large genetic variation among parents within each sex for all traits examined. An important finding of this experiment was that the effects of eastern white pine female parents were significant ($p < 0.05$) and highly significant ($p < 0.01$; $p < 0.001$) for all growth traits and for the three traits related to blister-rust resistance (Table 3, row 3). This suggests that (a) an additive genetic control in all traits including growth and blister-rust resistance and (b) high *gca* parents could be selected for breeding. Our finding concerning the existence of resistance to the blister-rust disease within *P. strobus* confirm the results reported by RIKER *et al.* (1943), RIKER & PATTON (1954), PATTON & RIKER (1958), PATTON (1966). However, it should be stressed that HEIMBURGER (1972a) mentioned a possible lack of certain resistance genes in this species but his hypothesis was not confirmed.

The Balkan pine as male parents had highly significant ($p < 0.001$) effects on three out of four growth traits but had no significant effects on stem straightness and on the three traits connected with blister-rust resistance (Table 3, row 4). It is important to note that Balkan pine male parents exhibited the same level of resistance and, consequently, all of them could be selected for further blister-rust resistance breeding works. Similar results, i. e. no significant differences among *P. peuce* parent trees were found at an earlier stage of this trial (BLADA 1989). Opposite, PATTON (1966) found differences in blister-rust resistance among his *P. peuce* selected parents.

Male x female interaction effects were significant ($p < 0.05$) and highly significant ($p < 0.01$; $p < 0.001$) for all traits except for stem straightness (Table 3, row 5), suggesting a non-additive gene action on most traits. A large variation among means of hybrid family was found (Table 4). The poorest group (X_2) had an average

Table 3. Analysis of variance of the hybrid traits.

Source of variation	d.f	*BRR*	*TS*	*TFBR*	*H*	*D*	*BA*	*V*	*SS*	*BT*
Block	2	0.4761	24.25	17.30	25.66	0.100	0.1050	4.4	0.0750	0.0550
Hybrids	27	1.524***	186.89***	171.92***	51.38***	3.569***	0.1267***	1264.4***	0.1393	0.1704***
Females (*F*)	(6)	2.4819***	365.35***	402.65***	103.73***	5.395***	0.1863***	2330.8***	0.2767*	0.1933**
Males (*M*)	(3)	0.0021	33.86	11.21	11.85	11.187***	0.3500***	2342.4***	0.1367	0.6067***
M × *F*	(18)	1.4605***	152.90***	121.80***	40.52***	1.691**	0.0756***	729.3***	0.0933	0.0900*
Pooled errors	54	0.1669	19.98	18.05	11.38	0.585	0.0163	5.1	0.1117	0.0494
Within plot [1]	420	15.1860	–	–	33.33	5.370	0.2380	2114.3	0.6284	0.2860

[1] Within plot variance was calculated by a separate analysis, by using six trees family / replication; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 4. Means of the five best and poorest families for each trait.

Family ranking	*BRR*	*TS*	*TFBR*	*H*	*D*	*BA*	*V*	*SS*	*BT*
1	9.7	98.9	98.9	80.3	15.7	1.97	150.3	3.8	3.4
2	9.5	98.9	97.4	79.1	15.1	1.80	144.5	3.7	3.3
3	9.5	98.8	95.4	79.0	15.1	1.80	140.6	3.7	3.3
4	9.4	98.7	95.2	78.3	15.0	1.80	139.8	3.4	3.2
5	9.4	96.0	94.9	78.3	15.0	1.77	139.7	3.4	3.2
X_1	9.5	98.3	96.4	79.0	15.2	1.83	143.0	3.6	3.3
24	8.2	80.8	78.0	69.7	13.1	1.40	102.7	3.1	2.7
25	7.8	74.5	74.5	68.0	12.5	1.33	79.3	3.1	2.7
26	7.3	71.4	69.8	66.9	12.5	1.30	78.9	3.1	2.7
27	7.2	71.4	68.7	66.5	11.4	1.13	78.9	3.0	2.5
28	7.2	68.8	68.2	66.5	11.4	1.07	76.3	2.8	2.4
X_2	7.5	73.4	71.8	67.5	12.2	1.25	83.2	3.0	2.6
X	8.8	8.7	86	73.7	14.0	1.58	118.0	3.3	3.0
D_1	27	34	34	17	25	46	72	2.0	27
D_2	8	13	12	7	9	16	21	9	10

D_1 = difference (%) between mean of the best (X_1) five families and the poorest (X_2) five families;
D_2 = difference (%) between mean of the best (X_1) five families and the test mean (X)

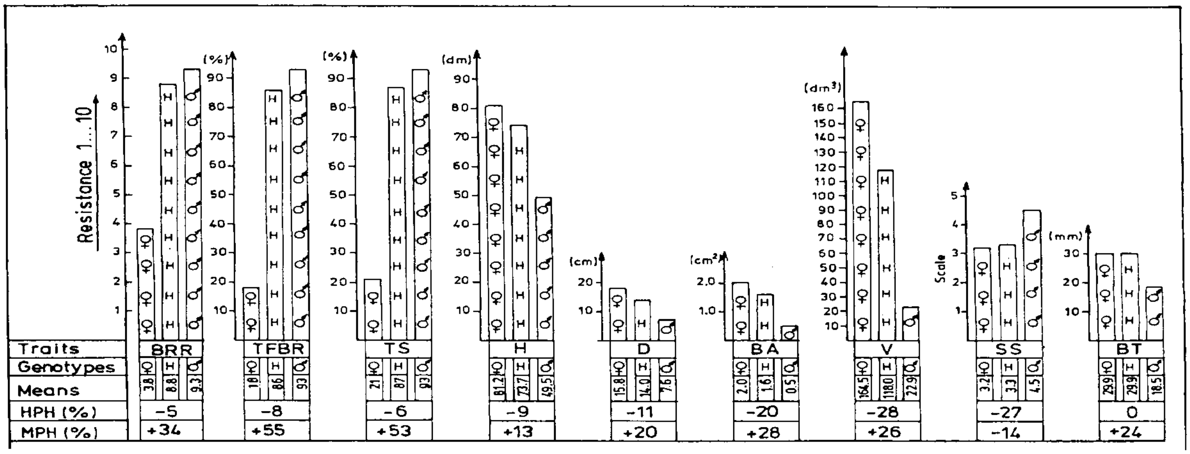

Figure 1. *Pinus peuce* hybrid performance, high-parent heterosis (HPH) and mid-paerent heterosis (MPH).

of 7.5 points in blister-rust resistance and 71.8% in trees free of blister-rust, while those for the best group (X_1) measured an average of 9.5 points and 96.4%, respectively, i. e. 27% and 34% more. At the same time, the difference between the two groups of families accounted for 17% in total height and 72% in stem volume growth. These figures demonstrated both the magnitude of family variation and large possibilities of selection at family level.

Hybrid performance and heterosis

Parent and hybrid performances and the two types of heterosis were illustrated in figure 1.

The Balkan pine was the best parent species for traits related to blister-rust resistance and for stem straightness, whereas the eastern white pine was the best parent species for all growth traits.

Mid-parent heterosis was positive for all but one trait.

Table 5. Phenotypic correlations among traits (d.f. = 26).

Traits	*TS*	*TFBR*	*H*	*D*	*BA*	*V*	*SS*	*BT*
BRR	0.92***	0.90***	0.33	0.02	0.01	0.25	0.38*	–0.26
TS		0.94***	0.30	0.05	0.03	0.22	0.38*	–0.24
TFBR			0.16	–0.04	–0.05	0.13	0.45*	–0.29
H				0.52**	0.57**	0.73***	0.30	0.27
D					1.02***	0.96***	0.31	0.81***
BA						0.96***	0.32	0.79***
V							0.32	0.64***
SS								0.34

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$;

Table 6. Analysis of variance of the hybrids

Parent	Traits								
	BRR	TFBR	TS	H	D	BA	V	SS	BT
					gca.-female effects				
1	–0.148	–3.238	–1.643	–0.608	0.679	0.114*	10.212***	–0.244	–0.035
2	–0.423*	–4.905**	–5.393**	–0.058	0.201	0.039	1.229	0.198	0.240*
3	0.386*	–0.071	–0.476	4.967*	–0.029	0.006	6.954***	0.056	–0.076
4	0.369*	3.012	1.857	–2.142	–0.763*	–0.127*	–8.613***	–0.011	–0.143
5	–0.714***	–8.155***	–7.310**	–3.275*	–0.863**	–0.169**	–23.921***	–0.119	–0.026
6	0.036	7.679***	4.274*	–1.775	–0.146	–0.019	–4.438***	–0.027	–0.051
7	0.494**	5.679**	8.690***	2.892*	0.921**	0.156**	18.579***	0.148	0.090
					gca.-male effects				
14	–0.012	–1.095	–1.690	0.261	0.570*	0.118*	3.962***	0.040	0.136
18	0.012	0.381	–0.262	–1.120	0.691**	0.104	8.290***	0.093	0.155*
20	–0.002	0.333	0.929	0.427	–0.633**	–0.130*	–8.914***	–0.083	–0.179*
21	0.002	0.381	1.024	0.432	–0.628*	–0.092	–9.338***	–0.050	–0.112

* p <0.05; ** p <0.01; *** $p < 0.001$;

This heterosis accounted for 34% for blister-rust resistance, 55% for trees free of blister rust, and 53% for tree survivors. Substantial positive mid-parent heterosis was also found in most growth traits, such as: 20% in diameter, 28% in basal area and 26% in volume growth. The total height had the lowest (13%) positive mid-parent heterosis, while the stem straightness was the only trait displaying a negative mid-parent heterosis.

High-parent heterosis was negative for all traits. For example, at age 17, this heterosis accounted for –5% for blister-rust resistance, –8% for trees free of blister-rust and –9% for total height. It is important to stress that, more or less, similar results were obtained on the same material at age 5 (BLADA 1989). In conclusion, hybrids showed intermediate performances between the two parent species (Fig. 1).

Correlations

Phenotypic correlation coefficients among traits were presented in Table 5.

Highly significant ($p < 0.012$ $p < 0.001$) and positive phenotypic correlations were found among growth traits, such as total height, diameter, basal area and volume growth. Such positive correlations imply positive simultaneous genetic gain in these traits even if selection was practiced on only one trait.

Phenotypic correlations between stem straightness and growth traits were low, ranging from 0.30 to 0.32. But correlations between branch thickness and growth traits (except total height) were highly significant ($p < 0.001$) and positive. This indicates that branch thickness was consistently dependent of stem diameter; in other words, trees with large diameters produced thicker branches. This means that an effort should be made to select larger trees with thin branches.

Highly significant ($p < 0.001$) correlations were obtained among the three traits connected with blister-rust resistance (0.90 to 0.94), but low correlations (0.01 to 0.33) were obtained between blister-rust resistance

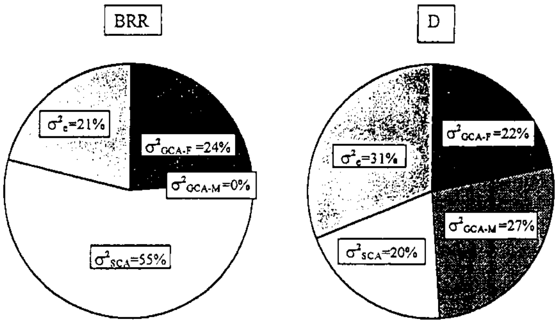

Figure 2. General combining ability effects (%) for volume growth rate (V) and blister rust resistance (BRR).

and growth traits; this suggests that the two categories of traits were inherited independently, and hence, tandem selection can not be applied.

Combining abilities

The general combining ability effects (*gca*) estimated for 11 parents and 9 traits were presented in table 6.

Both positive and negative *gca* effects which significantly ($p < 0.05$) and highly significantly ($p < 0.01$; $p < 0.001$) differed from zero were generally found for both male and female parents for most traits. The range of estimated *gca* effects among parents suggested that it may be possible to select parents with superior breeding values for blister-rust resistance and growth traits.

The eastern white pine female Parent 7 had the largest positive *gca* effects for blister-rust resistance, (*gca* = 0.494 points), and volume growth rate, (*gca* = 18.58 dm^3), whereas the female Parent 3 was the second highest for blister rust resistance and the fourth for volume growth rate. It meant that an increase in blister-rust resistance was accompanied by an increase in volume growth rate. Similarly, among Balkan pine parents, the male Parent 18 had the largest positive *gca* effects for both volume growth, *gca* = 8.290 dm^3, and blister-rust resistance, *gca* = 0.012. On the other hand, the female Parent 5 was the worst because of its negative *gca* effects for all tested traits.

The four Balkan pine male parents had no significant effects on blister-rust resistance as these parents exhibited the same level of resistance to blister-rust and, consequently, all of them could be selected for further breeding works.

A primary importance in tree breeding involves choosing proper parents for mating, especially when the trait to be improved is quantitatively inherited such as growth traits. Hence, the parents 7, 3 and 4 should be selected as good *gca* parents for blister-rust resistance and, on the other hand, 7, 1, 3, 14 and 18 should be selected as good parents for volume growth. It should be noted that parents 3 and 7 were good general combiners for both blister rust resistance and volume growth (Fig. 2). Also, taking into consideration that blister-rust resistance trait had the first priority in improvement, the male parents 14, 18, 20 and 21 may also be used for resistance breeding, as they have the similar level of resistance (Fig. 2). No similarities were found between the parent *gca* effects from age 5 and those from age 17 (BLADA 1989)[1].

Estimated specific combining ability (*sca*) effects for blister-rust resistance and volume growth are shown in tables 7 and *8*.

Of the 28 crosses, resulting from the 7 × 4 factorial crosses, five for blister-rust resistance and 11 for volume growth had positive significant ($p < 0.05$) or highly significant ($p < 0.01$; $p < 0.001$) *sca* effects.

The best specific crosses for blister-rust resistance were 5 × 18 and 6 × 14 followed by 5 × 14, 1 × 20 and 1 × 21. Surprisingly, none of these five crosses involved any parent with significant *gca* effects for blister-rust resistance. Similarly, the best specific crosses for volume growth were 4 × 18, 4 × 14, 2 × 21, 2 × 20, 7 × 21, 1 × 14 and 7 × 20, followed by 3 × 18, 5 × 18, 6 × 18 and 5 × 14. As can be seen, these crosses involved five parents (1, 3, 7, 14 and 18) with significant *gca* effects.

Once desirable crosses were identified, several techniques exist to exploit them for increased genetic gain. For example, large quantities of seed from full-sib families could be produced by supplemental mass pollination. Then vegetative propagation, including somatic embryogenesis, provide the potential of (a) expanding the number of seedling produced from a small number of full-sib seed and (b) selecting superior individuals within full-sib families for propagation.

In an improvement programme the most desirable parents would be ones that had both high *gca* effects and combined with other parents to consistently produce families with high *sca*. The high *gca* would insure a high expected full-sib family mean when the parents were crossed, and the high *sca* potential would provide the possibility of producing better than expected specific crosses.

Genetic gain

Genetic gain calculated as twice the average of the

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[1] The female code numbers used at age 5, i. e.1, 2, 62, 63, 65, 6-1, and 6-2 become 1, 2, 3, 4, 5, 6 and 7, respectively, at age 17.

Table 7. General (*gca*) and specific (*sca*) combining ability estimates for blister-rust resistance of the male and female parents, observed (*OGV*) and predicted genetic (*PGV*) values of full-sib families.

Female trees ($gca_{i.}$)		Male trees			
		($gca_{.j}$)			
		18 (0.012)	21 (0.002)	20 (–0.002)	14 (–0.012)
7 (0.494)	*OGV*	0.730 [2]	0.630 [5]	0.630 [6]	–0.070
	PGV	0.506 [1]	0.496 [2]	0.492 [3]	0.482 [4]
	sca_{ij}	0.224	0.134	0.138	–0.552
3 (0.386)	*OGV*	0.430 [8]	0.330	0.330	0.430 [10]
	PGV	0.398 [5]	0.388 [6]	0.384 [7]	0.374 [9]
	sca_{ij}	0.032	0.058	–0.054	0.056
4 (0.369)	*OGV*	–0.570	0.730 [3]	0.730 [4]	0.530 [7]
	PGV	0.381 [8]	0.371 [10]	0.367	0.357
	sca_{ij}	–0.951**	0.359	0.363	0.173
6 (0.036)	*OGV*	–0.070	–0.370	–0.370	0.930 [1]
	PGV	0.048	0.038	0.034	0.024
	sca_{ij}	–0.118	–0.408	–0.404	0.906**
1 (–0.148)	*OGV*	–0.970	0.330	0.330	–0.270
	PGV	–0.136	–0.146	–0.150	–0.160
	sca_{ij}	–0.834*	0.476*	0.480*	0.110
2 (–0.423)	*OGV*	0.030	–0.170	–0.070	-1.470
	PGV	-0.411	-0.421	–0.425	–0.435
	sca_{ij}	0.441	0.251	0.355	-1.035**
5 (–0.714)	*OGV*	0.430 [9]	-1.570	-1.570	–0.170
	PGV	–0.702	–0.712	–0.716	–0.726
	sca_{ij}	1.132***	–0.858*	–0.854*	0.556*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

[1] The top 10 full-sib families according to observed and predicted genetic values are identified by ranking in square brackets.

gca's, *i.e.* the average of breeding values, of the best parents is presented in table 9.

The best three parents for blister-rust resistance were female trees 7, 3 and 4 (Table 9, column 1), and their average breeding value was 0.833 points, which would represent an increase of 9.5% in the general mean (8.8 points) for blister-rust resistance. Similarly for volume growth, the best five parent trees were 7, 1, and 3 of eastern white pine and 14 and 18 of Balkan pine (Table 9, column 5) and their average breeding value was 21.6 dm^3, which would represent a genetic gain of 18.3 % in the overall mean (118.0 dm^3) for volume growth.

It is also possible to use genetic values estimated to calculate gains expected from specific combiner selection followed by limited test cross (CARSON 1986). If the best cross *i.e.* 6 x 14 was used for mass vegetative propagation, the genetic gain in blister-rust resistance would be 0.93 points which represents the observed genetic value of 6 x 14 (Table 7). This gain represents a 10.6% increase in the general mean for blister-rust resistance of hybrid population. If the four best crosses (6 x 14, 7 x 18, 4 x 20 and 4 x 21) were mass vegetatively propagated, the gain would be 0.78 points (the average of the observed genetic values of the four families) or an increase of 8.9%.

A much higher genetic gain in volume growth could be achieved. Thus, if the best cross 1 x 14 was used for vegetative mass propagation, the genetic gain would be 32.29 dm^3, *i.e.* the observed genetic value (Table 8). This gain represents a 27.4% increase in the general mean for volume growth of hybrid population. If all the

Table 8. General (*gca*) and specific (*sca*) combining ability estimates for stem volume , growth rate of the male and female parents, observed (*OGV*) and predicted genetic (*PGV*) values of full-sib families.

Female trees (gca_i)		Male trees (gca_j)			
		14 (9.962)	18 (8.290)	20 (–8.914)	21 (–9.338)
7	*OGV*	18.56 [7]	12.33 [8]	21.73 [4]	21.96 [5]
(18.579)	*PGV*	28.54 [1]	26.87 [2]	9.67 [9]	9.24 [10]
	sca_{ij}	–9.98***	–14.54***	12.06***	12.45***
1	*OGV*	32.29 [1]	4.26	2.13	2.16
(10.212)	*PGV*	20.17 [3]	18.50 [4]	1.30	0.87
	sca_{ij}	12.12***	–14.24***	0.83	1.29
3	*OGV*	–0.24	26.49 [2]	0.76	0.76
(6.954)	*PGV*	16.90 [5]	15.23 [6]	–1.97	–2.40
	sca_{ij}	–17.14***	11.26***	2.73	3.16
2	*OGV*	3.09	–10.54	6.29 [10]	6.16
(1.229)	*PGV*	11.19 [7]	9.52	–7.68	–8.11
	sca_{ij}	–8.10***	–20.06***	13.97***	14.27***
6	*OGV*	2.36	10.59 [9]	–15.37	–15.34
(–4.438)	*PGV*	5.52	3.85	–13.35	–13.78
	sca_{ij}	–3.16	6.74***	–2.02	1.56
4	*OGV*	22.59 [3]	21.19 [6]	–39.14	–39.11
(–8.613)	*PGV*	1.35	–0.32	–17.52	–17.95
	sca_{ij}	21.24***	21.51***	–21.62***	–21.16***
5	*OGV*	–8.94	–6.34	–38.71	–41.74
(–23.921)	*PGV*	–13.96	–15.63	–32.83	–33.26
	sca_{ij}	5.02**	9.29***	–5.88**	–8.48***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

1) The top 10 full-sib families according to observed and predicted genetic values are identified by ranking in square brackets.

10 best crosses (1 x 14, 3 x 18, 4 x 14, 7 x 20, 7 x 21, 4 x 18, 7 x 14, 7 x 18, 6 x 18 and 2 x 20) were mass propagated the gain would be 19.38 dm^3, *i.e.* an increase of 16.4% in the overall mean volume growth.

In summary, such genetic gains in blister-rust resistance and volume growth represented a considerable increase in both blister-rust resistance and timber production. Even smaller increases in the two traits would result in appreciable improvement in yield, especially when considered in relation with large-scale afforestation programmes. It should be noted that the genetic gain in blister-rust resistance was higher at age 5 than that from age 17 i.e. 14.5% as compared to 9.5 % (BLADA 1989)

Implications for breeding strategy

This *P. strobus* x *P. peuce* factorial experiment enabled the production and testing of F_1 hybrids that incorporated desired characteristics from both parent species, *i.e.* blister-rust resistance and fast growing.

The practical objective of the experiment was to produce F_1 hybrids on a large scale to be used for operational planting programmes. The hybrid planting stock can be produced both by sexual and vegetative propagation. For sexual reproduction, the best and the simplest method for obtaining large amount of hybrid seed is to select, by genetic tests, the best general combining ability parents, multiply them by grafting and then to establish hybrid seed orchards. For example, such a seed orchard should consists of female clone

Table 9. General combining ability effects (*gca*), breeding values (*BV*), and genetic gains (ΔG).

Blister-rust resistance				Volume growth			
Parents	*gca* (Points)	*BV* (Points)	ΔG[1] %	Parents	*gca* (dm^3)	*BV* (dm^3)	ΔG[1] %
7	0.494	0.988	11.2	7	18.579	37.158	31.5
3	0.386	0.772	8.8	1	10.212	20.424	17.3
4	0.369	0.738	8.4	14	9.962	19.924	16.9
–	–	–	–	18	8.290	16.580	14.0
–	–	–	–	3	6.954	13.908	11.8
Σ	1.249	2.498	28.4	Σ	53.997	107.994	91.5
x	0.416	0.833	9.5	x	10.799	21.598	18.3

[1] Calculated against general mean, i.e. 8.8 points for blister-rust resistance and 118.04 dm^3 for volume growth.

from Parent 7 of eastern white pine and the four male clones from parents 14, 18, 20 and 21 (and perhaps some others) of Balkan pine. The female parent was a good combiner for both blister-rust resistance and volume growth, whereas the four male parents exhibited a similar level of blister-rust resistance. In addition, the males 14 and 18 had highly significant *gca* effects for volume growth. It is expected that this bi-species seed orchard will be successful because, in Romania, the two species flower in a partial synchrony (BLADA, unpublished data). The lack of full flowering synchrony, *i.e.* partial open pollination, can be compensated by supplemental artificial pollination with mixed pollen sources. The hybrid seed that incorporated additive genes will be collected only from maternal clone. Non-hybrid progenies will be removed during the nursery stage.

Hybrid seed could also be produced through controlled pollination among the best parent trees.

According to ZOBEL & TALBERT (1984), there are two major ways to make uses of specific combining ability in a tree improvement programme. The first is to make crosses to mass-produce seed from specified parental combinations; this can be done by control pollinations or by developing two-clone seed orchards. The second way is to produce commercial quantities of planting stock by vegetative propagation.

The most effective means for producing hybrid-planting stock is controlled pollination followed by mass vegetative propagation using juvenile donor plants. PARK *et al.* (1998) consider three possible breeding-cloning options that incorporate breeding and vegetative propagation schemes, depending on which type of genetic variability are to be exploited: (i) general combining ability (*GCA*) variance; (ii) specific combining ability (*SCA*) variance; (iii) clonal variance. The first two options involve crossing of *GCA*-tested parents and "the best tested full-sib crosses", followed by vegetative propagation. The third option involves the direct deployment of tested clones.

According to the first option or strategy, called *backward GCA selection and polycrossing*, the breeding value or *GCA* of the parents can be determined by evaluating the performance of their open-pollinated, polycross or half-sib progeny in a progeny test. The best *GCA*-parents are selected for producing improved progeny. Limited quantities of seeds from the best *GCA*-parents could be obtained by controlled pollination with other selected *GCA* parents. In this specific case, the crosses should be carried out between the female parents 7 and 3 of eastern white pine and male parents 14, 18, 20 and 21 of Balkan pine.

These controlled crosses could be carried out both in seed orchard or directly on the parent trees. Then, simple vegetative propagation techniques for rooting of cuttings using juvenile seedlings or somatic embryogenesis techniques could produce planting stock for field deployment.

According to the second strategy called *backward SCA selection and repeat crossing*, selection of full-sib families based on the progeny test derived from controlled crosses utilizes the specific combining ability (*SCA*) of pairs of parents in addition to their individual *GCA*'s. Since it usually takes a long time to identify superior crosses, vegetative propagation directly from the test progeny may not be possible. It will, therefore, require repeating the controlled crossing, in order to obtain seeds for the best tested full-sib combinations. If the aim is to improve the blister-rust resistance, as in this case, it is recommended to obtain the following combinations: 5 x 18, 5 x 14, 6 x 14, 1 x 20 and 1 x 21 (Table 7). If the improvement of the volume growth has the first priority then the following combinations should be repeated, such as: 4 x 18, 4 x 14, 2 x 21, 2 x 20, 7 x 21, 7 x 20, 1 x 14, 3 x 18, 6 x 18, 5 x 18 and 5 x 14 (Table 8). The resulting quantities of seeds would provide zygotic embryos for somatic embryogenesis or juvenile seedling donor plants for seedling production. This approach, which has been referred to as a *family forestry*, has been developed for radiata pine in New Zealand (CARSON 1986; SHELBOURNE *et al.* 1989; CARSON & BURDON 1991), where vegetative propagation was combined with the control-pollinated orchard concept (SWEET & KRUGMAN 1977; CARSON *et al.* 1992).

According to the third strategy called *forward clonal selection and clonal deployment*, progenies of controlled crosses will be field-tested. The best individuals in the test will be selected as clones for deployment.

CONCLUSIONS

Significant genetic variation was detected in hybrid population to warrant improvement for blister-rust resistance and growth traits using additive as well as non-additive genetic effects.

Mid-parent heterosis was positive whereas de high-parent heterosis was negative for all traits.

The significant magnitude of variation in *gca* effects suggested that it may be possible to detect parents with high breeding value for both growth traits and blister-rust resistance.

Good *gca* parents were found within *P. strobus*, not only for growth traits but also for blister-rust resistance whereas the *P. peuce* parents exhibited a similar level of blister-rust resistance.

Phenotypic correlations indicated that genetic gain in any growth trait will be obtained if selection is applied to only one trait; however, because of a lack of significant correlation between growth traits and blister-rust resistance, no tandem selection can be applied.

Planting *P. strobus* x *P. peuce* F_1 hybrids in operational programmes seems to be promising as an average of 86% hybrids was free of blister-rust after a heavy artificial inoculation at age 2 then after 17 years of growing under a heavy natural blister-rust incidence. Also, a genetic gain of 9.5% in blister-rust resistance and 18.3% in volume growth could be achieved.

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