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References

Akopyanz, N. N., Bukanov, T. u., Westblom and Berg, D. E.: PCR based RFLP analysis of DNA squence diversity in the gastric pathogen *Helicobacter pylori.* Nucleic Acids Res. **20**: 6221-6225 (1992). — Bueno, M. A., Grau, J. M. and Garcia-de-los-Rios, M. D.: Micropropagación de árboles de *Populus tremula* e identificación de clones en rodales mediante electroforésis. Congreso Forestal Español. Tomo II., 177–182 (1993). — Castiglione, S., Wang, G., Damiani, G., Bandi, C., Bissofi, S. and Sala, F.: RAPD Fingerprintints for identification and for taxonomic studies of elite poplar (*Populus* spp.) clones. Theor. Appl. Genet. **87**: 54–59 (1993). — Dayanandan, S., Rajora, O. P. and Bawa.: Isolation and Characterization of microsatellites Tremblin aspen (*Populus tremuloides*). Theor. Appl. Genet. **96**: 950–956 (1998). — D'Ovidio, R., Mugnozza, G. S. and Tanzarella, O. A.: rDNA clonig and rapid hybrid identification in *Populus* spp. (Salicaceae). Pl. Syst. Evol. **177**: 165–174 (1991). — Doyle, J. J. and Doyle, J. L.: Isolation of DNA from fresh tissue. Focus. **12**: 13–15 (1991). — Erlich, H. A., Gelfand, D. and Sninsky, J. J.: Recent advances in the polimerase chain reaction. Science **252**: 1643–1650 (1991). — Faivre-Rampant, P., Jeandroz, S., Lefevre, F., Lemoine, M., Villar, M. and Bervillé, A.: Ribosomal DNA studies in poplar *P. deltoides, P. nigra, P. trichocarpa, P. maximowiczii,* and *P. alba.* Genome **35**: 733–740 (1992). — Hearne, C., Choshs and Todd, J.: Microsatellites for linkage analysis of genetic traits. Trends. Genet. **8**: 288–294 (1992). — Heinze, B.: PCR-based chloroplast assays for the identification of native *Populus nigra* and introduced poplar hybrids in Europe. Forest Genetics (Slovak Republic), International Journal of Forest Genetics. ISSN 1335–048X. **5**: 31–38 (1998). — Heybroek, H. M.: Clones in Forestry and in Nature. Arboricultural Journal **8**: 275–286 (1984). — Huntley, B. and Birks, H. J. B.: An atlas of past and present pollen maps for Europe, 0–13,000 Years ago. Cambridge University Press, Cambridge, U.K. (1983). — Keim, P., Paig, K. N., Whitham, T. G. and Lar, K. G.: Genetic analysis of an interspecific hybrid swarm of *Populus:* accurrence of unidirectional introgression. Genetics **123**: 557–565 (1989). — Kloosterman, A. D., Budowle, B. and Daselaar, P.: PCR-amplification and detection of the human D1S8 VNTR locus. Int. J. leg. Med. **105**: 257–264 (1993). — Lanham, P. G. and Brennan, R. M.: Characterization of the genetic resources of redcurrant (*Riber rubrum;* subg. Ribesia) using anchored microsatellite markers. Theor. Appl. Genet. **96**: 917–921 (1998). — Lin,V., Hubbes, M. and Zsuffa, L.: Differentiation of poplar and Wilow clones using RAPD fingerprinting. In: Klofenstein, N. B., Chun, Y. W., Kim, M. S. and Ahuja, M. R. eds. Micropopagation, Genetic Engineering and Molecular Biology of *Populus.* Gen. Tech. Rep. RM-GTR-297. Fort Collins, Department of Agriculture, Forest Service, Rocky Mountain Research Station. 326 p. (1997). — Liu. Z. and Furnier, G. R.: Comparison of allozyme, RFLP, and RAPD markers for revealing genetic variation within an between trembling aspen and bigtooth aspen. Theor. Appl. Genet. **87**: 97–105 (1993). — Nei, M.: Estimation of avarage heterozigosity and genetic distance from a small number of individuals. Genetics **89**: 583–590 (1978). — Rahman, M. H., Dayanandan, S. and Rajora, O. P.: Microsatellite DNA Markers in *Populus tremuloides.* Genome (in Press) (1999). — Rani, V., Parida, A. and Raina, S. N.: Random amplified polymorphic DNA (RAPD) markers for genetic analysis in micropropagated plants of *Populus deltoides* Marsh. Plant. Cell. Reports. **14**: 459–462 (1995). — Saiki, R. K., Gelfand, S., Stoffel, S., Scharf, S. T., Higuchi, R., Horn, G. T., Millis, K. B and Erlich, H. A.: Primer-detected enzimatic amplification of DNA with a thermostable DNA polymerase. Science **239**: 487–491 (1988). — Sánchez, N., Grau, J. M., Manzanera, J. A. and Bueno, M.A.: RAPD markers for the identification of *Populus* species. Silvae Genetica **47**: 67–71 (1998). — Sigurdsson, V., Anamthawat-Jonsson, K. and Sigurgeirsson, A.: DNA fingerprinting of *Populus trichocarpa* clones using RAPD markers. New Forests **10**: 197–206 (1995). — Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic. Acids. Res. **18**: 6531–6535 (1990). — Winfield, M. O., Arnold, G. M., Cooper, F., Leray, M., White, J., Karp, A. and Edwards, K. J.: A study of genetic diversity in *Populus nigra* subsp. *betulifolia* in the Upper Severn area of U.K. using AFLP markers. Molecular Ecology **7**: 3–10 (1998). — Zabeau and Vos.: Selective restriction fragment amplification a general method for DNA fingerprinting, European patents. Application. No: 0534858 A1 (1992). — Zsuffa, L., Mosseler, A. and Raj, Y.: Prospects for interspecific hybridization in willows for biomass production. In: Perttu, K. (ed.). Ecology and Management of Forest Biomass Production Systems. Dept. Ecol. and Environ. Res., Swed. Univ. Agric. Rep. **15**: 261–281 (1984).

Genetic Variation in Blister-Rust Resistance and Growth Traits in *Pinus strobus* x *P.peuce* Hybrid at Age 17: (Experiment 2)

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Summary

The major objective of this paper was to provide basically information about genetic variation within a 17 years old *P. strobus* x *P. peuce* F_1 hybrid population. The progeny trial involved factorial mating among 5 female x 5 male parents. The 25 families and the 2 parent species controls were artificially inoculated at age 2, 3 and 4, by using leaves of *Ribes nigrum* L. heavily infected by the *Cronartium ribicola*. At age 6, the families were planted out at 3 m x 3 m spacing using a randomized complete block design with 10 trees row-plot in each of the three blocks. Eleven traits (blister-rust resistance and growth traits among them) were assessed at age 17 and the major results are presented here. (1). Highly significant ($p<0.01$; $p<0.001$) differences among hybrid families for all but one trait were found; (2) The male, female and male x female effects were highly significant ($p<0.01$; $p<0.001$) for both blister-rust resistance and growth traits; (3) The high parent heterosis was negative whereas the mid-parent heterosis was positive for all but one trait; mid-parent heterosis accounted for 27.3% for blister-rust resistance and 27.7% for volume growth; (4) Tree survivors accounted for 86% for hybrid, 93% for Balkan pine and only 21% for eastern white pine; (5) Good general combiners, not only for growth, but for blister-rust resistance, too, were found among eastern white pine parents as four of 10 parents had positive significant *gca* effects for blister-rust resistance and six for volume growth; (6) Highly significant positive correlations were found among growth traits, but no significant correlations between any growth trait and blister-rust resistance; (7) A genetic gain of 11.5% in blister-rust resistance and 8.9% in volume growth could be expected.

Key words: Pinus strobus, P.peuce, heterosis, factorial mating, correlations, breeding value, genetic gain.

Introduction[1])

Intensive introduction in Romania of eastern white pine (*Pinus strobus* L.) has rapidly increased after 1960. A few years later, coincident with that increase has been an increase in infection by the blister-rust caused by *Cronartium ribicola* FISCH. ex RABENH. It was reconized that the only way to controll losses to the fungus in white pine plantations is to improve genetic resistance, instead of wasting money, energy and time with conventional control methods.

Because of potential importance of eastern white pine and the danger the blister-rust represents, a genetic improvement program was started in Romania, in 1977; this program included both intra- and interspecific hybridizations and has for a final objective the establishment of seed orchards composed of selections with high general combining ability for resistance to blister-rust (BLADA, 1982). But, in the meantime because of financial reasons the research project has been continuing on a restricted base taking into account mainly interspecific crosses. Some progress reports on this subject were previously published (BLADA, 1987, 1989, 1990, 1992, 1994) or are in press (BLADA, 1999, in press).

This paper provides new information about genetic variation within a 17 years old *P. strobus* x *P. peuce* F_1 hybrid population. These information is based on data from what it is reported as Experiment 2, which is closely related with the Experiment 1, whose results will soon be published as earlier mentioned.

Materials and Methods

Parents, mating design and progenies

Pinus strobus x *P.peuce* progeny trial included a 5 x 5 factorial or Design II (COMSTOCK and ROBINSON, 1952) mating pattern with all 25 possible crosses completed. All crosses were carried out in Dofteana Arboretum, and unimproved parent trees, of unknown origin, could be considered a random selection with respect to any trait, except reproductive fertility. The crosses were completed in 1979, cone colection in 1980 and seed sowing took place in 1981, in individual polyethylene pots (22 cm x 18 cm) in a potting mixture consisting of 70% spruce humus and 30% sand. The progenies grew in pots throughout the first six years and they represented the subject of the nursery test (BLADA, 1987).

Inoculation and experimental design

The progenies were artificially inoculated three times, i.e. in late August 1982, 1983 and 1984 when they were two, three and four years old, respectively. During each inoculation period, the potted trees were introduced into a polyethylene tent and arranged in randomized complete block design with 14 seedlings row-plot in each of the three blocks. Two open pollinated progenies, representing the mean of the parents of the two species were included as controls. Inoculum consisted of heavily infected leaves of *Ribes nigrum* L. collected from a single plantation. All the other details concerning inoculation and inoculation tent were approximately similar to those described by BINGHAM (1972).

At age six, the hybrids and controls were outplanted at a 3 m x 3 m spacing, in the Caransebes Forest District (at about 45°27' N latitude and 22°07' E longitude and 310 m altitude) by using a randomized complete block design. The field test design was similar to that from inoculation tent. The only one exception was that this time only 10 seedlings per row-plot were planted. No thinning was carried out by age 17.

Measurements

The basic measurements were completed when the progenies were 17 years old (*Table 1*). The blister-rust resistance (BRR), as a major trait, reflects the economical and biological impact as well as the incidence of disease. It was assessed on a 10 point scale; this scale took into account both the number and severity of the lesions. Its numerical values assigned, was: 1= dead tree or total susceptibility (all trees killed by rust in previous years were included in this cumulative catagory); 2 = four or more serious stem lesions; 3 = three severe stem lesions; 4 = three more or less stem severe lesions; 5 = two severe stem lesions; 6 = two more or less stem severe lesions; 7 = one severe stem lesion; 8 = one more or less stem severe lesion; 9 = branch or very light stem lesions; 10 = free of lesions or total resistance.

Table 1. – Measured traits.

Traits	Units	Symbol
1. Blister rust resistance	Scale 1...10	BRR
2. Trees free of blister-rust	%	TFBR
3. Trees survivors	%	TS
4. Total 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	Scale 1...5	SS
9. Branch thickness	cm	BT

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

Stem straightness was assessed using a 5 point visual scale where 1 = croocked and 5 = very straight.

Branch thickness was measured at the six major branches within each of the two whorls nearest the point where the stem diameter at 1.30 m was taken. To avoid the swelling of stem, the branch diameter was taken, with a caliper, a 3 cm away from the stem.

The other traits do not require supplementary explanations.

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

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.

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[1]) A state of knowledge concerning *P. strobus* x *P. peuce* hybrids was given in a previous article (BLADA, 1999, in press at Forest Genetics).

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 and ROBINSON, 1952). But, in this case a fix model was chosen because as previously stated, the question as to whether or not the parent populations were random mating populations, can not be answered.

The high-parent-heterosis (HPH) and the mid-parent heterosis (MPH) were calculated according to HALAUER and MIRANDA (1981) formula:

$$\mathrm{HPH} = [(\mathrm{H_y} - \mathrm{HP})/\mathrm{HP}] \times 100 \qquad (2)$$

$$\mathrm{MPH} = [(\mathrm{H_y} - \mathrm{MP}) / \mathrm{MP}] \times 100 \qquad (3)$$

where: H_y, HP and MP are the hybrid mean, the high-parent mean and the mid-parent value, respectively. As shown above, two estimates of heterosis were computed, one that compared to the best parent (HP) and the other that 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.

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 tree and five parents respectively, selected for the next breeding works (*Table 8*).

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

$$OGV_{ij} = x_{ij} - X... \qquad (7)$$

$$PGV_{ij} = gca_{i.} + gca_{.j} \qquad (8)$$

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

Genetic variation

Statistical analysis indicated highly significant ($p<0.01$; $p<0.001$) differences among *P. strobus* x *P. peuce* F_1 hybrid families for all but one traits (*Table 2,* row 2), suggesting that selection at family level within hybrid population could be carried out for most important traits including blister-rust resistance. As a complete list with all full-sib families could not be displayed here, the means of the five best and poorest hybrid families for each trait was given in *table 3*. A large variation among means of hybrid family was found. The poorest group had an average of 6.8 points in blister-rust resistance and 63.6% in trees free of blister-rust, while the best group measured an average of 9.5 points and 96.1%, respectively. At the same time the difference between the two groups of families accounted for 16% in total height and 36% in stem volume growth. Similarly, the difference between the two groups accounted for 24% for stem straightness and 17% for branch thickness. *Table 3* showed also that the differences between the test mean (X) and the top group (X_1) were much smaller than differences between test mean and the poorest group (X_2) in all traits. The figures demostrated both the magnitude of family variation and large possibilities of selection at family level.

There was found a large genetic variation among parents within each sex for all traits analysed. A remarkable result of this test was that the effects of eastern white pine female parents were highly significant ($p<0.01$; $p<0.001$), not only for all growth traits but for the three traits related to blister-rust

Table 2. – Analysis of variance of the hybrid traits.

Source of variation	Df	BRR	TS	TFBR	H	D	BA	V	SS	BT
Blocks (B)	2	0.599	1.5	7.00	5.80	0.109	0.088	5.1	0.037	0.004
Hybrids	24	3.060***	358.3***	261.15***	50.22***	1.150***	0.066***	523.4***	0.226	0.102**
-Females (F)	(4)	7.337***	1028.2***	618.58***	105.04***	1.259***	0.059***	389.3***	0.552**	0.321***
- Males (M)	(4)	3.306***	335.8***	268.98***	87.65***	3.343***	0.215***	1149.8***	0.356*	0.133*
-MxF	(16)	1.929***	196.4***	169.84***	27.15***	0.575**	0.031***	400.4***	0.111	0.040
Pooled errors	48	0.247	5.9	4.26	5.21	0.237	0.0097	4.6	0.133	0.041

Table 3. – Means of the five best and poorest hybrid families for each trait.

Family ranking	Traits...								
	BRR	TS	TFBR	H	D	BA	V	SS	BT
1	9.5	99.3	97.4	81.2	15.7	1.97	150.3	3.7	3.4
2	9.5	99.2	96.1	80.3	15.0	1.83	144.5	3.7	3.4
3	9.5	98.9	96.0	79.0	15.0	1.80	139.8	3.7	3.4
4	9.4	98.6	95.9	78.3	14.9	1.77	136.6	3.6	3.4
5	9.4	95.6	95.2	77.9	14.9	1.77	130.4	3.4	3.3
X_1	9.5	98.3	96.1	79.3	15.1	1.83	140.3	3.6	3.4
21	7.0	67.1	67.1	69.8	13.6	1.47	107.5	3.0	2.9
22	7.0	67.1	66.6	68.1	13.6	1.47	106.4	3.0	2.9
23	7.0	66.6	66.5	68.0	13.4	1.43	103.0	3.0	2.9
24	6.5	59.2	59.2	68.0	13.3	1.40	99.1	2.8	2.8
25	6.5	58.7	58.7	66.9	13.3	1.37	99.1	2.7	2.7
X_2	6.8	63.7	63.6	68.2	13.4	1.43	103.0	2.9	2.9
X	8.4	86.0	83.8	73.6	14.2	1.61	119.7	3.3	3.1
D_1	40	54	51	16	13	28	36	24	17
D_2	13	14	15	8	6	14	17	9	10

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

resistance, as well (*Table 2*, row 3). This suggests: (a) an additive genetic control in both blister-rust resistance and growth traits and (b) eastern white pine parents with a good general combining ability could be selected for breeding. The Balkan pine as male parents had significant ($p<0.05$) and hyghly significant ($p<0.001$) effects on all traits including blister-rust resistance and height growth (*Table 2,* row 4). It is important to note that Balkan pine male parents exhibited variable level of blister-rust resistance; consequently, selection of individuals within a resistant species (Balkan pine) is as important as the selection of individuals within the susceptible species (eastern white pine).

Male x female interaction effects (*Table 2,* row 5) were highly significant ($p<0.01$; $p<0.001$) for most growth traits and for the three traits related to blister-rust. This suggests a non-additive gene action that operated on phenotypic expression of the mentioned traits. However, no significant interaction effects were found for stem straightness and branch thickness.

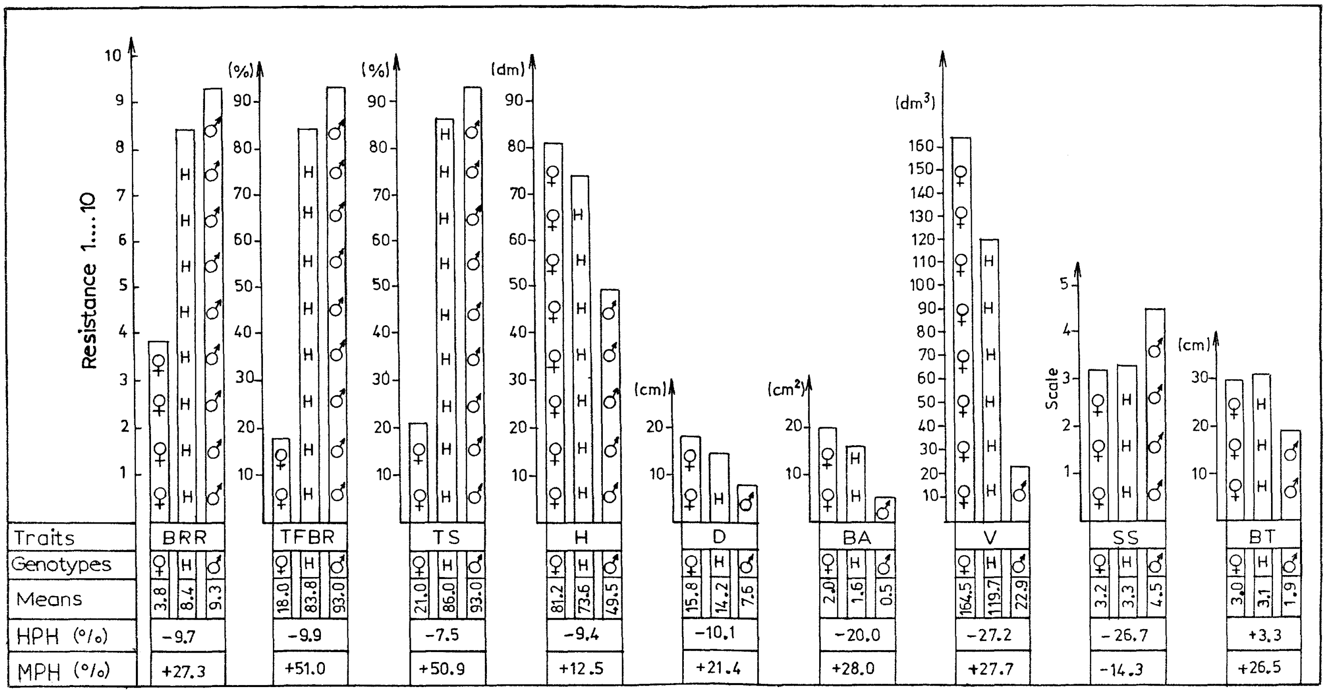
Hybrid performance and heterosis

Comparative hybrid and parent performances and the two types of heterosis were pictured graphically in *figure 1.*

As heterosis was calculated by comparison with both high-parent and mid-parent performances it should remembered that the eastern white pine was the best parent species for growth traits, whereas the Balkan pine was the best parent species for blister-rust resistance.

High-parent heterosis for all but one trait was negative, i.e. the hybrids exhibited an intermediate performance. Branch thickness was the only trait with a positive high-parent heterosis, but unfortunately this is an undesirable heterosis. Therefore it was indicated that hybrids combined their parental genes for both blister-rust resistance and growth traits.

Mid-parent heterosis was positive with the exception of stem straightness. This heterosis accounted for 27.3% for blister-rust resistance, 51.0% for trees free of blister-rust and 50.9%



Traits	BRR			TFBR			TS			H			D			BA			V			SS			BT		
Genotypes	♀	H	♂	♀	H	♂	♀	H	♂	♀	H	♂	♀	H	♂	♀	H	♂	♀	H	♂	♀	H	♂	♀	H	♂
Means	3.8	8.4	9.3	18.0	83.8	93.0	21.0	86.0	93.0	81.2	73.6	49.5	15.8	14.2	7.6	2.0	1.6	0.5	164.5	119.7	22.9	3.2	3.3	4.5	3.0	3.1	1.9
HPH (%)	-9.7			-9.9			-7.5			-9.4			-10.1			-20.0			-27.2			-26.7			+3.3		
MPH (%)	+27.3			+51.0			+50.9			+12.5			+21.4			+28.0			+27.7			-14.3			+26.5		

Figure 1. – *Pinus strobus* (♀) x *P. peuce* (♂) hybrid (H) performance and heterosis effect: HPH = high-parent heterosis and MPH = mid-parent heterosis.

Table 4. – Phenotypic correlations among traits (Df = 23).

Traits	TS	TFBR	H	D	BA	V	SS	BT
BRR	0.96***	0.98***	0.11	0.10	0.13	0.17	0.30	-0.39
TS		0.98***	0.07	0.04	0.03	0.09	0.30	-0.36
TFBR			0.08	-0.08	0.07	0.12	0.34	-0.36
H				0.54***	0.56***	0.63***	0.09	-0.39
D					0.98***	0.88***	0.04	0.30
BA						0.83***	0.02	0.25
V							0.06	0.01
SS								0.16

***p<0.001

Table 5. – Estimates of general combining ability (*gca*) effects.

Parent	Traits							
	BRR	TFBR	TS	H	D	BA	V	SS	BT
gca.-female effects									
1	0.436*	4.147***	4.760***	-1.731*	0.387*	0.100**	4.589***	-0.335*	-0.099
2	-0.764***	-6.253***	-7.640***	-1.511	-0.133	-0.033	-5.484***	0.038	0.121
3	0.143	-0.320	-1.440	4.529***	-0.340*	-0.060*	2.069*	0.072	-0.192*
7	0.849***	8.480***	11.693***	0.189	0.200	0.020	4.296***	0.152*	0.015
8	-0.664***	-6.053***	-7.373***	-1.477	-0.113	-0.027	-5.471***	0.074	0.155*
gca.-male effects									
13	-0.437*	-3.787***	-2.573**	-1.697*	-0.307	-0.093**	-7.957***	-0.142*	0.088
14	-0.124	-3.187***	-4.840***	-0.131	0.580**	0.153***	9.749***	0.085	0.075
15	-0.424*	-2.187***	-2.640**	-0.411	-0.567**	-0.140***	-10.351***	-0.148*	-0.072
18	0.423*	5.080***	4.027***	-1.864*	0.367*	0.073*	2.783***	0.214*	0.035
20	0.563**	4.080***	6.027***	4.103***	-0.073	0.007	5.776**	-0.008	-0.125

for trees survivors. Also, substantial positive mid-parent heterosis was found in most growth traits, such as: 21.4% in diameter, 28.0% in basal area and 27.7% in volume growth. Total height had the lowest (12.5%) positive mid-parent heterosis, while the stem straightness was the only trait displaying a negative heterosis.

The hybrids inherited a high blister-rust resistance. For example, the mean of hybrid population surpassed the mean of the eastern white pine female parent species in the three traits related to blister-rust. Thus, the eastern white pine measured an average of 3.8 points in blister-rust resistance and 18% in trees free of blister-rust while the hybrid measured 8.4 points and 83.8%, respectively, i.e. 121% and 366%, more. On the other hand, the means blister-rust resistance and trres free of blister-rust were only 9.7% and 9.9%, respectively, lower than the mean of Balkan pine. Therefore, the mean blister-rust resistance of hybrid population was very close to the mean of Balkan pine.

The hybrids inherited a rapid growth. The mean of hybrid exceeded the mean of the Balkan pine male parent species in all growth traits and branch thickness but not in stem straightness. For example, the Balkan pine measured 49.5 dm in total height and 22.9 dm^3 in volume growth, while the hybrid measured 73.6 dm and 119.7 dm^3, respectivelly, i.e. 49% and 423% more. For the total height and volume growth, the mean of hybrids was only 9.4% and 27.2%, respectively lower than the mean of eastern white pine. It was clear that the hybrids incorporated in their genotype genes controlling both blister-rust resistance and growth traits.

Phenotypic correlations

Phenotypic correlations coefficients were presented in *table* 4.

Highly significant ($p<0.01$; $p<0.001$) and positive correlations were found among growth characteristics, i.e. total height, diameter, basal area and volume growth; this indicate that selection for one trait, easily measurable as diameter, will cause a simultaneous improvement of the others, especially at the same age.

Phenotypic correlations between growth traits and stem straightness and between growth traits and branch thickness were positive but insignificant.

Highly significant ($p<0.001$) correlations were obtained among the three traits related to blister-rust, ranging from 0.96 to 0.98. But, low and insignificant correlations were found between blister-rust resistance and growth traits indicating no genetic relationship between these two categories of traits. Consequently, concurent improvement in these traits will be difficult.

Combining abilities

Estimates of general ability effects (*gca*) were computed to determine the contribution of individual parents to each trait (*Table 5*).

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 parents 2 and 8 and the Balkan pine male parents 13 and 15 had the largest negative effects for blister-rust resistance. On the contrary, the female parents 1 and 7 and the male parents 18 and 20 exhibited the largest positive effects indicating that the use of these parents in breeding could increase blister-rust resistance. Similarly, the parents 1, 3 and 7 of eastern white pine and 14, 18 and 20 of Balkan pine had the largest positive effects for volume growth suggesting their importance for improving this trait.

The previous data demonstrated that parents 1 and 7 of eastern white pine and 18 and 20 of Balkan pine were the best

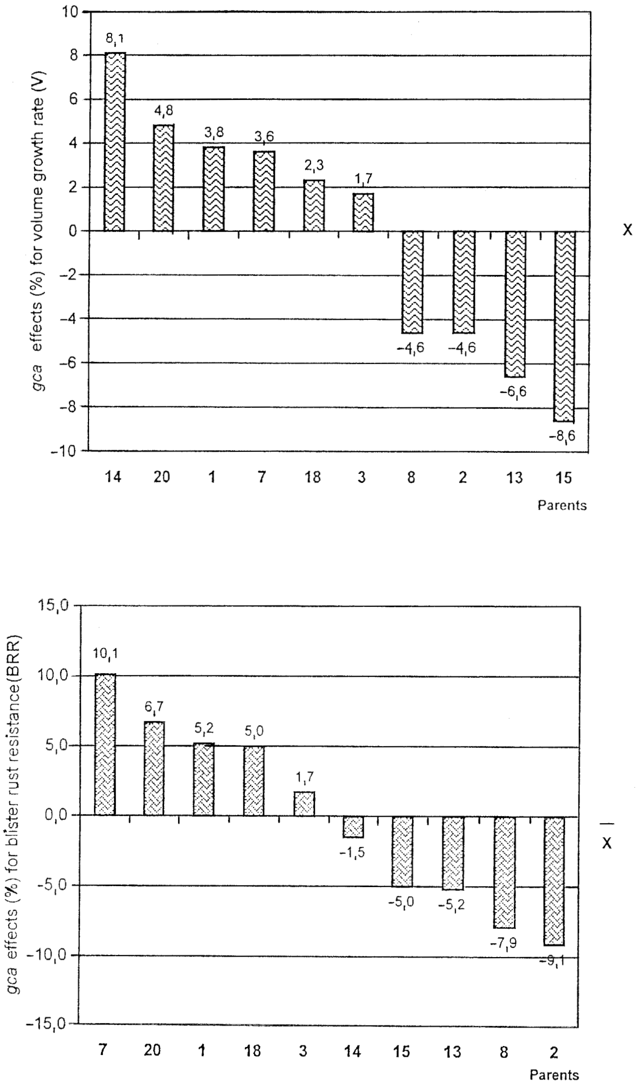


Figure 2. – General combining ability (gca) effects for volume growth (V) and blister-rust resistance (BRR).

combiners for both blister-rust rersistance and volume growth (*Fig. 2*) and consequently they should be selected and used for advanced generation selection.

Estimated specific combining ability (sca) effects for blister-rust resistance and volume growth were shown in *tables 6* and *7*. Of the 25 crosses, resulting from the 5 x 5 factorial mating design, two for blister-rust resistance and nine for volume growth had significant ($p<0.05$) or highly significant ($p<0.01$; $p<0.001$) positive *sca* effects.

The best specific crosses for volume growth (*Table 7*) were 3 x 18, 1 x 14, 8 x 13, 2 x 13, 7 x 20 and 3 x 15 followed by 8 x 20, 2 x 20 and 7 x 18. Five of these nine crosses, involved parents with a good general combining ability, i.e. parents 1, 7 and 3. Similarly, the best specific crosses for blister-rust resistance were 1 x 13 and 1 x 15: both of these crosses involved a good general combiner.

Once desirable crosses were identified, general techniques of vegetative propagation exist to exploit them for increasing genetic gain.

In an important programme, the most desirable parents would be ones that had both *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 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 *gca*'s, i.e. the average of breeding values of the best parents was presented in *table 8*. The best five parents for blister-rust resistance were female trees 7, 1 and 3 and male trees 20 and 18 (*Table 8*, column 1), and their average breeding value was 0.966 points which represent a genetic gain of 11.5% in the overal mean (8.4 points) for blister-rust resistance. Similarly, for volume growth, the best five parents were 14 and 20 of Balkan pine and 1, 7 and 3 of eastern white pine (*Table 8*, column 5) and their average breeding value was 10.6 dm^3 which would represent a genetic gain of 8.9% in the overall mean (119.7 dm^3) for volume growth.

Table 6. – General (gca) and specific (sca) combining ability estimates for blister-rust resistance of the male and female parents, observed (OGV) and predicted (PGV) genetic values of full-sib families¹).

Female trees (gca_i)		Male trees (gca_j)				
		20 (0.563)	18 (0.423)	14 (-0.124)	15 (-0.424)	13 (-0.437)
7 (0.849)	OGV	0.996 [4]	1.129 [1]	0.263	1.129 [2]	0.729 [8]
	PGV	1.412 [1]	1.272 [2]	0.725 [5]	0.425 [8]	0.412 [9]
	sca_{ij}	-0.416	-0.143	-0.462	0.704	0.317
1 (0.436)	OGV	0.663 [9]	-0.604	0.096	1.063 [3]	0.963 [5]
	PGV	0.999 [3]	0.859 [4]	0.312 [10]	0.012	-0.001
	sca_{ij}	-0.336	-1.463***	-0.216	1.051*	0.964*
3 (0.143)	OGV	0.663 [10]	0.829 [6]	0.796 [7]	-1.537	-0.037
	PGV	0.706 [6]	0.566 [7]	0.019	-0.281	-0.294
	sca_{ij}	-0.043	0.263	0.777	-1.256**	0.257
8 (-0.664)	OGV	0.229	0.363	-0.637	-1.371	-1.904
	PGV	-0.101	-0.241	-0.788	-1.088	-1.101
	sca_{ij}	0.330	0.604	0.151	-0.283	-0.803
2 (-0.764)	OGV	0.263	0.396	-1.137	-1.404	-1.937
	PGV	-0.201	-0.341	-0.888	-1.188	-1.201
	sca_{ij}	0.464	0.737	-0.249	-0.216	-0.736

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

¹) The top 10 full-sib families according to observed and predicted genetic values can be recognized by rankings in square breackets.

Table 7. – General (*gca*) and specific (*sca*) combining ability estimates for volume growth of the male and female parents, observed (OGV) and predicted (PGV) genetic values of full-sib families[1]).

Female trees (gca_i)		Male trees (gca_j)				
		14 (9.749)	20 (5.776)	18 (2.783)	13 (-7.957)	15 (-10.351)
1 (4.589)	OGV	30.669 [1]	0.503	2.636 [8]	-8.264	-2.597
	PGV	14.338 [1]	10.365 [4]	7.372 [7]	-3.368	-5.762
	sca_{ij}	16.331***	9.862***	-4.736**	-4.896**	3.165
7 (4.296)	OGV	16.936 [4]	20.103 [3]	10.703 [5]	-16.664	-9.597
	PGV	14.045 [2]	10.072 [5]	7.079 [8]	-3.661	-6.055
	sca_{ij}	2.891	10.031***	3.624*	-13.003***	-3.542*
3 (2.069)	OGV	-1.864	-0.897	24.869 [2]	-13.297	1.536 [9]
	PGV	11.818 [3]	7.845 [6]	4.852 [9]	-5.888	-8.282
	sca_{ij}	-13.682***	-8.742***	20.017***	-7.409***	9.818***
8 (-5.471)	OGV	1.469	4.603 [6]	-12.131	-0.764	-20.531
	PGV	4.278 [10]	0.305	-2.688	-13.428	-15.822
	sca_{ij}	-2.809	4.298*	-9.443***	12.664***	-4.709**
2 (-5.484)	OGV	1.536 [10]	4.569 [7]	-12.164	-0.797	-20.564
	PGV	4.265	0.292	-2.701	-13.441	-15.835
	sca_{ij}	-2.729	4.277*	-9.463***	12.644***	-4.729**

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

[1]) The top 10 full-sib families according to observed and predicted genetic values can be recognized by rankings in square breackets.

Table 8. – General combining ability (gca) estimates breeding values (BV) and genetic gains (△G).

Blister-rust resistance				Volume growth			
Selected parents	*gca*	BV	ΔG[1]	Selected parents	*gca*	BV	ΔG[1]
	Points		%		dm^3		%
7	0.849	1.698	20.2	14	9.749	19.498	16.3
20	0.563	1.126	13.4	20	5.776	11.552	9.7
1	0.436	0.872	10.4	1	4.589	9.178	7.7
18	0.423	0.846	10.1	7	4.296	8.592	7.2
3	0.143	0.286	3.4	3	2.069	4.138	3.5
Mean	0.483	0.966	11.5	Mean	5.3	10.6	8.9

[1]) Calculated against general mean, i.e. 8.4 points for blister-rust resistance and 119.7 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 two crosses, i.e. 7 x 18, and 7 x 15 were subsequently used for mass vegetative propagation, the average genetic gain in blister-rust resistance would be 1.129 points which represents the mean observed genetic value of 7x18 and 7x15 (*Table 6*). This genetic gain represents 13.4% increase in the general mean blister-rust resistance of hybrid population. If the ten best crosses (7 x 18, 7 x 15, 1 x 15, 7 x 20, 1 x 13, 3 x 18, 3 x 14, 7 x 13, 1 x 20 and 3x20) were mass vegetative propagated, the gain would be 0.896 points (the average of the observed genetic values of the ten families) or an increase of 10.7%.

A higher genetic gain in volume growth could achieved. If the best cross 1 x 14 was used for mass vegetative propagation, the genetic gain would be 30.7 dm^3, i.e. the observed genetic value (*Table 7*). This gain represents a 25.6% increase in the general mean (119.7 dm^3) for volume growth of hybrid population. If the best five best crosses (1 x 14, 3 x 18, 7 x 20, 7 x 14, 7 x 18) were mass vegetative propagated, the genetic gain would be 19.9 dm^3 (the average of the observed genetic values of the five families), i.e. an increase of 16.6% in the general mean for volume growth.

The above presented figures demonstrated that substantial genetic gain could be achieved in both blister-rust resistance and volume growth by using vegetative propagation of the best specific crosses.

Implications for breeding strategy

The objective of this experiment was to produce blister-rust resistant and fast growing F1 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 or rooting and then to establish hybrid seed orchards. In this case such a seed orchard should consists of a female clone-from Parent 7 (or Parent 1) of eastern white pine-and the best two male clones-from parents 20 and 18 of Balkan pine. All these parents were good combiners for both blister-rust resistance and volume growth. It is expected that this seed orchard will be successfully because, in Romania, the eastern white pine and Balkan pine generally flower in a partial syncrony (BLADA, unpublished data). The lack of full flowering syncrony can be compensated by suplemental artificial pollination with mixed pollen sources. The hybrid seed that incorporated additive genes, will be collected from maternal clone only. Non-hybrid progenies will be removed during the nursery stage.

Hybrid seed could also be produced through controlled pollination among the best general combining ability parents.

There are two major ways to make use of specific combining ability in a tree improvement programme (Zobel and TALBERT, 1984). 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 vagetative propagation.

One of the most effective ways for producing planting material is controlled pollination followed by mass vegetative propagation using juvenile donor seedlings. Park et al. (1998) consider three possible breeding-cloning options that incorporate breeding and vegetative propagation schemes, depending on which types of genetic variance are to be exploited: (1) general combining ability (GCA) variance; (2) specific combining ability (SCA) variance; (3) clonal variance. The first two options involve *crossing of GCA tested parents and the best tested full-sib crosses,* followed by vegetatative 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. 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 controlled-pollination should be carried out between female parents 7, 1 and 3 of eastern white pine and male parents 20 and 18 of Balkan pine. These controlled crosses could be carried out in seed orchard or directly on the selected parents. Then, 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 idetify superior crosses, vegetative propagation directly from the test progeny may not be possible. It will, therefore, require repeating the controlled crossings to obtain seeds for the best tested full-sib combinations. If the first purpose is to improve the blister-rust resistance, as in this case, it is recommended to obtain the following combinations: 1 x 15 and 1 x 13 (see *Table 6*). But, if the improvement of volume growth would have the first priority, the following combinations should be repeated: 1 x 14, 7 x 20, 7 x 18, 3 x 18, 3 x 15, 8 x 20, 8 x 13, 2 x 20 and 2 x 13 (see *Table 7*). The resulting quantities of seeds would provide zygotic embryos for somatic embryogenesis or juvenile seedling donor plants for steckling production. This approach, which has been referred to as *family forestry,* has been developed in New Zealand (Carson, 1986; Shelborune et al., 1989; Carson and Burdon, 1991) where vegetative propagation was combined with the control-pollinated orchards concept (Sweet and 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

Blister-rust resistance and rapid growth of the Balkan pine and eastern white pine, respectively, were successfully combined in an intermediate F_1 hybrid.

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

The improvement of blister-rust resistance and growth traits by using both additive- and non-additive genetic effects is possible.

Parents with a good general combining ability were found within both *P. strobus* and *P. peuce,* not only for growth traits but also for blister-rust resistance.

Because of a lack of significant correlation between blister-rust resistance and growth traits, no tandem selection can be applied.

The significant genetic gain that could be achieved, suggests that planting F_1 hybrids seems to be promising.

Both sexual and vegetative propagation could be employed for mass production of hybrid planting stock.

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Literature

Bingham, R. T.: Artificial inoculation of large number of *Pinus monticola* seedlings with *Cronartium ribicola.* In: Bingham, R. T. et al. (Eds.): Biology of rust-resistance in forest trees: USDA For. Serv., Misc. Publ., 1221, 357–372 (1972). — Blada, I.: Relative blister-rust resistance of native and introduced white pines in Romania. In: Heybroek, H. M. et al. (Eds.): Resistance to diseases and pests in forest trees. Workshop Gen. of Host-Parasite Inter. in For, Wageningen, Holland. pp. 415–416 (1982). — Blada, I.: Genetic resistence to *Cronartium ribicola* and height growth of some interspecific hybrids. Ph. D. Thesis. Agricultura-land Forestry Acad. of Sci., 146 p. (1987). — Blada, I.: Juvenile blister-rust resistance and height growth of *Pinus strobus* x *P. peuce* F_1 hybrids. Silvae Genetica **38**(2): 45–59 (1989). — Blada, I.: Blister-rust in Romania. Eur. J. Path. **20**, 55–58 (1990). — Blada, I.: Nuclear and extranuclear genetic effects in F_1 reciprocal hybrids between *Pinus strobus* and *P. peuce.* Silvae Genetica **41**(1): 34–38 (1992). — Blada, I.: Interspecific hybridization of Swiss stone pine (*Pinus cembra* L.). Silvae Genetica **43**(1): 14–20 (1994). — Blada, I.: Genetic variation in blister-rust resistance and growth traits in *Pinus strobus* x *P. peuce* hybrid at age 17 (Experiment 1). (In press at Silvae Genetica). — Carson, M. J.: Control-pollinated seed orchards of best general combiners. A new strategy for radiata pine improvement. Proc. Plant. Breeding Symposium, Lincoln, New Zealand (1986). — Carson, M. J. and Burdon, R. D.: Relative advantages of clonal forestry and vegetative multiplication. In: Miller, J. T. (Ed.): FRI Buletin No. 160, Forest Research Institute, Rotorua, N.Z., pp. 41–43 (1991). — Carson, M. J., Vincent, T. G. and Firth, H.: Control pollinated and meadow seed orchards of radiata pine. In: Proc. of IUFRO – AFOCEL Symposium, Bordeaux. pp. 100–109 (1992). — Comstock, R. E. and Robinson, H. F.: Estimation of average dominance of genes. In: Gowen, J. W. (Ed.). Heterosis. Iowa State Coll. Press, Ames. 522 p. (1952). — Griffing, B.: Concept of general and specific combining ability in relation to diallel crossing systems. Austral. J. Biol. Sci. **9**: 463–493 (1956). — Halauer, A. R. and Miranda, F. O.: Quantitative genetics in maize breeding. Iowa State University, Ames. 468 p. (1981). — Mac Key, J.: Genetic and evolutionary principles of heterosis. Eucarpia **VII**: 17–33 (1976). — Park, Y. S., Bonga, J. M. and Mullin, T. J.: Clonal forestry. In: Mandal, A. K. and Gibson, G. L. (Eds.): Forest Genetics and Tree Breeding. CBS Publishers and distributors, 4596/1A, New-Delhi. pp. 143–157 (1998). — Sherlbourne, C. J. A., Carson, M. J. and Wilcox, M. D.: New techniques in the genetic improvement of radiate pine. Commonw. For. Rev. **68**: 191–201 (1989). — Sweet, G. B. and Krugman, S. L.: Flowering and seed production problems and a new concept of seed orchards. In: Proc. of the Third World Consultation on Forest Tree Breeding, Canberra, Australia, FAO / IUFRO. pp. 749–759 (1977). — Zobel, B. and Talbert, J.: Applied forest tree improvement. John Wiley and Sons, New-York. 505 p. (1984).