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Growth, straightness and survival at age 32 in a *Pinus strobus* x *P. wallichiana* F_1 hybrid population (Experiment 2)

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Abstract. By using an incomplete factorial mating design between twenty *Pinus strobus* L. female and seven *P. wallichiana* Jack. male trees, a number of 34 full-sib families were obtained. The objective of this experiment was to combine the fast growing of the former species with moderately to high resistance to blister rust (*Cronartium ribicola* Fich. in Rabenh.) of the latter one. The hybrid families were artificially inoculated at age two, and field planted at age four. The plantation took place within an old black current (*Ribes nigrum* L.) heavy infected by blister rust. In order to provoke new infections, this time naturally, the pine rows were planted in between the black currant ones. Diameters at breast height, tree height, tree growth rate volume, stem straightness and tree survivals were the traits measured at age 32 from seed. The first trial thinning was simultaneously applied with the present measurements. The average tree survival was 74.8% in hybrids, 8.3% in *Pinus strobus* and 27.8% in *P. wallichiana*. Highly significant ($p < 0.01$) differences were found between hybrid families for all traits except stem straightness. Genetic coefficient of variation at family level was 13.7% for tree volume growth rate and 15.9% for tree survival, but only 2.1% for tree straightness. Broad-sense family heritability estimates were 0.530 for diameter at breast height, 0.596 for stem height, and 0.564 for stem volume growth rate, 0.166 for stem straightness, and 0.539 for tree survivals. Similarly, the individual tree narrow-sense heritability estimates were 0.138 for diameter at breast height, 0.209 for stem height, 0.149 for stem volume growth rate, and 0.022 for stem straightness. If the best 5, 10 and 15 of 34 families were selected, a genetic gain of 17.7%, 13.4% and 10.2%, respectively, may be achieved in tree survival or blister rust resistance. Similarly, if the best 5%, 10% and 15% individuals within the best hybrid families were selected, a genetic gain of 4.7%, 4.0% and 3.6% in diameter at breast height and 10.7%, 9.1% and 8.1% in tree volume growth rate could be made. The estimated genetic gains indicated that a program aimed at improving growth traits and survival through interspecific hybridization could be successfully achieved.
Keywords *Pinus strobus, P. wallichiana, Cronartium ribicola*, F_1 hybrids, survival, growth traits, genetic resistance, heterosis, heritability, genetic gain.

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Introduction

Owing to its remarkable growth performance, eastern white pine (*Pinus strobus* L.) was introduced to Romania in 1861, where it exhibited good wood qualities and adaptation to the extremes of the continental climate (Radu 1974). In an extensive survey made across most of the country 51% of eastern white pine plantations, of unknown origin, supported blister rust infections ranging from a 1 to 5 index score, with a mean of 3.9 points (Blada 1990). The alternate host, black currant (*Ribes nigrum* L.), distributed from sea level to about 700 m elevation, showed 92% infection in investigated populations (Blada 1990).

Because of the importance of eastern white pine in Romanian forestry and because of the potential danger from blister rust (*Cronartium ribicola* Fisch. in Rabenh.), a genetic resistance improvement program was launched in 1977 (Blada 1982). Interspecific hybridization between the susceptible but fast growing eastern white pine and moderate to highly resistant Balkan pine (*P. peuce* Griseb.) and blue pine (*P. wallichiana* Jacks.) was the main program objective (Blada 1982). Results to date concerning the F_1 hybrid populations were published before (Blada 1982, Blada 1992a, 1992b, 1994, 2000a, 2000b, 2004, Blada & Popescu 2004, 2008).

Attempts to introduce blister rust resistance genes from relative blister rust resistant species to eastern white pine have been ongoing for more than half a century. Those breeding efforts, mainly in the United States and Canada, have generated a lot of information useful in the current breeding programs. Thanks to that information, it is well known that eastern white pine has very limited blister resistance genes; consequently, introduction of such genes from other species, like blue and Balkan pines, seems to be a realistic procedure for developing resistant planting stock (Riker et al. 1943, Riker & Patton 1954, Heimburger 1962, 1972, Patton 1967, Patton & Johnson 1970).

Relatively resistant white pine genotypes were selected with various resistance mechanisms, such as ontogenetic resistance, slow rusting, and bark reactions (Patton 1967, Patton & Johnson 1970, Jurgens et al. 2003), and genetic gains were shown in some field tests (Zsuffa 1981, Sinclair 2003). However, in cases where blister rust inoculum density is high, such as with artificial inoculation, evidence suggests that the levels of resistance in eastern white pine may be insufficient to withstand heavy blister rust attack, especially at seedling stages (Heimburger 1972, Zsuffa 1981, Sniezko & Kegley 2002, Lu et al. 2005). An alternative strategy in developing stronger genetic resistance to blister rust in eastern white pine is to integrate resistance genes from Eurasian white pine species that have co-evolved with blister rust and demonstrate strong natural resistance to the pathogen (Heimburger 1962, 1972, Bingham 1972, Kriebel 1983, Garrett 1985, Zsuffa 1985).

In Canada, interspecific hybrids of *P. strobus* x *P. wallichiana* were developed, followed by backcrossing to *P. strobus* (Heimburger 1972). Breeding efforts indicated that these two species were highly compatible and some backcross hybrid seedlings survived despite exposure to massive blister rust inoculum under artificial inoculations (Heimburger 1972, Lu et al. 2005). More details about Ontario's breeding program were given by Lu & Sinclair

(2006) and Lu & Derbowka (2009). In Ontario the first-generation interspecific hybrids (F_1) and the first generation backcross hybrids (B_1) of *P. strobus* and *P. wallichiana* had higher survival rates than the pure *P. wallichiana*. For example, in trials 74-02579 and 84-02579 survival rates were 83.1 and 45.6%, respectively, for *P. wallichiana* x *P. strobus* six years after trial establishment, compared with only 5.6 and 27.7% for *P. wallichiana*. In the south-eastern Ontario trials, some F_1 interspecific hybrids had comparable survival rates to those of *P. strobus*. At more northerly colder sites, the F_1 hybrids had comparable survival rates to *P. strobus* 9 years after planting, although with inferior growth (Lu & Sinclair 2006). At present, the main objective of the Laurentian Forestry Centre, Quebec, Canada, hybrid white pine breeding program is to identify and characterize the white pine blister rust resistance mechanisms displayed by the hybrids pines. White pine blister rust resistant F_1 *P. strobus* x *P. wallichiana* hybrids were backcrossed with *P. strobus*. Somatic embryogenesis was initiated from immature zygotic embryos originating from these crosses. Somatic seedlings were obtained from over 600 cell lines, and screened under controlled inoculation, for white pine blister rust resistance. Few cell lines showed a hypersensitive reaction resulting in a rapid and synchronous bleaching and dropping of infected needles right after the appearance of needle infection spots. In other resistant cell lines, the pathogenic fungus reached the stem but its growth is greatly reduced compare to growth in susceptible lines, and the disease is not developing (Philippe Tanguay, pers. com.).

Breeding for resistance to the blister rust fungus in western white pine (*P. monticola* Dougl.) began in Northern Idaho in 1949 (Bingham et al. 1953). Three programs were developed in the western United States to breed for resistance: one directed at northern Rocky Mountain western white pine, a second for western white pine and sugar pine (*P. lambertiana* Dougl.) in Oregon and Washington, and a third for sugar pine in California. These breeding programs have created large seed banks, several seed orchards, and numerous additional plantings of pedigreed material (McDonald et al. 2004).

Our *P. strobus x P. wallichiana* first trial, located close to the Valiug village (45º 13' 16" north latitude, 22º 00' 54" east longitude and 620 m a.s.l. elevation) consisted of a factorial mating design, where seven eastern white pine female trees were mated to four blue pine male trees. At age two, the resultant 28 F_1 full-sib families and two open pollinated parent offspring controls were artificially inoculated according to the Bingham's 1972 procedure then the progenies were out planted at age six. Survival at age nine after seed (three after field planting) was 87.4% in the F_1 hybrid population, 24.1% in eastern white pine female population and 38.7% in blue pine male populations (Blada 1992b). In the same Văliug trial, but at age 17 after seed (11 after planting), the survival performances were 81.9% in F_1 hybrids, 15.0% in eastern white pine and 35.0% in blue pine parents. At age 17, the mean branch thickness was 30.6 mm for hybrids, 28.8 mm for eastern white pine female parent and 20.5 mm for blue pine; from these data has resulted a positive (6.3%) *high-parent-heterosis* for branch thickness. The Văliug hybrid trial is currently producing flowers and therefore, controlled pollinations to produce an F_2 generation can be made. Also the already exiting naturally occurring F_2 hybrid seed and hybrid seedlings can be used in somatic embryogenesis to rapidly exploit this material. In addition, backcrossing with the eastern white pine to introduce new resistance genes into eastern white pine can be launched (Blada 2004). Thick and long branches are specific characteristics of the eastern white pine x blue pine F_1 hybrids not only for the Coşteiu present study but also for the Văliug one (Blada 1992b 2004).

In this paper we evaluate age 32 (from seed) survival, growth and stem straightness of *P. strobus* x *P. wallichiana* F_1 hybrids tested in the Costeiu field trial. At the above mentioned age, the first trial thinning took place, so hybrid population volume per hectare is reported for the first time in a Romanian interspecific hybrid trial of *P. strobus* x *P. wallichiana*.

Materials and methods

Initial material and mating design. Both parent species are not native in Romania. Eastern white pine, of eastern North America origin, was selected as for growth traits, whereas blue pine, native across the Himalayan Mountains, was selected as the best parent species for high resistance to blister-rust. The objective was to combine the fast growth of the former species with high blister-rust resistance of the latter one.

By using an incomplete factorial mating design (with many missing cells) mating design between twenty eastern white pine female and seven blue pine male trees, 34 full-sib families were obtained (Table 1). All parents, located in planted Romanian populations of unknown origin, were selected at random without regard to any trait except female strobili production. The seeds were stratified according to Kriebel's (1973) methodology and then sown (spring 1981) in individual polyethylene pots (22 x 18 x 18 cm) in a potting mixture consisting of 70% spruce humus and 30% sand.

Inoculation. At age two, hybrid and open pollinated parent progenies of eastern white pine and blue pine, as controls, were arranged in a randomized block design inside a 20 m long x 8 m wide x 3 m tall tent and artificially inoculated with blister-rust. Each family was

Table 1 Parent trees, incomplete factorial mating design and the resulted families

♀	♂						
	21	22	23	24	25	26	28
	Families						
1	613		614			615	
2	616	617					
3	619						
4	620						
5	630					631	
6	632						
7						634	
8	621					622	
9	495B		496	497			
10	624						
11	627						
12				507			
13						511	
14	512B		513	514	515	516	517
15				519			
16				524	525	526	
17						530	
18							535
19				538			
20						629	

Note. ♀ and ♂ indicate the identity of female and male trees, respectively, used in hybridization. By crossing female with male trees, 34 families have resulted. For example, the family 613 resulted from the female 1 to male 21 mating and so on.

represented by a 12-seedling plot in each of the three replications. The inoculum consisted of heavily infected leaves of black currant collected from an old plantation located near the Coşteiu Experimental Field where the present F_1 hybrid trial was planted. Inoculation procedures and inoculation tent construction were similar to those described by Bingham (1972).

Field trial. At age four, the hybrid seedlings and the two open-pollinated parent seedling controls were field planted in rectangular plots, each plot containing six seedlings arranged in two rows. The trial (Figure 1) was placed in the Coşava Forest District, close to the Coşteiu Village (45º 53' 35" north latitude, 22º 22' 04" east longitude, 215 m a.s.l. elevation). The experiment was laid out within an existing old black currant plantation heavy infected by blister-rust, where inoculum for the artificial inoculation came from. The hybrid and control progenies were planted between black currant rows so that good conditions for natural infection occurred. This second exposure to the rust was applied in order to be sure that the selected material did not escape from infection. As previously mentioned, this local source of blister rust was used for inside the tent controlled inoculation. It should be stressed that no studies regarding blister rust virulence were carried out, by now, in Romanian populations.


Figure 1 The trial at age 20 (Photo I. Blada)

Assessments and observations. Diameter at breast height (1.30 m), stem height, stem volume growth, stem straightness and tree survival were measured or recorded (Table 2) at age 32 from seed. A subjective 1 to 5 index was used for the assessment of the stem straightness, where the 5 represents the best straightness.

The measurements were made simultaneously with the trial thinning; thus precision was increased.

Tree stem volume (v), in m^3, of each assessed tree was estimated using the bi-factorial logarithmic equation (Giurgiu et al. 2004) as:

$$\log v = a_0 + a_1 \log d + a_2 \log^2 d + a_3 \log h + a_4 \log^2 h, \quad (1)$$

where a_0, a_1, a_2, a_3, a_4 are regression coefficients for *Pinus strobus*, d, and h are the tree diameter at breast height (in cm) and tree height (in m), respectively.

Statistical analysis. A two-way analysis of variance based on plot means was performed (Becker 1984). The following mathematical model was applied:

$$X_{ik} = m + a_i + b_k + e_{ik} \quad (2)$$

where: X_{ik} - individual observation in the i^{th} pollinated family in the k^{th} replication; m - the general mean of the whole hybrid population; a_i - the random effect of the i^{th} full-sib progeny (i = 1, 2…I); b_k - the effect of the k^{th} replication (k = 1,2…K); e_{ik} - the random error. Replications and hybrid families were considered to be random effects. Variance components of the random effects were estimated by equating mean squares to expected mean square.

Table 2 Recorded traits at age 32

Row	Trait	Unit	Symbol
1	Stem diameter at 1,30 m	cm	D.32
2	Stem height	m	H.32
3	Stem volume	m³	V.32
4	Stem straightness [1)]	1 to 5	SS.32
5	Tree survival	%	SV.32

Note: [1)] 5 means the best straightness.

Finally, the Fischer's & Yates (1963) and Duncan (1955) tests were applied to establish the significance level between families.

Since data on individual trees were available, a separate analysis was performed in order to estimate the within plot variance Becker (1984). Because of unequal survival within plots, at age 32, only three hybrid trees were taken at random to estimate within plot variance.

To estimate effectiveness of selection, two types of heritabilities were calculated. The first heritability estimate (h^2_f) is the one commonly used for estimating the ratio of genetic to total variance which is appropriate for estimating gain from selection among families when they are vegetativelly propagated (Hallauer & Miranda 1981):

$$h^2_f = \sigma^2_g/\sigma^2_{ph1} = \sigma^2_g/(\sigma^2_g + \sigma^2_{er}/k) \qquad (3)$$

where: σ^2_g - genetic variance at family level; σ^2_{ph1} - the phenotypic variance which refers to family means;

Genetic gain (ΔG_1) was estimated by formula (Falconer 1981):

$$\Delta G_1 = (i_1 \cdot h^2_f \cdot \sigma_{ph1} \cdot 100)/X \qquad (4)$$

where: i_1 - the selection intensity for family selection, taken from Becker (1984); σ_{ph1} - phenotypic standard deviation which refers to hybrid family means; X - the general hybrid population mean.

The second heritability estimate (h^2_i) is individual tree heritability, which is commonly used for estimating genetic gain from mass selection among randomly placed best trees within the best families (Hallauer & Miranda 1981) as:

$$h^2_i = \sigma^2_g/\sigma^2_{ph2} = \sigma^2_g/\sigma^2_g + \sigma^2_p + \sigma^2_w) \qquad (5)$$

where: σ^2_{ph2} - individual tree phenotypic variance; σ^2_w - within plot variance; σ^2_p - plot error variance - $\sigma^2_{er} - \sigma^2_w / n$; n - number of trees per plot.

The mass selection genetic gain (ΔG_2) was estimated (Falconer 1981) by:

$$\Delta G_2 = (i_2 \cdot h^2_i \cdot \sigma_{ph2} \cdot 100)/X \qquad (6)$$

where: i_2 - the selection intensity for individual tree selection within hybrid family , taken from Becker (1984); σ_{ph2} - phenotypic standard deviation which refers to individual hybrid tree within plot; X = the general hybrid population mean.

Genetic coefficient of variation at the family (GCV_f) and the individual hybrid tree level (GCV_i) were calculated by formulas:

$$GCV_f = (\sqrt{\sigma^2_g}/X) \cdot 100 \qquad (7)$$

$$GCV_i = (\sqrt{\sigma^2_w}/X) \cdot 100 \qquad (8)$$

Heterosis. MacKey (1976) suggests that both positive and negative heterosis can be found for luxuriant, adaptive, selective or reproductive growth. Three types of heterosis were estimated in this study: high- (*HPH*), mid- (*MPH*) and low- (*LPH*) parent heterosis were calculated for each trait (Hallauer & Miranda 1981):

$$HPH\ (\%) = ((Hy - HP)/HP) \cdot 100 \quad (9)$$
$$MPH\ (\%) = ((Hy - MP)/MP) \cdot 100 \quad (10)$$

$$LPH\ (\%) = ((Hy - LP)\ /LP) \cdot 100 \quad (11)$$

were: *Hy, HP, MP* and *LP* - hybrid, high parent, mid-parent and low parent, respectively.

Results

Genetic variation. The analysis of variance indicated highly significant ($p < 0.01$) differences among hybrid families for diameter at breast height, total height, stem volume and survival, but not for stem straightness (Table 3). Hence, effective selection at the family level within the hybrid population could be carried out for these economically important traits i. e. survival and growth.

The Duncan Multiple Range Test shows large or very large variation at the hybrid family level for four of the five tested traits, as follows: between 25.1 and 38.9 cm in stem diameter, 21.5 and 26.3 m in stem height, 0.716 and 1.411 m³ stem volume, 28 and 100 % in tree survival. In contrast, the stem straightness exhibited a very low level of variation at the family level i. e. between 4.22 and 5.00 index score (Table 4, row 35). Differences between the best and the poorest family were 54.9% for diameter, 22.5% for height, 97.1% for volume, 260% for survival, and 18.4% for stem straightness (Table 4, row 36). With these large or very large ranges of variation, families' selection for all but straightness should be effective.

The means of the best and the poorest five hybrid family groups and the differences between them are given in Table 5. The poorest group (X_2) averaged 28.0 cm in diameter, 22.4 m in stem height, 0.760 m³ in tree volume growth, 4.22 stem straightness and 46.7% in survival (Table 5, row 12). For the same traits, the best group (X_1) averaged 36.7 cm, 26.0 m, 1.273 m³, 4.91 index score, and 96.7%, in tree survival, respectively, i. e. a superiority or difference (D1) of 31.2%, 16.0%, 67.5%, 16.3% and 107.1% (Table 5, row 14). Similarly, the difference (D2) between the best group and the general mean of the hybrid population (X) was 11.3% for diameter, 6.8% for stem height, 23.3% for tree volume, 7.0% for stem straightness and 29.2% for survival (Table 5, row 15).

These data demonstrate both the magnitude of family mean variation and the possibility of effective selection among families, especially for survival and the three growth traits but not for stem straightness which showed low variability.

The genetic coefficient of variation on a family basis was high for stem height (24.3%), moderate for stem volume (13.7%) and survival (15.9%), low for diameter (6.3%) and very low (2.1%) for stem straightness (Table 6, row 11). The genetic coefficient of variation on an individual hybrid tree basis was very high (30.8%) for stem volume growth rate, moderate for stem diameter (14.5%) and stem straightness (14.2%) and low for stem height (6.0%) (Table 6, row 12). Therefore, the traits stem height, stem volume and survival offer a

Table 3 Analysis of variance of the recorded traits of the hybrid population

Source of variation	*df*	Traits									
		D.32		H.32		V.32		SV.32		SS.32	
		MS	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*	*MS*	*F*
1	2	3	4	5	6	7	8	9	10	11	12
Replications	2	0294	0.03	12.831	7.78	0.030	0.66	337.691	0.92	0.249	1.73
Hybrid families	33	24.082	2.13**	4.082	2.48**	0.106	2.30**	792.566	2.17**	0.173	1.20 ns
Error	66	11.315		1.647		0.046		365.749		0.144	

Note. Abbreviations: *df* - degree of freedom, *MS* - mean squre, *F* - Fischer and Yates test.

Table 4 Distribution of the hybrid families into homogeneous groups according to the Duncan's multiple range test

Row	D.32						H.32						V.32					SV.32						SS.32			
	Fam	X	$p < 0.01$				Fam	X	$p < 0.01$				Fam	X	$p < 0.01$			Fam	X	$p < 0.01$				Fam	X	$p<0.05$	
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	511	38.9	a				620	26.3	a				511	1.411	a			513	100	a				511	5.00	a	
2	627	36.7	a	b			627	26.3	a				627	1.345	a	b		516	100	a				517	4.89	a	b
3	513	36.3	a	b			614	25.8	a	b			620	1.263	a	b	c	525	100	a				615	4.89	a	b
4	629	36.2	a	b			622	25.7	a	b	c		630	1.175	a	b	c	615	94	a				512	4.89	a	b
5	512	35.4	a	b			526	25.6	a	b	c		513	1.171	a	b	c	627	89	a	b			514	4.89	a	b
6	517	35.2	a	b	c		630	25.4	a	b	c		629	1.156	a	b	c	496	83	a	b	c		496	4.89	a	b
7	620	35.1	a	b	c		613	25.3	a	b	c		622	1.143	a	b	c	497	83	a	b	c		497	4.78	a	b
8	525	35.0	a	b	c		615	25.3	a	b	c		525	1.141	a	b	c	512	83	a	b	c		495	4.78	a	b
9	632	34.8	a	b	c		621	25.2	a	b	c		517	1.136	a	b	c	515	83	a	b	c		614	4.78	a	b
10	630	34.4	a	b	c		617	25.0	a	b	c		632	1.129	a	b	c	613	83	a	b	c		622	4.78	a	b
11	622	34.4	a	b	c		631	24.9	a	b	c	d	526	1.125	a	b	c	617	83	a	b	c		629	4.78	a	b
12	507	34.3	a	b	c		525	24.9	a	b	c	d	624	1.090	a	b	c	629	83	a	b	c		526	4.78	a	b
13	495	34.2	a	b	c		530	24.7	a	b	c	d	507	1.079	a	b	c	630	83	a	b	c		513	4.67	a	b
14	624	34.0	a	b	c		516	24.6	a	b	c	d	512	1.075	a	b	c	519	78	a	b	c	d	519	4.67	a	b
15	526	33.7	a	b	c	d	514	24.5	a	b	c	d	614	1.074	a	b	c	526	78	a	b	c	d	524	4.67	a	b
16	631	33.3	a	b	c	d	517	24.5	a	b	c	d	631	1.061	a	b	c	530	78	a	b	c	d	525	4.67	a	b
17	619	33.2	a	b	c	d	511	24.4	a	b	c	d	615	1.044	a	b	c	535	78	a	b	c	d	530	4.67	a	b
18	535	33.0	a	b	c	d	515	24.4	a	b	c	d	495	1.033	a	b	c	614	78	a	b	c	d	538	4.67	a	b
19	615	33.0	a	b	c	d	632	24.1	a	b	c	d	516	1.030	a	b	c	616	78	a	b	c	d	613	4.67	a	b
20	634	32.9	a	b	c	d	634	24.1	a	b	c	d	613	1.020	a	b	c	619	78	a	b	c	d	620	4.56	a	b
21	614	32.9	a	b	c	d	624	24.0	a	b	c	d	617	1.015	a	b	c	620	78	a	b	c	d	624	4.56	a	b
22	516	32.8	a	b	c	d	513	23.9	a	b	c	d	535	1.014	a	b	c	631	78	a	b	c	d	634	4.56	a	b
23	616	32.7	a	b	c	d	616	23.8	a	b	c	d	616	0.984	a	b	c	634	78	a	b	c	d	515	4.44	a	b
24	617	32.5	a	b	c	d	507	23.7	a	b	c	d	530	0.976	a	b	c	495	67	a	b	c	d	516	4.44	a	b
25	613	32.4	a	b	c	d	495	23.6	a	b	c	d	619	0.961	a	b	c	624	67	a	b	c	d	631	4.44	a	b
26	530	32.3	a	b	c	d	535	23.6	a	b	c	d	634	1.009	a	b	c	632	67	a	b	c	d	632	4.44	a	b
27	514	31.8	a	b	c	d	629	23.4	a	b	c	d	514	0.949	a	b	c	511	61	a	b	c	d	616	4.33	a	b
28	538	30.1	a	b	c	d	496	23.3	a	b	c	d	515	0.852	a	b	-c	514	61	a	b	c	d	621	4.33	a	b
29	515	30.0	a	b	c	d	619	23.0	a	b	c	d	621	0.840	a	b	c	517	61	a	b	c	d	507	4.22		b
30	496	29.8		b	c	d	497	22.8		b	c	d	496	0.817		b	c	524	61	a	b	c	d	535	4.22		b
31	519	29.7		b	c	d	512	22.8		b	c	d	538	0.783		b	c	621	61	a	b	c	d	617	4.22		b
32	621	29.0		b	c	d	524	22.5		b	c	d	519	0.762			c	622	44		b	c	d	619	4.22		b
33	497	26.4			c	d	519	22.3			c	d	497	0.721			c	538	39			c	d	627	4.22		b
34	524	25.1				d	538	21.5				d	524	0.716			c	507	28				d	630	4.22		b
35	X	33.0					X	24.3					X	1.032				X	74.8					X	4.59		
36	Diff	54.9					Diff	22.5					Diff	97.1				Diff	260					Diff	18.4		

Note. Abbreviations: X - trait mean; Diff - difference between the first and the last family in the rank.

promising opportunity for effective selection at family level, while stem volume, stem diameter, and stem straightness are traits more favorable for selection at the individual tree level.

Heritability. Estimates of broad-sense heritability at the family level (h_f) as well as estimates of individual hybrid tree heritability (h^2_i) and their standard errors (*SE*) are presented in Table 6. The broad-sense heritability esti-

Table 5 Means of the best and the poorest five hybrid families and the differences between them

Row	Rank	Traits									
		D.32		H.32		V.32		SS.32		SV.32	
		Fam	X	Fam	X	Fam	X	Fam	X	Fam	X
0	1	2	3	4	5	6	7	8	9	10	11
1	1	511	38.9	620	26.3	511	1.411	511	5.00	513	100.0
2	2	627	36.7	627	26.3	627	1.345	517	4.89	516	100.0
3	3	513	36.3	614	25.8	620	1.263	615	4.89	525	100.0
4	4	629	36.2	622	25.7	630	1.175	512	4.89	615	94.4
5	5	512B	35.5	526	25.6	513	1.171	514	4.89	627	88.9
6	**X_1**		**36.7**		**26.0**		**1.273**		**4.91**		**96.7**
7	30	496	29.8	497	22.8	496	0.817	535	4.22	524	61.1
8	31	519	29.7	512B	22.8	538	0.783	617	4.22	621	61.1
9	32	621	29.0	524	22.5	519	0.762	619	4.22	622	44.4
10	33	497	26.4	519	22.3	497	0.721	627	4.22	538	38.9
11	34	524	25.1	538	21.5	524	0.716	630	4.22	507	27.8
12	X_2		28.0		22.4		0.760		4.22		46.7
13	**X**		**33.0**		**24.3**		**1.032**		**4.59**		**74.8**
14	**D_1 (%)**		**31.2**		**16.0**		**67.5**		**16.3**		**107.1**
15	**D_2 (%)**		**11.3**		**6.8**		**23.3**		**7.0**		**29.2**

Note. Fam - family; X - general trait mean; X_1 and X_2 are the trait means of the best and the poorest five family group, respectively; $D_1(\%)$ - the difference between the mean of the best five family group and the mean of the poorest five family group, i. e. $D_1(\%) - ((X_1 - X_2)/X_2) \cdot 100$; $D_2(\%)$ - the difference between the mean of the best five family group and the general trait mean, i. e. $D_2(\%) - ((X_1 - X)/X) \cdot 100$.

mates at the family level were high for stem diameter (0.530), stem height (0.596), and stem volume (0.564) and tree survival (0.539) but low (0.166) for stem straightness. Similarly, the individual hybrid tree heritability estimates were 0.138 for diameter, 0.209 for height, 0.149 for volume, and 0.022 for stem straightness. As expected, the broad-sense heritability estimates were greater than the narrow-sense ones. Four out of five heritability estimates at both hybrid family and individual hybrid tree level were associated with standard errors less than the magnitude of the respective estimates, i. e. they are reliable. In contrast, the standard errors of heritability estimates for stem straightness for both family and individual tree within family were greater than the estimates themselves, thus they are not reliable.

The magnitude of heritabilities ensures significant genetic progress is possible in improving tree growth and tree survival.

Phenotypic correlations. Phenotypic correlations are presented in Table 7. Highly significant ($p < 0.001$) positive phenotypic correlations were found between stem diameter and stem volume (0.96) and between stem height and stem volume (0.61), as well. On the other hand, no statistically significant correlations were found between any other traits. Even though the above mentioned phenotypic correlations were significant, they may not be applied in indirect selection. The lack of any relationship between growth traits and survival, i. e. rust resistance may be worth mention as a good thing.

Performance and hybrid heterosis. The estimates of hybrid and parent means and hybrid heterosis are presented in the Table 8. At 32 years of age, the mean performance of the hybrid population was 33.0 cm in diameter, 24.3 m in height, 1.032 m^3 in volume, a 4.59 index score in stem straightness, and 74.8% in survival or blister rust resistance. The mean performance of the eastern white pine female open pollinated trees averaged 36.0 cm in diameter, 25.0 m in stem height, and 1.199 m^3

Table 6 Estimates of the genetic and non-genetic parameters of the F_1 hybrid population

Row	Parameters	Traits / Estimates				
		D.32	H.32	V.32	SS.32	SV.32
0	1	2	3	4	5	6
1	$\sigma^2 g$	4.255	0.812	0.020	0.010	142.3
2	$\sigma^2 er$	3.772	0.549	0.015	0.048	121.9
3	$\sigma^2 w$	22.993	2.138	0.101	0.425	-
4	$\sigma^2 p$	3.651	0.935	0.012	0.002	-
5	$\sigma^2 ph1$	8.027	1.361	0.035	0.058	264.2
6	$\sigma^2 ph2$	30.900	3.884	0.134	0.437	-
7	$\sigma\ ph1$	2.833	1.166	0.188	0.240	16.3
8	$\sigma\ ph2$	5.559	1.971	0.366	0.661	-
9	$h^2_f \pm SE$	0.530 ± 0.470	0.596 ± 0.404	0.564 ± 0.436	0.166 ± 0.234	0.539 ± 0.461
10	$h^2 i \pm SE$	0.138 ± 0.122	0.209 ± 0.141	0.149 ± 0.115	0.022 ± 0.110	-
11	GCV_f (%)	6.3	24.3	13.7	**2.1**	**15.9**
12	GCV_i (%)	14.5	6.0	30.8	**14.2**	-

Note. $\sigma^2 g$ and $\sigma^2 w$ - family and within plot genetic variances; $\sigma^2 er$ and $\sigma^2 p$ - family and plot error variances; $\sigma^2 ph1$ and $\sigma^2 ph2$ - plot mean and individual tree phenotypic variances; σ ph1 and $\sigma ph2$ - family and individual tree phenotypic standard deviations; $h^2 f$ and $h^2 w$ - family broad-sense and individual tree narrow-sense heritability; *SE* - standard error; *GCVf* and *GCVi* - genetic coefficient of variation at the family and individual tree level, respectively.

Table 7 Phenotypic correlations between traits

Row		D.32	H.32	V.32	SS.32	SV.32
0	1	2	3	4	5	6
1	D.32	1.00	0.38	0.96***	0.06	0.10
2	H.32		1.00	0.61***	-0.14	0.24
3	V.32			1.00	-0.01	0.12
4	SS.32				1.00	-0.02
5	SV.32					1.00

in volume, 4.73 stem straightness index score, and 8.3% survival. Similarly, for the same traits, the blue pine male tree open pollinated trees performance was 29.7 cm, 22.0 m, 0.744 m^3, 3.30 and 27.8%, respectively (Table 8, rows 1, 2, 3). Consequently, a 74.8% survival in a hybrid population artificially inoculated versus 8.3% survival in the open pollinated eastern white pine control, definitely pleads in favor of hybrid plantations wherever there is a rust hazard.

According to Wright (1976), the term of hybrid vigor or high parent heterosis is reserved for those cases (i. e. traits) in which the hybrids outperform the better of the two parents while Zobel & Talbert (1984) have reported that the hybrid vigor term refers to size superiority over both parents.

In this experiment, the eastern white pine is the best parent species for growth traits whereas the blue pine is the best parent species for blister rust resistance. Average estimate of high-parent-heterosis was positive and very high for tree survival (88.9%) but negative for diameter (-8.3%), height (-2.9%), volume (-13.9%), and stem straightness (-2.9%)(Table 8, row 4). Thus, the hybrids showed high-parent-heterosis in tree survival but not in all growth traits and stem straightness, compared to the eastern white pine open pollinated female parent trees. Estimates of mid-parent-heterosis were positive for all five traits, i. e. 0.5% for diameter, 3.3% for height, 6.3% for volume growth rate, 13.9% for stem straightness and 75.9%, for survival (Table 8, row 5). Estimates of low-parent-heterosis were positive for all traits (Table 8, row 6).

Wood productivity. The growth measurements made simultaneously with the thinning ensured a correct evaluation very accurate estimate of wood productivity of the hybrid trial at 32 years of age. The average volume per tree was 1.032 m^3 for hybrids, 1.199 m^3 for the eastern white pine parent open pollinated offspring and 0.744 m^3 for the blue pine male-parent open pollinated offspring (Table 8, rows 1-3, col. 4). Taking into account the volume per tree, wood yield per hectare was estimated as 689 m^3 for eastern white pine open pollinated offspring, 574 m^3 for F_1 hybrids and 427 m^3 for the blue pine open pollinated offspring. Eastern white pine parent species open pollinated offspring had a higher yield than the hybrids and the blue pine open pollinated offspring, as well. The hybrid performance was intermediate between the two open pollinated parent offspring, but hybrid productivity is much closer to that of eastern white pine.

The authors found no comparable estimates of volume per individual tree and wood yield per hectare for similar white pine hybrid populations However, comparisons with sessile oak (*Quercus petraea* Liebl.) and beach (*Fagus sylvatica* L) as local natural species are possible. Thus, at age 32, in similar environment conditions, the sessile oak and beech have yielded 209 and 216 m^3 per hectare, respectively (Giurgiu et al. 2004). If increasing wood volume per hectare is the economic objective, planting fast growing, blister rust resistant white pine hybrids is preferable to the slower growing sessile oak and European beech, or any other slower growing species.

Selection and genetic gain. In a breeding program selection is based upon the principle that genetic value of selected families or individuals within families will be better than the average value of families or individuals

Table 8 Parent open pollinated family and hybrid families mean performances and hybrid

Row.	Genotype	D.32	H.32	V.32	SS.32	SV.32
0	1	2	3	4	5	6
1	P. *strobus* (♀)	36.0	25.0	1.199	4.73	8.3
2	Hybrids (♀ x ♂)	33.0	24.3	1.032	4.59	74.8
3	P. *wallichiana* (♂)	29.7	22.0	0.744	3.30	27.8
4	*HPH* (%)	- 8.3	- 2.9	-13.900	-2.90	88.9
5	*MPH* (%)	0.5	3.3	6.300	13.90	75.9
6	*LPH* (%)	11.1	10.4	38.700	37.80	62.9

Note. *HPH, MPH, LPH* - high-, mid- and low-parent heterosis, respectively (%).

Table 9 Expected genetic gain (%) if selecting the best hybrid families (ΔG *1*) and the best individuals within the best families (ΔG *2*)

Row	Trait	ΔG *1*			ΔG *2*		
		Family selection			Individual selection		
		5 / 34	10 / 34	15 / 34	5%	10%	15%
0	1	2	3	4	5	6	7
1	D.32	6.90	5.20	4.00	4.70	4.00	3.60
2	H.32	4.30	3.30	2.50	3.40	2.90	2.60
3	V.32	15.50	10.90	8.40	10.70	9.10	8.10
4	SS.32	1.31	0.99	0.76	0.63	0.54	0.48
5	SV.32	17.70	13.40	10.20			

Note. 5/34, 10/34, 15/34 represents intensity of selection at family level, i. e. 5 and 10 and 15 best families selected out of the 34 tested oes. Similarly, 5%, 10% and 15% represents best individual trees selected within the best hybrid families.

Table 10 The best and the poorest specific combining ability parents and their families, for survival

Row	Fam	♀ x ♂	X (%)
0	1	2	3
The best			
1	513	14 x 23	100.0
2	516	14 x 26	100.0
3	525	16 x 25	100.0
4	615	1 x 26	94.4
5	627	11 x 21	88.9
The poorest			
30	524	15 x 24	61.1
31	621	8 x 21	61.1
32	622	8 x 26	44.4
33	538	19 x 24	38.9
34	507	12.24	27.8

Note. X (%) - family mean.

in the population as a whole (Zobel & Talbert 1984). Data from Table 9 show that substantial genetic gain in hybrid population at both hybrid family and individual hybrid tree level can be achieved.

If the best 5, 10 or 15 out of 34 families were selected and vegetativelly propagated, a genetic gain of 6.9%, 5.2% and 4.0% in diameter at breast height and 15.5%, 10.9% and 8.4% in tree volume, and 17.7%, 13.4% and 10.2% in hybrid tree survival, respectively, could be expected at age 32. Selection on an individual hybrid tree basis could make an additional gain. So, selecting the best 5%, 10% and 15% of individual hybrid trees (Figure 2) within the best hybrid families would result in a genetic gain of 4.7%, 4.0% and 3.6% in diameter and 10.7%, 9.1% and 8.1% in tree volume. The best families and their specific combining ability parents to be selected for survival or blister rust resistance are presented in Table 10.

These estimated genetic gains indicate that a program aimed at improving growth and survival (blister rust resistance) in Romanian white pine plantings through interspecific hybridization would be successful.

Discussion

Survival. It should be taken into account that the trees of this experiment were exposed to two heavy infections, one artificial at age two while the next one was a heavy natural exposure to blister rust infections across field testing. Therefore, in this specific case, the term survival is mostly attributable to blister rust resistance of trees within the trial.

In the Coşteiu white pine hybrid trial, the subject of this paper, the rate of survival was 74.8% in the hybrid population, while in the eastern white pine open pollinated offspring and the blue pine open pollinated offspring controls, survival was 8.3% and 27.8%, respectively. The high-parent-heterosis for survival computed between hybrid and eastern white pine mean survival was 88.9% (Table 8, row 4, col. 6). Such high heterosis estimate encourages the development and planting of *P. strobus* x *P. wallichiana* F1 hybrids. On the other hand, substantial variation in survival rate among hybrid families was detected. The best hybrid family survival averaged 100% while the poorest one only 28%, a huge difference of 260% (Table 4, row 36, col. 19). Such a range of variation offers the opportunity to improve survival at the family level. Selection for survival may be considered valuable from the blister rust resistance viewpoint because the F_1 hybrid population, including the parent open pollinated offspring, were heavy artificially inoculated at age two then exposed to the high

Figure 2 Within the F_1 hybrid trial such plus tree can be selected (Photo I. Blada)

natural blister rust infections across the field testing period. Consequently, high survival should relate to high rust resistance, and all 23 hybrid families whose survival mean surpassed the population mean should have above average rust resistance, and might be selected for resistance breeding work.

Growth. The mean volume growth rate of 1.032 m³ per tree and wood yield of 574 m³/ha was the second important result of the Coşteiu trial. Relatively high productivity plantings are possible.

Relative similar results were achieved in the previously mentioned *P. strobus x P. wallichiana* F_1 hybrid trial from Văliug. The height mean growth performances recorded at age nine after seed (three after planting) were 1.4 m in hybrid population, 1.3 m in eastern white pine and 0.6 m in blue pine open pollinated parents. The estimates of high-parent-heterosis were positive for tree survival (126%), stem height (1%), diameter (25%), basal area (58%), volume growth rate (63%) and numbers of branches per whorl (3%)(Blada 1992b).

In the same trial, but at age 17 after seed (11 after planting), the mean performance of hybrid population was 7.6 m in height, 15.4 cm in diameter at breast height, 0.151 m³ volume per tree, 3.1 cm in branch thickness and 3.1 in stem straightness according to a 1 to 4 index score. The estimates of high-parent-heterosis was negative for height (-8.7%), diameter at breast height (-5.5%) and volume per tree (-14.7%)(Blada 2004).

Specific literature about survival, growth wood specific gravidity in eastern white pine x blue pine F_1 and backcrossed hybrids was published in Canada (Lu & Sinclair 2006). Eastern white pine outperformed its interspecific hybrids with blue pine in Ontario trials with more extreme climate. Across the trials with annual mean temperature below -6.8ºC or minimum temperature below -12.5ºC, eastern white pine averaged significantly greater height growth than its interspecific hybrids with blue pine regardless of age. However, interspecific of eastern white pine x blue pine hybrids grew as well as eastern white pine in southeastern Ontario, where climatic conditions were milder. In a few trials where minimum temperature was above -12.5ºC, some of the F_1 and B_1 interspecific hybrids had greater height and diameter growth than eastern white pine at 20–44 years post-trial establishment. For example, in the H-7 trial, eastern white pine trees averaged 10.7 m and 16 cm, while *P. strobus* x (*P. strobus* x *P. wallichiana*) averaged 14.4 m and 20.8 cm and (*P. strobus* x *P. wallichiana*) x *P. strobus* averaged 12.9 m and 20.2 cm, and *P. wallichiana* x *P. strobus* averaged 13.7 m and 18.7 cm in height and diameter at breast height, respectively, 30 years post-establishment (Lu & Sinclair 2006).

Branching. The branching features are very specific to these hybrids and they seem to be entirely inherited from the blue pine male parent, i. e. they are thick and long. These results are consistent with those reported for the Văliug trial (Blada 1992b, 2004).

Cold hardiness. The Romania climate is a continental one with winter temperature frequently under -32ºC, consequently, it may cause severe frost damage. However, across field testing the eastern white pine x blue pine F_1 hybrid trees were never injured by winter frost, neither in this study nor the Văliug planting (Blada 1992b, 2004), so they exhibited a clear cold hardiness. But, early leader bud flushing of some hybrid trees from the Coşteiu trial (215 m elevation) were subject to late frost susceptibility which caused multi stem formation and deviation from usually good stem straightness. Such phenomenon did not occur in the Văliug trial (620 m elevation) where the climate is much cooler, and consequently, the leader bud flushing takes place much later than in the Coşteiu one (Blada, unpublished data).

Implications for breeding strategy. Breeders may make genetic progress through many generations of recurrent selection aiming at exploiting differences among trees in general combining ability, whereas other programs have been devised to utilize specific combining ability or to use both types of genetic effects (Zobel & Talbert 1984).

The mating design suggested for application in this present study (Table 1), would utilize recurrent selection aiming at exploiting differences among families and trees in specific combining ability effects. The best specific combining ability (*s.c.a*) parents and their families which may be used for developing F_2 breeding generation were listed in Table 10. Zobel & Talbert (1984) stated that the key to success in the future use of improved genotypes, including hybrids, will be the degree to which vegetative propagation can be used operationally, because obtaining seed of hybrids is usually difficult and expensive. Somatic embryogenesis is a relatively recently developed biotechnology whereby genetically identical trees can be mass produced using tissue-culture technics. This biotechnology is already available for eastern, western and whitebark (*Pinus albicaulis* Eng.) white pines and attempts to induce it in limber pine (*Pinus flexilis* James) are in progress in New Brunswick, Canada (Park 2008). Also, application of somatic embryogenesis for *P. strobus* x *P. wallichiana* F_2 hybrids propagation is underway in Quebec, Canada (Daoust, et.al. 2008, Tanguay, pers. com. 2013). The F_1 hybrids from both the Coşteiu and Văliug trial have been abundantly

Figure 3 Flowers in the hybrid trial (Photo I. Blada)

flowering (Figure 3) after 14 years in the field and now their crown are full of cones; germinating seeds have been frequently recorded, giving rise to F_2 hybrids, naturally regenerated. Consequently, application of somatic embryogenesis for *P. strobus* x *P. wallichiana* F_1 hybrids propagation in Romania is suggested.

Conclusions

Thanks to the existence of highly significant differences among hybrid families and among individual trees within them, effective selection on both the family and the individual tree basis could be made to improve growth and survival of hybrid white pine in Romania.

The high family survival in hybrid population is mostly attributable to the rust resistance genes which were introduced from resistant blue pine to the F_1 hybrid genotype.

Owing to the incorporated resistance genes to F_1 hybrids, plantations in high blister-rust hazard areas, or anywhere else, are highly protected from blister rust attack.

Individual tree and family heritability estimates are high enough that significant genetic progress in improving growth traits and tree survival may be expected.

A high-parent-heterosis estimate of 88.9%, which represents the difference between the 74.8% survival in hybrid population and 8.3% survival in the eastern white pine open pollinated offspring control, definitely argues in favor of hybrid plantations, even in a high blister rust hazard areas.

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