

# Growth, straightness and survival at age 32 in a *Pinus strobus* x *P. wallichiana* F<sub>1</sub> hybrid population (Experiment 1)

I. Blada, Ș. Tănăsie, C. Dinu, I. Bratu

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**Abstract.** An interspecific factorial hybridization was made, where seven *P. strobus* female parent trees were mated to four *P. wallichiana* male trees, in order to transfer genes for resistance to blister-rust from the blue pine to the F<sub>1</sub> hybrids. The hybrid seedlings and the open pollinated families of the two parents were three times consecutively inoculated with blister-rust. The six years old seedlings were transplanted in the field in the so called Văliug Experiment 1. The experimental design consisted in 28 hybrid full-sib families and two open pollinated families of the parent species. Plantation took place in a complete randomized block design with three blocks, with 6 to 12 seedlings per linear plot and 3 x 3 m spacing. This paper is based on the measurements made at age 32, just after the first thinning. Diameter at breast height, tree height, tree volume, stem straightness and tree survival were the five measured traits. According to ANOVA, significant ( $p < 0.05$ ) and highly significant ( $p < 0.001$ ) differences for diameter at breast height, tree height, volume growth rate, stem straightness and tree survival were detected between hybrid families. Highly significant ( $p < 0.01$ ,  $p < 0.001$ ) differences were found between eastern white pine female parents for survival, stem straightness and all growth traits, suggesting that nuclear additive genes are controlling the respective characters. Consequently, high survival or genetic resistance to blister-rust can be found within the *P. strobus* species even though it is highly susceptible to this pathogen. The *P. wallichiana* parents exhibited significant additive effects on straightness and breast height diameter, but no significance in height, volume, and tree survival. The contribution of the additive variance to the phenotypic one was 65.7% for diameter, 58.6% for height, 50.5% for volume, 70.4% for stem straightness and 73.4% for tree survival. Hence, additive variance should be employed in a breeding program. The narrow-sense family heritability estimates were 0.657 for diameter, 0.586 for height, 0.505 for volume, 0.705 for stem straightness and 0.734 for tree survival. The individual-tree narrow-sense heritability estimates were 0.336 for diameter, 0.253 for height, 0.205 for volume and 0.121 for stem straightness. Assuming selection of 5, 10, or 15 families out of the 28 tested ones and sexual propagation, a genetic gain of 9.5%, 6.8% and 4.8% in diameter, and 11.2%, 8.1% and 5.7% in volume and 16.4%, 11.8% and 8.4% in tree survival, respectively, might be achieved. Selecting the most outstanding 5%, 10%, or 15% individual F<sub>1</sub> hybrids would yield a genetic progress of 9.7%, 8.3% and 7.4% in diameter and 10.2%, 8.8% and 7.8% in volume growth rate. The

hybrid population mean surpassed in tree survival the open pollinated eastern white pine mean by 70.7% while the eastern white pine, surpassed the hybrid in all growth traits. The  $F_1$  hybrid and *P. strobus* open pollinated parent species averaged 0.993 and 1.069 m<sup>3</sup> volume per tree, respectively. By extrapolation the yield results from the hybrid trial area to 1 ha results in a yield of 559.9 m<sup>3</sup> per hectare for hybrids and 602.5 m<sup>3</sup> for *P. strobus* female parent species. In conclusion, hybrids should be taken into consideration and used in plantation programs in high-blister-rust-hazard areas.

**Keywords** *Pinus strobus*, *P. wallichiana*,  $F_1$  hybrids, *Cronartium ribicola*, blister-rust resistance, wood yielding, heterosis, additive variance, additive effects, heritability, genetic gain.

**Authors.** Ioan Blada (ioan\_blada@icashd.ro), Cristiana Dinu - Forest Research and Management Institute, Bd.Eroilor 128, Voluntari, Romania; Ștefan Tănăsie - Forest Research and Management Institute, Caransebeș Station; Iulian Bratu - Lucian Blaga University, Sibiu.

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## Introduction

In the pre-1860's, the blister-rust (*Cronartium ribicola* Fisch. in Rabenh.) was transported via infected stock of Siberian pine (*Pinus sibirica* Du Tour) from Siberia to Europe (Lepik 1967). Next, on infected nursery stock, the blister-rust was shipped from Europe across the Atlantic into indigenous stands of eastern white pine (*Pinus strobus* L.), western white pine (*P. monticola* Dougl.), sugar pine (*P. lambertiana* Dougl.), and white bark pine (*P. albicaulis* Eng.) where it caused severe damage (Bingham & Gremmen 1971). Because blister rust control through classic treatments failed, many breeding programs for improving blister rust resistance were launched and developed in Canada and United States (Riker et al. 1943, Hirt 1948, Bingham et al. 1953, Heimburger 1956, Patton R.F. 1966, Zsuffa 1981, Meagher & Hunt 1985, Snieszko & Kegley 2002, Sinclair 2003, Jurgens et al. 2003, McDonald et al. 2004, Lu et al. 2005, Lu & Sinclair 2006, Park Y.S. 2008, Daoust et al. 2008). The main objectives of these programs were to exploit the existing, both within and between, species variation in resistance from both *P. strobus* and *P. monticola* and from blister rust resistant Euro-Asian species (Bingham & Gremmen

1971, Bingham 1972a, Hoff et al. 1980). A comprehensive discussion and analysis on the American and Canadian literature on breeding blister-rust resistance programs in five needle white pines was made in a recently papers (Blada & Tănăsie 2013) hence, it is not repeated here.

Taking into account that eastern white pine exhibited fast growing and adaptability in plantations from many countries (Kriebel 1983), massive introductions took place in Romania after 1961 (Radu 1974). But the forester's enthusiasm was soon lost because of the high blister rust frequency and intensity of attacks that occurred in black currant (*Ribes nigrum* L.) and eastern white pine plantations (Blada 1982, Blada 1990).

To fight against the blister-rust disaster, a program aimed at improving the genetic resistance to this rust was started in 1977. One of the objectives of this program was to introduce genes for resistance to blister-rust from the blue pine and from Balkan pine (*P. peuce* Griseb.) into the  $F_1$  hybrid genotypes (Blada 1982).

This article presents the performances in growth, stem straightness and tree survival (i.e. blister-rust resistance) traits measured at age 32 in the Văliug *Pinus strobus* x *P. wal-*

*lichiana* F<sub>1</sub> hybrid population artificially inoculated with blister rust at ages 2, 3 and 4, consecutively.

## Materials and methods

Parent trees, mating design and inoculation. A number of 7 *P. strobus* female trees and 4 *P. wallichiana* males were selected from non-improved planted stands. Parent selection was made at random to any trait, except reproductive fertility. To obtain the hybrid families, during July of 1979 a 7 x 4 factorial mating design (Table 1), according to Comstock & Robinson (1952) was completed and seed collection took place during September of 1980. It should be stressed that the *P. strobus* x *P. wallichiana* seed never matures completely its embryos before cone collection; thus the seed requires stratification. To avoid costly and difficult stratification, the fresh seed was sown according to our local experience, i.e. immediately after their collection, the seeds were sown in plastic pots, filled with a mixture of spruce humus and sand in a 3:1 proportion, respectively. During the winter of 1980/1981, the sown pots were covered with conifer sawdust and placed in the nursery, in natural climatic conditions. In such conditions, 87% of seeds have fully matured and, consequently germinated by the end of May, 1981. By this way, the difficult and costly stratification was avoided (Blada, unpublished data).

Three consecutive artificial inoculations

of the seedlings took place, at age two, three and four, and all relevant information concerning this subject can be found in our previous publications (Blada 1982, 1992a, 2004) and in Bingham (1972b).

Field trial establishment. The field trial was laid out in the Vāliug Forest District, at 45° 13' 16" north latitude, 22° 00' 54" east longitude and 620 m elevation. The trial consisted of 30 families (28 hybrid + 2 parent species), planted according to a complete randomized block design, three blocks, 6 to 12 seedlings per linear plot and 3 x 3 m spacing. In general, across its life, the trial exhibited very good development (Fig. 1). The nursery test took place between 1981 and 1986 and the field test between 1987 and 2012 years.

Trial thinning and measurements. Both trial thinning and trait measurements were made in October 2012, i.e. at age 32 from seed (Fig. 2).

The thinning took place two weeks before measurements, which greatly facilitated accurate trait recording. Diameters at breast height, tree height, stem straightness, tree volume growth rate and tree survival were the recorded traits (Table 2). It is important to stress that soon after 18-19 years of age, the blue pine parent open pollinated families were ultimately all killed by winter frosts (-31°C), while the hybrids were not affected by this phenomenon. This was the reason why the blue pine parent could not be involved in any analyses. As the hybrid trees were killed only by blister rust, in this paper, the tree survival has the same mean

**Table 1** Factorial mating design

♀ / ♂	21	26	27	28
2	X	X	X	X
3	X	X	X	X
5	X	X	X	X
9	X	X	X	X
10	X	X	X	X
11	X	X	X	X
12	X	X	X	X

Note. X - successful cross

**Table 2** Measured traits at age 32

Trait	Unit	Symbol
Diameter at breast height	cm	<i>D.32</i>
Tree height	m	<i>H.32</i>
Tree volume	m <sup>3</sup>	<i>V.32</i>
Stem straightness*	1 to 5	<i>SS.32</i>
Tree survival (i.e. blister-rust resistance)	%	<i>SV.32</i>

Note. \* Ranking: 5 - the best straightness.



**Figure 1** An outside view of the trial just before thinning



**Figure 2** An internal view of the trial just after thinning (age 32)

ing as the blister rust resistance. It should be stressed that the killed young trees by the rust while in the nursery, were taken into account when the trial was assessed, i. e. at age 9 (Blada 1992a) and age 17 (Blada 2004, Blada & Popescu 2004) and at age 32 (Blada et al. this paper).

By using the diameter and height data, the tree bole volume was calculated according to the bi-factorial logarithmic equation (Giurgiu et. al. 2004).

Analysis of variance, heritability and genetic gain. A random model

of variance analysis at the family level in one environment (Comstock and Robinson 1952, Experiment II) (Table 3) and a variance analysis on an individual tree basis (Becker 1984), were applied. As the number of survival trees within plots was variable between 6 and 12, only six trees per plot, taken at random, were involved in the above mentioned analyses. The standard error (SE) of variance components were estimated by using the Anderson & Bancroft (1952), formulas.

The broad sense heritability ( $H^2$ ) was calculated by the Grafius & Wiebe (1959) formula; this heritability is used for genetic gain in case of vegetative propagation:

$$H^2 = \sigma_G^2 / \sigma_{Ph.I}^2 = (\sigma_M^2 + \sigma_F^2 + \sigma_{MF}^2) / (\sigma_M^2 + \sigma_F^2 + \sigma_{MF}^2 + \sigma_e^2 / k) \quad (1)$$

where  $\sigma_G^2$  - genetic variance;  $\sigma_{Ph.I}^2$  - phenotypic variance at plot mean level;  $\sigma_M^2$ ,  $\sigma_F^2$ ,  $\sigma_{MF}^2$ ,  $\sigma_e^2$  and  $k$  - males, females,  $M \times F$  interactions and error variances and number of blocks, respectively.

The genetic progress or genetic gain ( $\Delta G_1$ ) from family selection was calculated by Falconer formula (1981):

$$\Delta G_1 = i_1 H^2 \sigma_{Ph.I} \quad (2)$$

where  $i_1$  - selection intensity at family level (Becker 1984);  $\sigma_{Ph.I}$  - phenotypic standard deviation at plot mean level.

The narrow-sense heritability estimate ( $h^2_A$ ) on a plot mean basis was calculated according to Grafius & Wiebe (1959); this heritability is used for genetic gain estimation in case of sexual propagation:

$$h^2_A = \sigma_A^2 / \sigma_{Ph.I}^2 = (\sigma_M^2 + \sigma_F^2) / (\sigma_M^2 + \sigma_F^2 + \sigma_{MF}^2 + \sigma_e^2 / k) \quad (3)$$

where:  $\sigma_A^2$  - additive variance.

The genetic gain ( $\Delta G_2$ ) from family selection was calculated by formula:



**Table 3** Random model analysis of variance of hybrid families

Source of variation	DF	MS	E(MS)
Blocks (B)	$k - 1$		
Males (M)	$m - 1$	$M_5$	$\sigma^2 + k \sigma^2_{mf} + k f \sigma^2_m$
Females (F)	$f - 1$	$M_4$	$\sigma^2 + k \sigma^2_{mf} + k m \sigma^2_f$
Males x Females (M x F)	$(m - 1)(f - 1)$	$M_3$	$\sigma^2 + k \sigma^2_{mf}$
Pooled errors (B x M x F)	$(k - 1)(mf - 1)$	$M_2$	$\sigma^2$
Total	$k m f - 1$		
Within plot	$k m f (n - 1)$	$M_1$	

Note.  $k$ ,  $m$ ,  $f$  and  $n$  - number of blocks, males, females and hybrid trees measured within plot, respectively;  $M_1$  - the within plot mean square which includes the within plot genetic variance and environmental variance;  $M_2$ ,  $M_3$ ,  $M_4$  and  $M_5$  - pooled errors, males x females interactions, female and male parent trees, respectively.

$$\Delta G_2 = i_1 h^2_A \sigma_{Ph1} \quad (4)$$

The individual tree narrow-sense heritability ( $h^2_i$ ), which is used for gain calculation from mass selection of trees within plot was estimated by the formula:

$$h^2_i = \frac{\sigma^2_A / \sigma^2_{Ph2}}{(\sigma^2_M + \sigma^2_F) / (\sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_p + \sigma^2_w)} \quad (5)$$

where:  $\sigma^2_{Ph2}$  - the phenotypic variance at individual tree level;  $\sigma^2_w$  - within plot variance;  $\sigma^2_p$  (plot error variance) =  $\sigma^2_e - \sigma^2_w / n$ ;  $n - 6$  measured trees per plot.

The genetic gain ( $\Delta G_3$ ) at individual tree level was calculated with the formula:

$$\Delta G_3 = i_2 h^2_i \sigma_{Ph2} \quad (6)$$

where:  $i_2$  - intensity of selection when selection is made at individual tree level;  $\sigma_{Ph2}$  - phenotypic standard deviation at individual level.

When the best parents of the best families are selected and crossed among them,  $i_1$  has to be multiplied by 2 and the genetic progress ( $\Delta G_4$ ) is calculated by formula:

$$\Delta G_4 = 2i_1 h^2_A \sigma_{Ph1} \quad (7)$$

Standard errors ( $SE$ ) for the heritability estimates were approximated by dividing the standard error of the  $\sigma^2_{gca}$  used as numerator, by the phenotypic variance of the selection unit used

as denominator (Hallauer & Miranda 1981).

The general combining ability ( $gca$ ) effects of each parent were calculated by the Griffing (1956) formula, adapted to this factorial crossing design, such as:

$$x_{ijk} = X... + g_i + g_j + s_{ij} + e_{ijk} \quad (8)$$

where:  $x_{ijk}$  is the mean of the  $i$ -th female parent crossed to the  $j$ -th male parent over  $k$  blocks;  $X...$  is the overall mean;  $g_i$  is the  $gca$  effect associated with the  $i$ -th female parent;  $g_j$  is the general combining ability ( $gca$ ) effect associated with the  $j$ -th male parent;  $s_{ij}$  is the specific combining ability ( $sca$ ) effect associated with the cross between the  $i$ -th female parent tree crossed to the  $j$ -th male parent;  $e_{ijk}$  is the error effect.

The calculation formulae for general combining ability ( $gca$ ) parent effects are:

$$gca_j = x_{.j} - X \quad (9)$$

$$gca_i = x_{i.} - X \quad (10)$$

where:  $gca_j$  and  $gca_i$  are the effects attributable to male and female parents, respectively;  $x_{i.}$  - the average of the  $F_1$  hybrid resulting from mating the  $i$ -th female parent with each of the male parent;  $x_{.j}$  - of the  $F_1$  hybrid resulting from mating the  $j$ -th male parent with each of the female parent;  $X$  - the overall trait mean.

Genetic coefficient of variation at family

( $GCV_p$ ) and individual tree ( $GCV_i$ ) level were estimated as follows:

$$GCV_{f( \% )} = (\sqrt{\sigma_g^2 / X}) \cdot 100 \tag{11}$$

$$GCV_{i( \% )} = (\sqrt{\sigma_w^2} / X) \cdot 100 \tag{12}$$

**Results**

Genetic variability. Significant ( $p < 0.05$ ) and highly significant ( $p < 0.01$ ) differences were detected among hybrid families for diameter, height, tree volume, stem straightness and tree survival (Table 4, row 2). These results indicate that improvement by selection at family level is possible for all tested traits.

One of the most prominent feature of this experiment was the existence of highly significant ( $p < 0.01$ ,  $p < 0.001$ ) genetic differences among eastern white pine female parent trees, not only for growth traits and stem straightness but for tree survival, as well (Table 4, row 3). This suggests that individual parent trees with significant general combining ability effects for growth and tree survival i. e. blister rust-resistance could be selected within eastern white pine species for breeding.

There are highly significant ( $p < 0.01$ ,  $p < 0.001$ ) genetic differences among blue pine male trees for diameter at breast height and stem straightness but no significance for height, volume, and survival (Table 4, row 4). The small male parent sample involved in crossing might be the cause of the absence of significant differences in hybrid survival. Almost identical results were found in previously reports (Blada 1992a, 2004).

The male x female interaction effects were insignificant for all traits (Table 4, row 5). This suggests the absence of non-additive gene action for the respective traits.

According to the analyzed trait, low to large variation at the hybrid family mean level, was found (Table 5). The mean of the best five family groups ( $X_1$ ) in tree survival was 95.6%, while the mean for the poorest five family group ( $X_2$ ) was 65.8%, i. e. a difference ( $D_1$ ) of 29.8% (Table 5, row 14, col. 6). Similarly, the difference ( $D_2$ ) between the best five family group ( $X_1$ ) and the overall mean ( $X$ ) in tree survival was smaller, i.e. 15.8% (Table 5, row 15, col. 6). Also, significant metrical differences ( $D_1$ ;  $D_2$ ) were found in several other traits. Such differences demonstrate not only the mag-

**Table 4** The results of the variance analysis

R Source of w variation	Df	Traits / Estimates											
		D.32		H.32		V.32		SS.32		SV.32			
		MS	F-test	MS	F-test	MS	F-test	MS	F-test	MS	F-test		
1 Blocks (B)	2	37.843	3.83	3.404	2.96	0.133	4.57	0.036	0.41	472.03	4.68		
2 Hybrid families (f)	27	32.603	3.30**	2.118	3.08**	0.063	2.15*	0.188	2.11*	272.87	2.71**		
3 Female trees (F)	(6)	61.963	4.94**	6.596	9.59***	0.149	4.10**	0.290	18.94***	1077.21	38.58***		
4 Male trees (M)	(3)	94.290	7.52**	1.744	2.54	0.098	2.70	1.022	66.86***	133.83	4.79		
5 Interaction (F x M)	(18)	12.535	1.27	0.688	0.60	0.036	1.25	0.015	0.17	27.92	0.28		
6 Error (E)	54	9.887		1.150		0.029		0.089		100.76			
7 Within plot (w)	420	14.004		1.466				0.580		--			

Note. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; DF, MS, F-test - degree of freedom, mean square, and Fischer and Yates test, respectively.

**Table 5** Means of the best and the poorest five family groups and the differences between them. Genetic coefficient of variation at family ( $GCV_f$ ) and individual tree ( $GCV_i$ ) level

Row	Rank	Traits				
		D.32	H.32	V.32	SS.32	SV.32
0	1	2	3	4	5	6
1	1	40.9	21.9	1.299	5.0	97.1
2	2	40.1	21.8	1.278	5.0	95.6
3	3	38.6	21.8	1.158	4.8	95.5
4	4	38.2	21.4	1.135	4.8	95.1
5	5	37.7	21.3	1.089	4.8	94.7
6	$X_1$	<b>39.1</b>	<b>21.6</b>	<b>1.191</b>	<b>4.9</b>	<b>95.6</b>
7	24	30.6	19.9	0.752	4.4	79.1
8	25	30.1	19.8	0.748	4.2	77.2
9	26	30.0	19.6	0.742	4.2	63.7
10	27	29.1	18.9	0.645	4.1	54.5
11	28	28.5	18.4	0.619	4.1	54.3
12	$X_2$	<b>29.7</b>	<b>19.3</b>	<b>0.701</b>	<b>4.2</b>	<b>65.8</b>
13	$X$	<b>34.0</b>	<b>20.7</b>	<b>0.927</b>	<b>4.5</b>	<b>79.8</b>
14	$D_1$	<b>9.4</b>	<b>2.3</b>	<b>0.490</b>	<b>0.7</b>	<b>29.8</b>
15	$D_2$	<b>5.1</b>	<b>0.9</b>	<b>0.264</b>	<b>0.4</b>	<b>15.8</b>
16	$GCV_f$ (%)	8.7	3.6	12.200	5.9	13.6
17	$GCV_i$ (%)	11.0	5.9	20.900	16.8	-

Note.  $X$  - overall trait mean,  $X_1$  and  $X_2$  - 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,  $D_1 = X_1 - X_2$ ;  $D_2$  - the difference between the mean of the best five family group and the overall trait mean, i. e.  $D_2 = X_1 - X$ .

nitude of family variation but the possibilities of selection at the family level, as well. The family  $GCV_f$  was relatively high (12.2%) for volume growth rate and 13.6% for tree survival, and moderate (8.7%) for diameter at breast height and low (3.6%) for tree height (Table 5, row 16). Similarly, the individual  $GCV_i$  was high (20.9%) for volume growth rate and stem straightness (16.8%), moderate (11.0%) for diameter at breast height, and low (5.9%) for tree height (Table 5, row 17).

Hybrid performance. The performances of the *P. strobus* x *P. wallichiana* hybrid population and its *P. strobus* female parent species, are presented in Table 6. The mean performance of the hybrid population averaged 34.2 cm in diameter, 20.7 m in tree height, 4.54 index score in stem straightness, and 79.8% in tree survival. The average of open pollinated population of eastern white pine measured 36.5 cm in diameter at breast height, 21.8 m in stem height, 4.54 index score for stem straightness and 9.1% in tree survival.

The hybrid and the eastern white pine parent populations averaged 0.993 and 1.069 m<sup>3</sup> volume per tree, respectively. By extrapolation of the yield resulting from the hybrid trial to a 1.0 ha area, wood yields is 559.9 m<sup>3</sup>/ha for F<sub>1</sub> hybrids and 602.5 m<sup>3</sup>/ha for eastern white pine populations (Tab. 11, row 1, col. 3 and 6).

The previously presented data shows that (i) the F<sub>1</sub> hybrid growth traits are slightly inferior but closed to that of the eastern white pine parent species and (ii) the tree survival is very high in hybrid population but very small in eastern white pine population. Taking into account the relatively high wood yield of the hybrids and their very high tree survival compared to low tree survival in mother species, the hybrids would be preferable for planting for wood production in high-blister-rust hazard areas.

Variances. The general combining ability variance from this experiment reached high level for most traits. Thus, the contribution of the general combining ability ( $\sigma^2_{GCA}$ ) vari-

ance to the phenotypic variance ( $\sigma^2_{Ph,1}$ ) was as follows: 65.7% for diameter at breast height, 58.6% for total height, 50.5% for volume growth rate, 70.4% for stem straightness and 73.4% for survival (Table 7, row 3). Hence, the additive variance should be used in the breeding program for growth and survival improvement. As compared to the  $\sigma^2_{GCA}$  variance, the

contribution of  $\sigma^2_{SCA}$  was very insignificant for diameter (7.3%) and volume (9.9%), and zero for all the other traits (Table 7, row 4) suggesting that this variance cannot be utilized in a breeding program.

The ratio of  $\sigma^2_{GCA-F}/\sigma^2_{SCA-M}$  showed that the estimates of the female GCA variance was greater than estimates of the male variance for

**Table 6** The hybrid and eastern white pine female parent performances and absolute differences between them calculated as against the hybrid mean

Row	Genotype	Traits / Means				
		D.32 (cm)	H.32 (m)	V.32 (m <sup>3</sup> )	SS.32 (1...5)	SV.32 (%)
0	1	2	3	4	5	6
1	<i>P. strobus</i> x <i>P. wallichiana</i>	34.2	20.7	0.993	4.54	79.8
2	<i>P. strobus</i>	36.5	21.8	1.069	4.54	9.1
3	Absolute differences	-2.3	-1.1	-0.076	0.00	70.7

Note. As all *P. wallichiana* parent trees were previously killed by winter frost, the respective species was not included in this table and, consequently, the heterosis could not be estimated.

**Table 7** General ( $\sigma^2_{GCA}$ ) and specific ( $\sigma^2_{SCA}$ ) combining ability variances (percents in brackets) and variance standard errors (*SE*)

Row	Parameter	Traits / Estimates				
		D.32	H.32	V.32	SS.32	SV.32
0	1	2	3	4	5	6
1	$\sigma^2_{GCA-F} \pm SE$	4.119(33.8) ±2.603	0.492(53.2) ±0.275	0.0094(38.5) ±0.0063	0.023(22.7) ±0.012	87.44(69.4) ±44.89
2	$\sigma^2_{GCA-M} \pm SE$	3.893(31.9) ±2.846	0.050(5.4) ±0.054	0.0029(12.0) ±0.0030	0.048(47.7) ±0.031	5.043(4.0) ±4.052
3	Total $\sigma^2_{GCA} \pm SE$	8.012(65.7) ±2.724	0.543(58.6) ±0.164	0.0124(50.5) ±0.0047	0.071(70.4) ±0.021	92.48(73.4) ±24.47
4	$\sigma^2_{SCA} \pm SE$	0.883(7.3) ±1.461	0.000(0.0) ±0.000	0.0024(9.9) ±0.0043	0.000(0.0) ±0.000	0.000(0.0) ±0.000
5	Total $\sigma^2_G \pm SE$	8.895(73.0) ±2.303	0.543(58.6) ±0.100	0.0148(60.4) ±0.0045	0.071(70.4) ±0.016	92.48(73.4) ±18.65
6	$\sigma^2_E \pm SE$	3.296(27.0) ±1.869	0.383(41.4) ±0.217	0.0097(39.6) ±0.0055	0.030(29.5) ±0.017	33.59(26.6) ±19.04
7	$\sigma^2_{Ph,1}$	12.191 (100)	0.926(100)	0.0245(100)	0.100(100)	126.07(100)
8	$\sigma^2_{GCA-F} / \sigma^2_{GCA-M}$	1.1	9.8	3.2	0.5	17.3
9	$\sigma^2_W$	14.004	1.466	0.0430	0.580	-
10	$\sigma^2_p$	0.962	0.139	0.0025	0.000	-
11	$\sigma^2_{Ph,2}$	23.860	2.147	0.0603	0.5837	-

Note.  $\sigma^2_G$ ,  $\sigma^2_W$ ,  $\sigma^2_p$ ,  $\sigma^2_E$  - total genetic variance, within plot variance, plot error variance and error variance, respectively;  $\sigma^2_{GCA-F}$  and  $\sigma^2_{GCA-M}$  general combining ability attributable to female and male parent trees, respectively;  $\sigma^2_{Ph,1}$  and  $\sigma^2_{Ph,2}$  - phenotypic variance which refers to family means and phenotypic variance at individual tree level, respectively.



diameter (1.1), height (9.8), volume (3.2) and survival (17.3) (Table 7, row 8) suggesting, once again, that the  $\sigma^2_{GCA-F}$  should be applied in a breeding program.

The results show that the greatest amount of additive variance for all growth traits and survival as well, was detected within the *P. strobus* parent population suggesting that the breeding program might be based on additive variance available within the eastern white pine species. The *P. wallichiana* male parent contribution to additive genetic variance was significant for diameter and stem straightness but insignificant for height and volume, and tree survival.

The variance components of the female parents have standard errors smaller than the variance estimates, thus making the heritability estimates reliable. The standard errors of the variances of the *P. wallichiana* male parent species were reliable for diameter and stem straightness, but not reliable for height, volume and tree survival.

**Heritability estimates.** The heritability estimates are presented in the Table 8. The family broad-sense heritability ( $H^2$ ) estimates, varied between 0.586 for tree height and 0.734 for tree survival. The family narrow-sense her-

itabilities ( $h^2_A$ ) were as great as 0.657 for diameter at breast height, 0.586 for height growth, 0.505 for volume growth rate, 0.705 for stem straightness and 0.734 for tree survival. The individual narrow-sense heritability estimates ( $h^2_i$ ) were 0.336 for diameter, 0.253 for height, 0.205 for volume and 0.121 for stem straightness. The broad-sense heritabilities may be used for vegetative propagation while the narrow-sense ones may be used in conventional breeding.

All but one heritability were associated with standard errors less than half the magnitude of the respective estimates, i.e. they are reliable.

**Combining ability.** Estimates of general combining ability (*gca*) effects are presented in Table 9. The following *P. strobus* female parents exhibited positive general combining ability effects, such as: parent 11 for diameter at breast height (*gca* = 3.37\*) and for tree volume (*gca* = 0.148\*), parent 3 for tree height (*gca* = 0.97\*), parent 10 for stem straightness (*gca* = 0.281\*), and parent 2 for tree survival (*gca* = 8.800\*). Therefore, within the eastern white pine species one can select parents whose offspring will exhibit additive genetic effects for survival and growth traits.

Two blue pine parents exhibited significant

**Table 8** Estimates of phenotypic variances ( $\sigma^2_{Ph}$ ), phenotypic standard deviations ( $\sigma_{Ph}$ ), heritabilities ( $h^2_G$ ,  $h^2_A$ ,  $h^2_i$ ) and the heritability standard errors (*SE*)

Row	Parameters	Traits / Estimates				
		D.32	H.32	V.32	SS.32	SV.32
0	1	2	3	4	5	6
1	$\sigma^2_{Ph.1}$	12.191	0.926	0.024	0.100	126.070
2	$\sigma^2_{Ph.2}$	23.860	2.147	0.070	0.584	-
3	$\sigma_{Ph.1}$	3.491	0.962	0.156	0.317	11.228
4	$\sigma_{Ph.2}$	4.885	1.465	0.265	0.764	-
5	$h^2_G \pm SE$	0.730 $\pm$ 0.189	0.586 $\pm$ 0.118	0.604 $\pm$ 0.185	0.705 $\pm$ 0.162	0.734 $\pm$ 0.266
6	$h^2_A \pm SE$	0.657 $\pm$ 0.223	0.586 $\pm$ 0.178	0.505 $\pm$ 0.190	0.705 $\pm$ 0.213	0.734 $\pm$ 0.266
7	$h^2_i \pm SE$	0.336 $\pm$ 0.157	0.253 $\pm$ 0.564	0.205 $\pm$ 0.038	0.121 $\pm$ 0.055	-

Note.  $\sigma^2_{Ph.1}$  and  $\sigma^2_{Ph.2}$  - phenotypic variance which refers to family means and phenotypic variance at individual tree level, respectively;  $\sigma_{Ph.1}$  - the phenotypic standard deviation at family level and  $\sigma_{Ph.2}$  - phenotypic standard deviation at individual tree level;  $h^2_G$  and  $h^2_A$  - broad- and narrow-sense heritability, respectively, at family level;  $h^2_i$  - narrow-sense heritability at individual tree level.

general combining ability (*gca*) effects, as follows: parent 26 for diameter and stem straightness and parent 27 for stem straightness. Of course, genes for tree survival or blister-rust resistance are present within the blue pine species, but probably because of the low number of parents involved in this experiment, significant *gca* effects for this trait were not found.

Genetic gain. According to the selection intensity, substantial genetic gain in hybrid population, at both family and individual level can be made (Table 10). Thus, assuming selec-

tion of 5, 10, or 15 families out of 28 tested and sexually propagated, a genetic gain ( $\Delta G_2$ ) of 9.5%, 6.8% and 4.8% in diameter at breast height and 11.2%, 8.1% and 5.7% in volume growth rate and 16.4%, 11.8% and 8.4% in tree survival, respectively can be made (Table 10, col. 4, 5, 6, rows 1, 3, 5). Similarly, using the same selection intensity and applying vegetative propagation, the obtained genetic gain ( $\Delta G_1$ ) may be 10.5%, 7.6% and 5.4% in diameter and 13.4%, 9.7% and 6.9% in volume (Table 10, col. 1, 2, 3, rows 1, 3). Selecting the

**Table 9** Estimates of general combining ability (*gca*) effects for the 11 parents

Row	Parent	Traits / Estimates				
		D.32	H.32	V.32	SS.32	SV.32
0	1	2	3	4	5	6
Female parents						
1	2	1.94	0.71	0.103	0.094	8.800*
2	3	-1.99	0.97*	-0.065	-0.086	2.561
3	5	-1.00	-0.19	-0.020	-0.072	3.517
4	9	-0.81	-0.01	-0.053	-0.253*	-19.807*
5	10	1.39	-0.30	0.062	0.281*	6.722
6	11	3.37*	0.14	0.148*	-0.008	-2.152
7	12	-2.88*	-1.31*	-0.175*	0.106	0.358
Male parents						
8	21	1.59	0.18	0.043	0.094	0.863
9	26	2.04*	0.30	0.063	0.265*	2.901
10	27	-2.16*	-0.31	-0.016	0.216*	-3.080
11	28	-1.46	-0.17	-0.089	0.143	-0.684

Note. \**p* < 0.05.

**Table 10** Expected genetic gain ( $\Delta G$  %) if selecting the best families ( $\Delta G_1$ ,  $\Delta G_2$ ), the best individuals within family ( $\Delta G_3$ ) and the best *gca* parents to be selected and intermated  $\Delta G_4$

Row	Trait	$\Delta G_1 = (i_1 \cdot h_G^2 \cdot \sigma p_1 \cdot 100)/X$			$\Delta G_2 = (i_1 \cdot h_A^2 \cdot \sigma p_1 \cdot 100)/X$			$\Delta G_3 = (i_2 \cdot h_i^2 \cdot \sigma p_2 \cdot 100)/X$			$\Delta G_4 = (2i_1 \cdot h_A^2 \cdot \sigma p_1 \cdot 100)/X$		
		Family selection			Family selection			Individual hybrid selection			Individual parent selection		
		5/28	10/28	15/28	5/28	10/28	15/28	5%	10%	15%	20./100	30./100	40./100
		1	2	3	4	5	6	7	8	9	10	11	12
1	D.32	10.5	7.6	5.4	9.5	6.8	4.8	9.7	8.3	7.4	18.7	15.5	12.9
2	H.32	3.8	2.8	2.0	3.8	2.8	2.0	3.6	3.1	2.8	7.6	6.3	5.2
3	V.32	13.4	9.7	6.9	11.2	8.1	5.7	10.2	8.8	7.8	22.0	18.3	15.2
4	SS.32	6.9	5.0	3.6	6.9	5.0	3.6	4.1	3.5	3.1	13.6	11.3	9.4
5	SV.32	16.4	11.8	8.4	16.4	11.8	8.4	-	-	-	-	-	-

Note. *X* - the general mean; see the other symbols at the bottom of the Table 8.

most outstanding 5%, 10%, or 15% individual  $F_1$  hybrids, a genetic gain ( $\Delta G_3$ ) of 9.7%, 8.3% and 7.4% in diameter at breast height and 10.2%, 8.8% and 7.8% in volume growth rate could be achieved (Table 10, col. 7, 8, 9, rows 1, 3). Assuming that the 20, 30 or 40 best *gca* tested parent trees were selected in the original population and crossed, a genetic gain ( $\Delta G_4$ ) of 18.7%, and 15.5%, and 12.9% in diameter at breast height and 22.0%, and 18.3%, and 15.2% in volume growth rate, respectively, could be made (Table 10, col. 10, 11, 12, rows



**Figure 3** Seed conelets with their long pedicels is specific to the blue pine parent which is the evidence that the hybridization was successful and that backcrossing is possible. In addition, backcrossing and other breeding works in  $F_2$  generation may be started.



**Figure 4** Since about age 15 the Vāliug  $F_1$  hybrid trial has been producing  $F_2$  hybrid offspring, suggesting that sexual and vegetative propagation can be applied.

1, 3).

In summary, such high genetic gains or even smaller ones would bring appreciable improvement in wood yielding and tree survival, especially if the plantation programs are applied on large areas. The previously mentioned genetic gains encourage production of *P. strobus*  $\times$  *P. wallichiana* hybrids to be employed in operational forest regeneration programs.

**Hybrid sexual maturation.** In this Vāliug Experiment 1, the  $F_1$  hybrids have started their flowering after 14-15 years from planting (Figure 3) and have reached their full sexual maturity and seed production after about 17-18 years; good seed crops have occurred every 3-5 years. The high quantity of currently producing  $F_2$  seed and hybrid offspring (Figure 4) suggests that such biological material can be widely used for forest regeneration, including through somatic embryogenesis.

## Discussion

Based on measurement data obtained from this Vāliug experiment, three progress reports were already published, such as: the first at age 9 (Blada 1992a), the second at age 17 (Blada 2004), the third at age 19 (Blada & Popescu 2008) and the fourth follows at age 32 (this paper). A short review of the above mentioned papers revealed that some major results were generally consistent at different ages, as follows: (i) the heterosis was negative for volume growth rate but positive and high for tree survival; (ii) the differences among eastern white pine female parents were significant ( $p < 0.05$ ) or highly significant ( $p < 0.01$ ,  $p < 0.001$ ) for volume growth rate and tree survival; (iii) the family narrow-sense heritabilities were 0.802, 0.777, 0.778, and 0.505 for volume at ages 9, 17, 19, and 32 respectively, and 0.603, 0.708, 0.861 and 0.734 respectively, for tree survival or

blister-rust resistance; (iv) the individual-tree narrow-sense heritability estimates for volume growth rate were 0.263 at age 17 and 0.205 at age 32. The above mentioned results encourage the breeding blister rust resistance, via *P. strobus* x *P. wallichiana* hybrids.

Perhaps, the most important outcome of this study was that the analysis of family means from 28 outcrosses showed highly significant general combining effects in eastern white pine female parent species for tree survival. It should be pointed out that in this experiment, the tree survival means blister-rust resistance which exists within the previously mentioned species. Also, blister rust resistance in eastern white pine at both family and individual level was previously reported (Blada 1992b).

It is interesting to compare the  $F_1$  hybrid and eastern white pine female parent performances achieved in the Văliug Experiment 1 (this paper) and Coșteiu Experiment 2 (Blada & Tănăsie 2013). The hybrid and parent families planted in these two trials have, more or less, the same parents and all of them were artificially inoculated then out planted and then measured at age 32. Table 11 shows that the Văliug and Coșteiu hybrids yields were 559.9 and 574.0 m<sup>3</sup> per hectare, respectively while the eastern white pine yields were 602 and 689 m<sup>3</sup> per hectare, respectively. Differences in yield could be attributable to the local environment. Similarly, the hybrid survival in Văliug and Coșteiu hybrid trials were 79.8 and 74.8%, respectively while the eastern white pine survival in the same trials were much lower, i.e. 9.1 and 8.3%, respectively. As the artificial

inoculations were made in similar conditions, the difference in survival seems to be normal. The blue pine male families, from Văliug were killed by winter frost, therefore, this parent species can not be discussed here.

Another comparison may be made between the hybrid yields and yields of two species which are naturally growing in the vicinity of the Văliug Experiment 1, i.e. Scotch pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.). According to Giurgiu et al. (2004), at 32 years of age the Scotch pine and the European beech yield 200 and 227 m<sup>3</sup> per hectare, respectively; therefore, the hybrid population mean yield surpassed the Scotch pine and the European beech yields by 179.9 and 146.6%, respectively. It should be stressed that in the 1970's, the Romanian forestry authorities have recommended to our Institute to develop hybrid programs, like this one. However, at present such high wood productivity including blister-rust resistance of hybrids is not taken into account. Perhaps, a future possible wood crisis will appreciate both hybrid and non-hybrid forest genotypes capable of a higher and a faster wood production.

## Conclusions

The existence of significant or highly significant differences between the tested hybrid families in growth traits, stem straightness and tree survival indicated that the selection at family level is possible.

The presence of significant or highly significant genetic effects for growth traits and tree survival in eastern white pine, suggests that: (i) the *P. strobus* is a very susceptible species to blister-rust, however, genes of resistance can be found within the species tree genotypes, (ii) there was detected the presence of an additive genetic control on all growth traits and survival, i.e. blister rust resistance, (iii) within the *P. strobus* species, parents with significant general combining ability effects could be selected

**Table 11** Performances in yield and survival in the Văliug Experiment 1 and Coșteiu Experiment 2 trials

Trial	Hybrids			<i>P. strobus</i>		
	Vol./tree (m <sup>3</sup> )	Vol./ha (m <sup>3</sup> )	SV (%)	Vol./tree (m <sup>3</sup> )	Vol./ha (m <sup>3</sup> )	SV (%)
1	2	3	4	5	6	7
Văliug	0.993	559.9	79.8	1.069	602	9.1
Coșteiu	1.032	574.0	74.8	1.199	689	8.3

Note. TS –tree survival.



for advance generation breeding.

Since the contribution of the general combining ability variance to the total phenotypic variance was the most important for all tested traits including tree survival, i.e. blister rust resistance, the respective variance should be employed in breeding programs.

The relatively high enough of both family and individual tree heritabilities will contribute to significant genetic gains achievement in growth traits and tree survival or blister-rust resistance.

The achieved such high genetic gains or even smaller ones would bring appreciable improvement in wood yielding and tree survival, mainly when the hybrids are planted on large areas.

The incorporated genes for blister-rust resistance into the  $F_1$  hybrid genotype, has given rise to a very significant positive difference (70.7%) between the hybrid mean and eastern white pine populations for tree survival; this is the major reason for promoting *P. strobus* x *P. wallichiana*  $F_1$  hybrids in forest regeneration programs to be implemented on high-blister-rust hazard areas.

At age 32 and at 3 x 3 m spacing, the  $F_1$  hybrid and *P. strobus* open pollinated parent species averaged 0.993 and 1.069 m<sup>3</sup> volume per tree, respectively. By extrapolation the yielding result from the hybrid trial area to 1 ha area, will result a wood yielding of 559.9 m<sup>3</sup> per hectare for *P. strobus* x *P. wallichiana*  $F_1$  hybrids and 602.5 m<sup>3</sup> per hectare for open pollinated *P. strobus* female parent species. Therefore, the  $F_1$  hybrid yielding is slightly inferior, but closed to that of the eastern white pine.

At similar 32 years of age and similar environment, as the hybrids in question, the natural Scotch pine and the European beech have yielded only 200 and 227 m<sup>3</sup> per hectare, respectively; therefore, the hybrid population yielding has surpassed the Scotch pine and the European beech ones by 179.9 and 146.6%, respectively.

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