

## RESULTS OF BLACK ALDER (*ALNUS GLUTINOSA* (L.) GAERTN.) IMPROVEMENT IN LATVIA

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**Abstract.** Black alder wood is suitable for production of high quality veneer and other uses, but due to its small share in total forest area (3%) in Latvia its genetic improvement have not been a priority. Set of progeny tests, together 22.8 ha, have been established only in the last decade. First of them have reached the age of 8 to 10 years and are suitable for first assessment. Aim of our study was to evaluate potential of improvement of black alder using selection, based on progeny testing. Data from 4 open pollinated progeny tests of phenotypically selected black alder plus trees, located in central part of Latvia, each containing from 15 to 21 families, are evaluated, using breeding value as a criterion. Results reveal that selection of parent trees with the practically possible intensity (10%) yields notable improvement in height at the age of 8 to 10 years: from 10 to 32%. Parent trees of open-pollinated families S9, S14, S16 and 84115 that have superior productivity and above-average quality can be recommended for establishment of second-round seed orchard. Estimates of genetic parameter suggest that selection in black alder trials could be carried out with high accuracy and improvements are possible both in productivity and quality (branch thickness, stem straightness, occurrence of spike knots).

**Key words:** tree breeding, selection differential.

### Introduction

Black alder natural distribution areal includes all European territory as well as parts of North Africa and West Siberia. It has been introduced and spread also in North America (Funk, 1992). Black alder distribution is limited by temperature (at least 6 frost-free months) in north and precipitation (at least 500 mm y<sup>-1</sup>; optimal 800-860 mm y<sup>-1</sup>) in south (Kajba and Gračan, 2003). Highest density of stands of this species are located in North-eastern part of Germany, North part of Poland, Baltic states, Byelorussia and Ukraine (Priedītis, 1997). In Latvia black alder is a minor species, that occupies 3% of the area (roughly 78 thousand ha) with the total yield 16.4 mil. m<sup>3</sup> (SFS, 2008). The figure is larger than total black alder stock in Sweden: 9.2 mil. m<sup>3</sup> (Johansson, 1999) and Finland 1.3 mil. m<sup>3</sup> (Kärki, 1999).

Black alder wood is relative easy to process and due to its colour is often used in carpentry products. Average wood density is similar to that of Scots pine: for a pair of samples 0.479 g cm<sup>-3</sup> and 0.464 g cm<sup>-3</sup> respectively, for other: 0.503 and 0.520 g cm<sup>-3</sup> respectively (Malkoçoğlu and Özdemir, 2006). Wood density of this species is slightly increasing by age: at the age of 4 years it is 0.45 - 0.48 g cm<sup>-3</sup>, at the age of 60 years: 0.49 g cm<sup>-3</sup> (Chow et al., 1999). In other study increase of wood density from 0.421 (at the age of 5 years) to 0.436 g cm<sup>-3</sup> (at the age of 11 years) was noted (Klevinska and Bikova, 1999). Described values of wood density and colour ensure the possibility to use black alder wood in veneer industry (Aydin and Colakoglu, 2005). It has been found, that wood properties (module of elasticity, density) of *Alnus* is not tightly correlated with radial increment (width of annual rings) suggesting high possibilities of breeding to simultaneously improve growth and wood quality (Aydin and Colakoglu, 2005; Lei et al., 1997). Wood properties, specifically – ash content – indicate that

black alder can be efficiently used as energy-wood (Chow et al., 1999; DeWald and Steiner, 1986, Johansson, 1999).

Due to fast juvenile growth alders can be used as pioneer species, forming shelter to introduce oak or spruce in frost prone sites; due to nitrogen-fixing properties alders can be used in re-cultivation of burned forest or former mining areas (Mangalis, 2004).

Due to rather small share of total forest area, occupied by alder, only few studies of genetic properties and improvement of this species have been carried out. Analysis of 48 provenances from different European regions in common garden experiment revealed, that bud-burst happened simultaneously (largest differences between 2 provenances does not exceed 2 weeks, in majority of cases it happened within 4 days), however time of bud set was strongly correlated with latitude of origin. Length of used vegetation period (time from bud burst to bud-set) for different provenances was from 96 to 155 days and was weakly correlated with total length of annual height increment. It indicates, that differences in growth intensity was the major factor determining differences in height growth (DeWald and Steiner, 1986), therefore selection of faster growing individuals would most likely not lead to increase of frequency of frost damages. Provenance experiments of other *Alnus* species reveal similar trends: length of used vegetation period correlates with latitude of origin and shorter length of it is related to higher intensity of photosynthesis (Benowicz et al., 2000).

Notable differences in frequencies of alleles were found between several populations of black alder, applying molecular markers. However, most of the genetic diversity was distributed within population and only small portion – among populations (Gömöry and Paule, 2002), suggesting possibilities for efficient selection of individual trees with desirable traits.

This conclusion have been supported by results from analysis of 17 black alder populations, consisting from separate open-pollinated families (family – group of progenies of the same mother tree), located in trials in 3 Lithuanian regions (Pliūra and Kundrotas, 2002). It was found, that family and family × trial site interaction have significant influence on tree height and time of development of frost hardiness, but population influence was not significant. Tree diameter was significantly influenced both by family and population. High phenotypic plasticity – genetically determined ability to adapt to different environmental conditions – was found for black alder.

Black alder breeding (improvement) was initiated in Latvia in middle of previous century, partly related to interest on superior growth and wood properties of hybrids between 2 alder species: gray and black alder (Kundziņš and Pīrāgs, 1963). Trees with high productivity and desirable branch and stem quality (phenotype), so called plus-trees, were selected within productive and qualitative stands. In total 70 trees were grafted into seed orchard that supplied (and still supplies) seeds for nurseries. Next period of activities was during last two decades: new set of plus trees were selected and 2 seed orchards established: in 1999 (3.2 ha 53 clones) and in 2003 (4.1 ha 70 clones). In the period between years 2000 and 2008 in total 17 progeny tests, including open pollinated families of more than 200 trees were established with total area 22.8 ha. Oldest of them now have reached the time of first evaluation.

Aim of the study is to evaluate potential of improvement of black alder using selection, based of progeny tests of individual trees.

### Materials and Methods

Study material consists of 4 trials, referred by the respective numbers in ‘Register of long term scientific experiments’ (Baumanis et al., 2006). Trials are established in former arable and fertile, drained forest land (Table 1).

Seed material for experiments No. 71, 72 and 590 has been collected from phenotypically selected plus trees in year 1997 in Jaundziras (Tukuma county, Irlava

forest district according to administrative division at seed collection time) and S – Sunākste (Aizkraukles county, Seces forest district).

Height has been measured for every tree in year 2006. Quality was visually assessed in 5 grade scale (experiments No. 72 and 590) or 3 grade scale (experiments No. 71 and 270) in comparison to trees with similar height at the same trial, where 1 – straight stem (sg), thin branches (bg), high overall quality (g), jointly considering previously mentioned traits and spike knots, branch angle, vitality; 5 – stem with more than 2 bends (larger than 5 cm), very thick branches, poor overall quality. Spike knot, double stems and lost tops have been noted in binary scale (yes/no).

Some of the trees have been damaged during early years of growth and regenerating by stump sprouts, forming bush-like structure with 3 and more stems. Notable differences among families in proportions of such trees have not been found, indicating environmental cause of this phenomenon. Therefore such trees have been excluded from further analysis.

Selection differential was calculated as deviation of family mean value from mean of experiment in respective traits. Since selections are made for establishment of seed orchards, in those selected clones contribute to genetic value of seedlings both as mother and father trees, breeding values were calculated as selection differential  $2^{-1}$ .

Probability, that progenies of certain parent tree will have spike knots were estimated based on methodology derived from Roff (2001).

Variance components have been calculated with SAS Proc mixed, using general linear model:

$$y_{irs} = \mu + F_i + B_r + FB(S)_{ir} + FS_{is} + E_{irsk} \quad (1)$$

where:  $y_{ir}$  – trait  $y$  measured;  $\mu$  – overall mean;  $F_i$  – the effect of family;  $B_r$  – the effect of block;  $FB(S)_{ir}$  – interaction among block (within site) and family (plot effect);  $FS_{is}$  – interaction between family and site;  $E_{irsk}$  – the error term. For calculations of single-site estimates site factor is not included.

Open pollinated families were assumed to consist mostly of half-sibs, consequently  $4\sigma_F^2$  were considered to be an estimate of the additive genetic variance ( $\sigma_A^2$ ).

Table 1

Description of black alder progeny trials used in the study

No.	Trial		Age	Land type	Spacing	Number of families	Number of replications	Survival, %
	Locality (county)							
72	Rembate		10	arable	2×2	21	34*	77
590	Taurene		10	arable	2×2.5	19	28	68
270	Cenu		8	forest	2×2.5	15	40*	86
71	Ukri		10	arable	2×2.5	16	35	86

\*average number of replications per family, particular family can be represented in 4 replications more or less than average

Narrow sense heritability ( $h^2_i$ ) and its standard error as well as half-sib family mean heritability ( $h^2_{fam}$ ) and its standard error were estimated as described by Falconer and Mackay (1996).

Coefficients of additive genetic variation ( $cv_a$ ) in percentage were estimated as:

$$cv_a = \frac{100 \cdot \sqrt{4 \cdot \sigma_F^2}}{\mu}, \quad (2)$$

where  $\mu$  – overall mean;  $\sigma_F^2$  – family variance.

Coefficients of phenotypic variation among family means ( $cv_{pfam}$ ) in percentage were estimated as:

$$cv_{pfam} = \frac{100 \cdot \sqrt{\sigma_F^2 + \frac{\sigma_{FB(S)}^2}{bs} + \frac{\sigma_{FS}^2}{s} + \frac{\sigma_E^2}{bns}}}{\mu}, \quad (3)$$

where:  $\sigma_F^2$  – family variance;  $\sigma_{FB(S)}^2$  – family-block (within site) interaction variance;  $\sigma_{FS}^2$  – family-site interaction variance;  $\sigma_E^2$  – error variance.

### Results and Discussion

Breeding values of families in experiments No. 72 and 590 are calculated based on data from trees that are not suppressed: 24 and 25 highest trees per family respectively are used. Notable differences in number of suppressed trees per family have not been found, indicating, that this selection will not bias the results of analysis.

Highest breeding values for trait, characterising productivity of trees – height – in experiment No. 72 have been found for families S15, S5, S8, in experiment 590 – for families J4, J7, S14. Selection of 2 best families that corresponds to intensity 10% (2 out of 21 or 2 out of 19) would guarantee 14% or 10% (respectively) superiority in tree height in comparison to trial average. Part of the difference among the trials could be explained by fact, that mean height of trees in experiment No. 590 exceeds that in experiment No. 72 by 2 m environmental factors

Trial average represents value of progenies of already phenotypically selected plus trees, therefore, if comparison would be made with un-selected material (corresponding to average result in natural regeneration), value of improvement would have been higher. This logical relationship has been proven to be true in assessments of trials of other species (Ståhl and Jansson, 2002). None of the analysed black alder trials contains control-lots of unimproved material, therefore exact calculation of increase in quality or productivity is not possible.

Selection intensity for establishment of second-round

(with progeny-tested clones) seed orchard depends on:

1) number of clones needed in seed orchard to ensure genetic diversity: for other wind-pollinated species it is estimated to be 16-25 (Lindgren and Prescher, 2005);

2) number of trees with progeny tests available.

Black alder progeny tests contains slightly more than 200 families, therefore selection intensity 10%, choosing 20 clones for establishment of seed orchard, can be used in praxis and is applied as a criteria for analysis of trials No. 71, 72, 270 and 590.

Establishment of plantation is a costly operation in comparison to natural regeneration. It is economically justified only with higher value of assortments from planted stand in future. Therefore in tree breeding process not only productivity, but also quality needs to be considered.

Notable differences in breeding value for branch quality (bg) have been found in experiment No. 72: it varies from -0.8 to +0.6, average quality grade being 2.6. Variation in breeding values for this trait is lower in experiment No. 590 (from -0.3 to +0.3). Slightly lower variation have been detected for breeding values for stem straightness (from -0.4 to +0.6), slightly higher – for joint quality grade (from -0.7 to +0.7). Variation according to all quality traits is larger in experiment No. 72 indicating possibilities to better detect and exclude inferior families. According to the result in these trial families with highest trees have at least average quality, and only two of them (S15 and S13) needs to be excluded due to poor quality. Especially low quality in both experiments has been found for family J5.

Spine knot or double leaders have been found in 40% of all trees in experiment No. 72 and in 74% in experiment No. 590. Highest possibilities to detect genetically determined differences in binary (yes/no; 1/0) coded traits are in the case, if frequency of trees with the trait (in this case – spike knot) are close to 50%. It is confirmed by the analysis: in experiment No. 72 families S3 and S5 have highest probabilities, that trees will be with spike knots (0.8 and 0.7 respectively); in experiment No. 590 none of the probability estimates exceeds 0.6. Spike knot lowers the value of assortment due to its larger cross-cut area and longer life span than branches and sometimes also causes stem defects. Besides it can indicate lower adaptability to some environmental factors. Therefore trees with high probability that there progenies will have spike knots, cannot be recommended for seed orchards.

Joint analysis of 18 families, represented in both trials (No. 72 and 590) has been carried out, based on data from dominant (10 highest per family in each experiment) trees (Table 2).

Table 2  
**Breeding values in open pollinated progeny tests  
of black alder (No.72, 590) at the age of 10 years**

Family	Trait				
	h	sg	bg	g	sp
J 2	-7.2	0.1	-0.4	0.3	0.5
J 3	3.5	-0.4	0.0	-0.2	0.5
J 4	2.7	-0.2	-0.4	-0.1	0.5
J 5	-1.8	1.4	0.1	0.7	0.5
J 6	-2.9	-0.5	-0.5	-0.1	0.5
J 7	-6.9	0.0	-0.1	0.1	0.4
S 3	-2.9	0.3	0.0	0.2	0.6
S 5	-2.3	0.2	0.8	0.6	0.6
S 7	0.8	-0.2	0.0	-0.4	0.5
S 8	0.6	0.0	-0.6	-0.4	0.4
S 9	4.3	-0.4	0.2	-0.2	0.5
S 10	-1.6	-0.3	-0.2	-0.2	0.5
S 13	1.6	-0.2	0.4	0.2	0.5
S 14	3.2	-0.2	0.0	-0.3	0.5
S 15	4.6	0.5	0.3	0.2	0.5
S 16	4.2	-0.3	0.1	-0.4	0.5
S 17	-3.5	-0.2	0.4	0.1	0.5
S 33	3.8	0.5	0.0	0.0	0.5
Average:	66.8	1.9	3.0	3.0	0.5

Note: h – height, dm; sg – stem straightness, grades; bg – branch thickness, grades; g – joint quality estimate, grades; sp – probability to have trees with spike knots; average – average values of traits in both experiments jointly.

Joint analysis of data from both experiments confirmed the results listed earlier. Families J3, S9, S14, S16 have high productivity and slightly increased quality and their parent-trees can be recommended for establishment of second-round seed orchard. Families J2, J5, J7, S17 have both low productivity and quality and could not be recommended for further breeding or propagation work.

Experiment No. 71 includes the same set of families as No. 72, but in this trial notably more trees have stem damages: spike knot or sprout from stump was found for 46% of trees, 2 stems or lost leader – for 43% of trees. Observed damages might partly be related to errors in early tending work, when some of the planted trees were cut. It can also be a consequence of substantial frost and/or browsing damages at the first years after establishment of experiment, reflecting genetic differences in some adaptive traits. Later statement is supported by estimates of probability, that tree will have a double stem or spike knot: for particular families it varies from 0.3 to 0.6.

Mean height of trees in the experiment is 4.5 m that is almost by 1m lower than in experiment No. 72. Breeding value of this trait for the best family is 9% compare to trial average. Notable differences

have been found in branch quality ( $\pm 16\%$ ), stem straightness ( $-12 - +27\%$ ) and joint quality estimate ( $-9 - +20\%$ ). According to all analysed traits highest quality has been found for families S7 and S9, lowest for families J5 and J7.

Based on data from experiments No. 71, 72 and 590, parent-trees of families S9, S14 and S16 can be recommended for establishment of seed orchard.

Seed production in total in 27 trees from 13 families has been assessed. It indicates, that controlled crossing for recombination of genes from selected trees to obtain seed material for establishment of next breeding cycle is possible at rather early age – just 10 years.

Experiment No. 270 includes different set of families than analysed previously and is located on drained, fertile forest land. Very low number of trees with spike knots (3%) indicates no adaptation problems. However, number of trees with double stems is relative high for such conditions (20%), pointing towards possible mechanical damages during the early cleaning work.

Height of dominant trees (20 highest per family), used for detailed analysis, reaches 4.5 m on average. Selection differential for best family is as high as 32% from the mean in the experiment. If all trees are included in analysis, selection differential reaches 23%. Survival (94%), stem and branch quality for this family (84115) exceeds the average in experiment, indicating a notable potential in use of it in forest regeneration. Differences in quality traits among the families are similar to those, reported in experiment No. 71: for stem quality  $\pm 12\%$ , for branch quality  $\pm 15\%$ .

Heritability of tree height, based on the data from all trees, is rather low in experiments No. 270:  $h^2_i=0.13$ . It is higher in experiments No. 72 and 590, where selection of 25 highest trees per family has been used:  $h^2_i=0.39$ . However, both estimates are within the range of values, published for other wind-pollinated species (Haapanen et al., 1997; Stener and Jansson, 2005; Jansons et al., 2008) are more than twice higher than respective standard errors and indicate, that accurate selection can be made in particular black alder experiments. Heritability of quality traits is slightly lower than for height in experiments No. 72 and 590: on average  $h^2_i=0.21$ .

Tree height, representing growth traits have higher narrow-sense heritability ( $h^2_i$ ) estimated across sites than branch traits (Table 3). This trend have been found also in earlier studies both if in single experiments and joint analysis of several experiments (Velling and Tigerstedt, 1984; Merrill and Mohn, 1985; Haapanen et al., 1997). As noticed in several studies, single site heritability values can be up to twice as large as across site estimates, indicating possibilities of serious bias in genetic gain estimates based on results from one test site. High narrow-sense heritability for across-site analysis, especially for tree height, in our study can be related to selection of just 10 highest individuals per family for this analysis.



Table 3  
**Values of genetic parameters in black alder open-pollinated progeny tests (No.72 and No.590) at the age of 10 years**

Trait	Genetic parameter					
	$h^2_i$	$se_i$	$h^2_f$	$se_f$	$cv_a$	$cv_{pfam}$
h	0.63	0.28	0.79	0.35	7.4	4.2
sb	0.48	0.23	0.73	0.35	26.1	15.2
zb	0.24	0.15	0.56	0.35	15.1	10.1
b	0.25	0.16	0.57	0.36	16.6	11.1

Note:  $h^2_i$  and  $se_i$  – narrow-sense heritability and its standard error;  $h^2_f$  un  $se_f$  – family heritability and its standard error;  $cv_a$  – coefficient of additive genetic variation;  $cv_{pfam}$  – coefficient of family mean phenotypic variation; other abbreviations as in Table 2.

Estimates of coefficients of variation indicate a considerable potential of improvement, especially for quality traits, both using selection of best parent-trees (among-families) and selection of best individuals within family.

Experiments, included in our analysis, have reached the age and height, recommended for selection in broadleaved tree species in southern part of Sweden

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(Stener and Jansson, 2005). Therefore the results of our study can be considered as first selections for second-round seed orchard of black alder in Latvia. Since seeds from such orchard could be used in all territory of Latvia (only one seed zone for black alder is delineated), results must be based on careful testing in different regions and selections, based on results of at least 3 experiments (like No. 71, 72, 590) considered.

## Conclusions

1. Selection of parent trees with the practically possible intensity (10%) yields notable improvement in height at the age of 8 to 10 years: from 10 to 32%.
2. Parent trees of open-pollinated families S9, S14, S16 and 84115 that have superior productivity and above-average quality can be recommended for establishment of second-round seed orchard.
3. Estimates of genetic parameter suggests, that selection in black alder trials could be carried out with high accuracy and improvements are possible both in productivity and quality (branch thickness, stem straightness, occurrence of spike knots).

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