5. Acknowledgements

We thank the owners and managers of the trial sites, Mr. JOHN DAVIS of Woodland Improvement and Conservation Ltd, Mr. GABRIEL HEMERY of The Northmoor Trust, Mr. W. ACKWORTH of Little Hidden Farm, and Mr. JIM MORTLOCK of Mulgrave Estates. They have financed the establishment and carried out all the maintenance of the trials. Professor PETER KANOWSKI (now of the Australian National University) was largely responsible for their conception. Many others, including the Forest Rescarch Agency's geneticist, Dr. NED CUNDALL and various students have been involved with assessing them in a variety of ways.

6. References

BARNES, R. D.: The breeding seedling orchard in the multiple population breeding strategy. Silvae Genetica **44**(2–3), 81–88 (1995). — BECKER, W. A.: Manual of quantitative genetics. 4th Edition. Academic Enterprises, Pullman, Washington (1984). — COTTERILL, P. P. and DEAN, C. A.: Successful free breeding with index selection. CSIRO, Melbourne (1990). — EVANS, J.: Silviculture of broadleaved woodland. Forestry Commission Bulletin **62**, HMSO, London (1984). — KANOWSKI, P. J.: Forest genetics and tree breeding. Plant Breeding Abstracts **63**(6), 717–726 (1993). — KERR, G.: Silviculture of ash in southern England. Forestry **68**(1), 63–70 (1995). — KLEINSCHMIT, J., SVOLBA, J., ENESCU, V., FRANKE, A., RAU, H. M. and RUETZ, W.: First results of provenance trials of *Fraxinus excelsior* established in 1982. Forstarchiv **67**(3), 114–122 (1996). — LOCKE, G. M. L.: Census of woodlands and trees 1979-1982. Forestry Commission Bulletin **63**, HMSO, London (1987). — MARINOV, I.: Heritability of stem form in progeny of *Fraxinus excelsior.* Gorskostopanska Nauka **16**(2), 10–16 (1979). — MARINOV, I.: Heritability of diameter growth in seedling progeny of *Fraxinus excelsior.* Nauka za Gorata **27**(1), 9–15 (1990). — MINITAB: Release 12. Minitab Inc., 3081 Enterprise Drive, State College, PA, USA (1998). — NAMKOONG, G., BARNES, R. D. and BURLEY, J.: A philosophy of breeding strategy for tropical forest trees. Tropical Forestry Paper, No. 16. Commonwealth Forestry Institute, Oxford (1980). — POTTER, M. J.: Tree shelters. Forestry Commission, Handbook 7, HMSO, London (1991). — ROBERTS, J. E.: The breeding seedling orchard: an innovation when applied to the temperate bruadleaf, common ash *(Fraxinus excelsior)*. Unpublished MSc thesis, Oxford Forestry Institute, University of Oxford (1996). — SAVILL, P. S.: The silviculture of trees used in British forestry. CAB International, Wallingford (1991). — SAVILL, P. S.: British Hardwoods Improvement Programme. Quarterly Journal of Forestry **92,** 217–222 (1998). — ZOBEL, B. and TALBERT, J.: Applied forest tree improvement. John Wiley and Sons, Inc., New York (1984).

Evidence for Long-term Heterosis Phenomenon in the *Alnus incana* x *glutinosa* F_1 Hybrids

By L. MEJNARTOWICZ

Institute of Dendrology, Polish Academy of Sciences, 62-035 Kórnik, Poland

(Received 26th August 1998)

Abstract

This report gives the result of investigation carried out with hybrid progenies of the phenotypically negative dwarf *Alnus incana* tree, pollinated with a pollen mixture sampled from 12 plus trees of *Alnus glutinosa*. The experiment covers growth rate, branching type, stem form and morphological character of leaves. Through twenty-eight years of studies the mean height and diameter growth of tested hybrid progenies *A. incana* x *A. glutinosa* were much better than those of *A. glutinosa* paternal population. At age 12 interspecific hybrids showed superior growth-rate over the paternal population on 90%. At age 28 hybrids surpassed the black alder paternal population by 22% in diameter. The trunks were straight with the vital twigs settled at an acute angle. Heterosis was also demonstrated in the bigger leaf size and more abundant production of leaf proteins. Flowering and seed production of hybrids was classified as poor.

Key words: Alnus glutinosa, Alnus incana, hybrids, heterosis, growth.

Introduction

This paper presents results of 28 years of observations of the heterosis (or luxuriance) occurrence in the hybrids between two *Alnus* species: *Alnus incana* (L.) MOENCH. – gray (or speckled) alder and *Alnus glutinosa* (L.) GAERTN. – black alder. In this context heterosis and hybrid vigor are synonymous. The aim was to produce interspecific hybrids to see, if these would maintain hybrid vigor and if the nondesirable characters such as poor shape and slight diameter growth would be transmitted from mother to F_1 hybrid generation. Such unwelcome traits like poor shape and stunted growth are frequently observed on the trees growing far away from their range of the massive distribution.

Both alder species belong to the important European trees of the genus *Alnus* (MILL.) occupying two different ecological niches. In the Poland the gray alder has two regions of the occurrence. The main one in southern Poland where it frequently grows on the slopes of Sudeten and Karpathian Mountains, used in the past for the agriculture purposes, and the second location in the northern Poland all along the Baltic see-shore. Sporadically isolated trees of gray alder appear along the rivers throughout Poland, producing viable seeds, also following selfing. In opposite to many plants selfed progenies of some alder species in CHIBA studies (1966), frequently didnít show apparent decrease of growth. Black alder is most abundant and commercially important alder species in Europe. It occupied moist sites in lowlands of Poland producing very valuable hardwood timber resistant to decay in moisture soil. Both species posses nonleguminous nitrogen-fixing root nodules that seem to be of considerable ecological significance on poor forest and farmland sites. This may contribute to the fertility of the forest soil and prevent erosion of soil suitable for agriculture.

The first controlled interspecific crosses in trees, that have yielded a progeny with heterosis, was probably done on alders by KLOTSCH (1854, after SVOBODA, 1957). Luxuriance however was not always observed at the alder interspecies hybrids

(CHIBA, 1966). Investigation of the interspecific alders crossing done by VACLAV (1969) has indicated the importance of the choice of the mother tree species. Twelve years old hybrids *A. incana* x *glutinosa* were about two times higher than corresponding reciprocal cross of *A. glutinosa* x *incana.* This author has also shown that the higher number of fertile seeds was obtained when a mixture of pollen from several trees of black alder was used for pollination. This phenomenon could be explained by the results of study conducted by LINARES (1985) and STEINER and GREGORIUS (1988). Their germination experiments with black and gray alders have shown the enormous variability of pollen germinating. The last authors have found also that the pollen mixture shows the lowest differentiation values, what explains the fact that different pollen types give in the mixture an intermediate germination behavior.

Material and Methods

In 1966 one isolated tree of gray alder was found in central Poland in Czeszewo on Warta black alder natural seed stand. It is very probable that this gray alder grew up from selfing because Czeszewo black alder stand is located far away, more then 150 km from natural range of gray alder distribution. Isolated trees of this species in this zone were found only very sporadically. This gray alder tree was about 15 years old, very small, six meters in height and seven cm in diameter measured at 1.3 meter above the ground. The stem was heavily carved with widely spreading, low positioned branches. Phenotypically it was a typical negative individual. This gray alder tree was emasculated and served as the mother tree. The female flowers of these trees were covered with isolation bags and then pollinated with the pollen mixture of the black alders of Czeszewo. This black alder population phenotypically belongs to the best natural stands in Poland. Twelve trees from Czeszewo stand were chosen to collect the pollen. Paternal trees at age 70 have had height of 26 m to 30 m and the diameter of 26 cm to 38 cm. The stems were straight and of high technical quality. Detailed description of paternal stand was presented elsewhere (MEJNARTOWICZ, 1972).

Hybrid seeds, received from controlled pollination and seeds from open pollination from paternal Czeszewo population and other 10 black alder seeds stands from Poland, have been sown in the same nursery of Institute of Dendrology with the purpose to establish provenance experiment on black alder. In all 11 seed stands ten to fifteen trees were randomly chosen to collect seeds for our experiment. Two years seedlings (1+1) were planted out onto experimental area in four blocks. The field trial was a randomized block design with 4 replicates and twenty-five trees were planted onto rectangular plots using 1.5 m x 2 m spacing. Each population was represented by 100 trees. Four to six hybrid seedlings were always planted randomly on the plots together with the offspring of paternal population of Czeszewo. The experiment was carried out on former agriculture soil.

The first cleaning was performed after 6 years, so the spacing changed to 2 m x 3 m. The next thinning was done when the trees were 12 and 25 years old to leave 12 trees on each plot. During these maintenance procedures hybrid trees were spared. In the first year and then years 4 to 12 of experiment the height of trees was measured yearly with the precision of +/– 10 cm. In the following years height measurements were discontinued because of inability to maintain the same precision of measurements. The diameter of trees is still measured periodically until this day. For morphological studies we used samples of 50 leaves from each tree both hybrid and paternal types. The design where all the trees were planted with regular spacing and at the same time in a very uniform habitat, yields accurate data for biometrical analysis of heterosis phenomenon. The field experiment details were described earlier (MEJNARTOWICZ, 1980a).

Results

The F_1 hybrids *A. incana* x *glutinosa* have many leaf characters, clearly distinguishing them from the parental species. The hybrid leaves don't have the characteristic luster of the paternal species, and are not as long and narrow as maternal species. Hybrids are intermediate in petiole length and number of vein pairs, and the distance between veins. The leaf shape is almost round, and never retused on the top, frequently blunt or with the small ligule. The hybrid leaf surface is bigger than the one of the parents *(Table 1).* The bark is smooth with the texture and color more similar to that of gray alder *(Figure 1* and *2).* These characters are inherited in a matroclinal manner (MEJNARTOWICZ, 1982).

The height of hybrids and of the adjacent offspring trees of black alder paternal population of Czeszewo was measured. Heterosis manifested very clearly in the height increment of the hybrids. This was much greater than that of paternal population until the age of 12 *(Table 2).* Diameter increment of the hybrid measured at 130 cm above the ground, throughout the 28 years was also much greater then that of paternal *Alnus glutinosa* population and all other black alder progeny stands in this experiment *(Table 3).* Hybrid stems are straight with vital branches. At younger age the branches of hybrids are pruned with a delay comparing with the *Alnus glutinosa,* but at age 30 all trees are self-pruned to the same height level.

Table 1. – Comparison of mean values of leaf characters measured in *A. incana x glutinosa (Ai x g), Alnus glutinosa* paternal trees and the maternal tree of *A. incana.*

Character Taxon	Petiole lenght [cm]	Blade lenght [cm]	Blade width [cm]	No. of vein pairs	Distance between 2-3 vein [cm]	Down	Apex type*	2:4
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Alnus gluti.	1.98	6.25	5.56	6.40	0.82	1	1.25	1.12
Ai x g	2.32	8.96	8.21	8.77	8.77	0	2.90	1.09
Alnus inca.	2.44	8.54	6.41	11.16	11.16	0	3.00	1.34

*) Apices defined as: 1 – retuse, 2 – obtuse, 3 – acuminate.

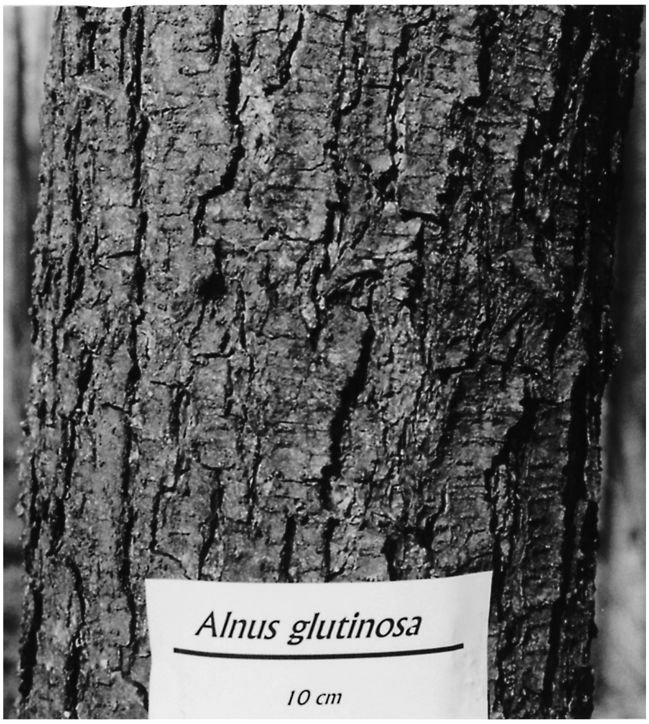

Figure 1. – Bark texture of the black alder tree from paternal population.

Figure 2. – Bark texture of the hybrid tree *A. incana x glutinosa.*

Discussion

Much information about heterosis effect is from the corn breeding or the short term tree breeding programs. There is a lack of information from the long term forest-tree breeding programs. Among the deciduous forests hybrid superiority has been demonstrated at the interspecies level in *Alnus* and *Populus* in Poland (BIAŁOBOK and BARTKOWIAK, 1965, 1966; MEJNARTOWICZ, 1982). The studied hybrids of *A. incana* x *glutinosa* demonstrated remarkably pronounced heterosis phenomenon in the morphological and physiological characters. Even though in the studied case the mother tree was a crooked dwarf nevertheless after pollination with the valuable *A. glutinosa* pollen it produced fast growing, straight, good shape hybrid trees. This effectiveness of hybrids could be connected to the bigger then the parental trees leaf blades, though the shape of hybrid leaves and leaf veins number was intermediate comparing with the parent species (MEJNARTOWICZ, 1982).

Dominance and overdominance is the most common explanation for heterosis phenomenon. The first hypothesis is based on the absence of the inbreeding depression. However NAMKOONG et al. (1988) propose that an inbreeding depression is not likely to be a substantial problem for the selection unless the epistatic effects and linkage are very strong. According to the overdominance hypothesis heterozygotes are able to produce an effect which is impossible for both types of homozygotes (WRIGHT, 1976). There is no direct information weather the vigor is due to the overdominance at a single or multiple loci, or is a phenomenon of complementary additive genes at different loci controlling the traits (NAMKOONG and KANG, 1990). The latest study did by LI and WU (1996) on interspecific hybrids of *Populus tremuloides* and *P. tremula* manifested heterosis of F_1 suggested that hybrid vigor might be due to overdominant interaction between alleles of parental species. In this study heterosis in aspen growth appeared to be under multigenic con-

Table 2. – Height of hybrids *A. incana x glutinosa (Ai x g)* trees and *A. glutinosa (Ag)* of paternal population of equal age growing jointly.

Age	*Alnus incana* x *glutinosa* Mean $X_{[cm^{-1}]}$	X_{min}	X_{max}	SSD	*Alnus glutinosa* Mean $X_{[cm^{-1}]}$	X_{min}	X_{max}	SSD	Ag / *Ai* x g[%]	$t_{0,01} = 2.98$
3	165	26	235	55	112	30	210	45	68	8.75
4	328	160	400	66	249	130	380	65	76	9.37
5	460	370	550	57	379	250	460	52	82	11.53
6	579	480	660	57	490	370	600	73	85	11.56
7	643	550	780	81	563	480	650	62	88	6.70
8	761	610	880	93	656	550	790	81	86	5.59
12	1103	900	1400	142	990	700	1200	123	90	9.42

Table 3. – Heterosis effect manifested in diameter (cm^{-1}) of controlled hybrids *Alnus incana x glutinosa*.

Year:	1973	1974	1975	1977	1978	1979	1980	1983	1986	1988	1990	1995	Mean	t
Tree age:	6	7	8	10	11	12	13	16	19	21	23	28		
Mean														
Ag. **	3.7	4.8	5.9	7.3	8.1	8.9	9.4	11.8	13.1	14.7	15.6	16.5	10.07	
Ag. Cze*	4.1	4.9	5.7	6.8	7.2	7.9	8.5	11.3	12.4	14.0	14.7	15.7	9.43	Ns
Ai x g	5.9	7.4	8.3	10.3	11.1	12.0	12.5	14.5	15.6	17.7	18.1	19.1	12.71	17**
(Ai x g):Ag%	43.9	51.0	45.6	51.5	54.2	51.9	47.1	28.3	25.8	26.4	23.1	21.7		

**) Mean for 10 *Alnus glutinosa* seed stands from whole Poland.
*) Mean for *Alnus glutinosa* of paternal population of Czeszewo.

trol of 9 to 10 loci for stem diameter and 6 to 8 loci for height. However this genetic parameters might be biased by linkage and epistasis among loci.

Since *A. incana* mother tree was found several hundred kilometers beyond the contiguous range of the species it is quite probable that the individual in question is selfed offspring of the similarly isolated individuals. Such putative inbreeding could explain well extremely poor shape and growth of mother tree. Thus the crossing of at least partially inbreed *A. incana* individual with genetically distant *A. glutinosa* has resulted in the high degree of heterozygosity of F_1 generation. Beside typical for parental species proteins hybrids produce as well proteins that are specific for the hybrids only (PRUS-GŁOWACKI and MEJNARTOWICZ, 1992). The volume of all proteins produced by the hybrid leaves were higher than that of parental species. These proteins consisted mostly of ribulose-1,5-biphosphate carboxylase-oxygenase catalyzing CO_2 assimilation in a photosynthesis process. Mentioned above authors assume it could be the reason for the fast hybrids growth in diameter and height. Higher enzyme activity in the hybrids of higher plants has been demonstrated by SRIVASTAVA (1972). This author considered also an intergenomic complementation of chloroplasts and mitochondria as an essential component of heterosis (SRIVASTAVA, 1983). In some hybrids there has been also observed the chloroplast heterosis with higher photosynthetic rates (SINGHA and KHANNA, 1975). Moreover there is another possible explanation of the hybrids vigor. This is a multiple (epistatic) effect or non-allelic interaction of genes where one locus effects the function of other towards heterozygote adaptive superiority (GOWEN, 1952). Currently there are still methodical difficultys in detecting epistasis.

In 28 years of investigation hybrids were always superior in the diameter in comparison to paternal population of *A. glutinosa*. Superiority was from 43.9% in the sixth year of life to 21.7% in the year 28 with the culmination (54.2%) in the year 11. In the last 8 years this superiority is diminishing. That could be connected with the selection done at the time of thinning at the age 12 and 25. At that time only black alder trees with the poorest growth rate were removed. There was no intervention within the hybrid trees. The phenotypically poor shape of mother tree did not effect the character of F_1 hybrid generation. Comparing the shape of stems in the F_1 generation with the mother *A. incana* tree one can say that this character is patroclinal in nature. Some of the heterosis effects manifested in *A. incana* x *glutinosa* hybrids were however not desirable. To those we should include longer vital twigs of the hybrids. This twigs are settled at the more acute angle (inherited in a matroclinal way). The hybrids are producing as well many fewer seeds than the parental trees. This is true specially comparing with the mother tree that was producing abundant seed crop every year.

Literature

BIAŁOBOK, S. and BARTKOWIAK, S.: The ratio of stem diameter to stem height in poplar hybrids as an index for the selection of heterotic individuals. (In Polish). Arbor. Kórnickie **10**: 175–181 (1965). — BIAŁOBOK, S. and BARTKOWIAK, S.: Morphological variability in artificial hybrids of *Populus* x *canescens* SMITH. (In Polish). Arbor. Kórnickie **11**: 10–151 (1966). — GOWEN, J. W.: Heterosis. Iowa State College Press, Ames, Iowa (1952). — LI, B. and WU, R.: Genetic causes of heterosis in juvenile aspen: a quantitative comparison across intra- and inter-specific hybrids. Theor. Appl. Genet. **93**: 380–391 (1996). — LINARES, B.: Male sterility in *Alnus glutinosa* (L.) GAERTN. Silvae Genetica **34**: 69–72 (1985). — MEJNARTOWICZ, L.: Studies on the racial variation of *Alnus glutinosa* (L.) GAERTN. population in Poland. (In Polish). Arbor. Kórnickie **17**: 43–120 (1972). — MEJNARTOWICZ, L.: Studies on the racial variation of black alder in Poland. (In Polish). Arbor. Kórnickie **25**: 167–180 (1980a). — MEJNARTOWICZ, L.: Provenance experiment on black alder [*Alnus glutinosa* (L.) GAERTN.] established in 1968. (In Polish). Arbor. Kórnickie **25**: 161–166 (1980b). — MEJNARTOWICZ, L.: Morphology and growth of *Alnus incana* x *glutinosa* F_1 hybrids. Arbor. Kórnickie **26**: 15–28 (1982). — NAMKOONG, G. and KANG, H. C.: Quantitative genetics of forest trees. In: Plant Breeding Reviews. Vol. 8. J. JANICK (ed.). Timber Press. 139–188 (1990). — NAMKOONG, G., KANG, H. C. and BROUARD, J. S.: Tree breeding: Principles and Strategies. Monographs on Theoretical and Applied Genetics. Vol. **11**. Springer-Verlag, New York (1988). — PRUS-GŁOWACKI, W. and MEJNARTOWICZ, L.: Serological investigations of *Alnus incana* x *glutinosa* hybrids and their parental species. Silvae Genetica **41**, 2: 65–70 (1992). — SINHA, S. K. and KHANNA, R.: Physiological, biochemical, and genetic basis of heterosis. Adv. Agron. **27**: 123–170 (1975). — SRIVASTAVA, H. K.: Mitochondrial complementation and hybrid vigour. Ind. J. Genet. Plant Breed. **32**: 215–228 (1972). — SRIVASTAVA, H. K.: Heterosis and intergenomic complementation. In: Monographs on Theor. App. Gene. Vol. **6**. FRANKEL, R. Heterosis. Reappraisal of theory and practice. Springer-Verlag, Berlin, Heidelberg. p. 260–286 (1983). — STEINER, W. and GREGORIUS, H. R.: *In vitro* pollen germination experiments in *Alnus* and their relevance for mating system analysis. Forest Genetics **5**(1): 47–60 (1988). — SVOBODA, P.: Lesni dreviny a jejich porosty. **3**: 165–218. Praha (1957). — VACLAV, E.: Height increment of birch and alder hybrids. Second World Consultations on Forest Tree breeding. Section II. Breeding for high yielding characters. FAO, Washington 7 to 16 August: 2/11 (1969). — WRIGHT, J. W.: Introduction to forest genetics. Academic Press, New York (1976).