Serological Investigation of Alnus incana x glutinosa Hybrids and their Parental Species

By W. PRUS-GLOWACKI*) and L. MEJNARTOWICZ**)

(Received 28th June 1990)

Abstract

Studies on antigenic properties of proteins from leaves of *Alnus incana, A. glutinosa* and their hybrids, indicate specificity of the protein spectrum of each parental species and intermediate character of proteins of the hybrid forms. The serological techniques identifying homology of individual protein fractions permit to define precisely the parental "input" into the protein spectrum of the hybrids. By quantitative analysis of the antigens the probable basis for heterosis effect is demonstrated.

Key words: Alnus incana, Alnus glutinosa, hybrids, antigenic proteins, heterosis.

Introduction

Alnus glutinosa L. (GAERTN.) and *Alnus incana* L. (MOENCH.) occur in Poland in the form of pure as well as mixed stands. Each of the alder species inhabits a specific ecological habitat, growing mainly in marshes and on humid sites. Apart from a compact range in the submountain region, Alnus incana occurs sporadically all over the Polish lowland, mainly along rivers which provide means of seed transport from the submountain regions (MEJNARTOWICZ, 1972). Alnus glutinosa, on the other hand, is common in the lowlands. Only the latter is of any significance for forestry, the former finding application only as a pioneer species in afforestation of industrially degraded areas (MEJNARTOWICZ, 1980, 1986; MEJNARTOWICZ et al, 1986). Trees of the two species can neighbour each other and occasionally give rise the hybrids. This is relatively infrequent since *Alnus incana* blooms approximately 2 weeks earlier than *Alnus glutinosa*. On the other hand, controlled hybrids of the two species have quite often been described in the literature (VACLAV, 1969; FER and SEDIVY, 1963; CHIBA, 1966; MEJNARTOWICZ 1982). Interspecific hybrids of the two trees, described by KLOTSCH in 1854, represent probably some of the first forest tree hybrids described in the world literature (after SVOBODA, 1957). The present study deals with immuno-chemical characteristics of leaf proteins in artificial hybrids obtained by pollinating *A. incana* with pollen from *A. glutinosa* trees and also leaf protein characteristics of both parental species.

Material and Methods

Seeds of *A. incana* x *A. glutinosa* from which the hybrid forms were grown had been obtained by pollinating flowers of a single *A. incana* tree with an *A. glutinosa* pollen mixture collected from 12 trees. The maternal *A. incana* tree was the only individual of the species throughout an *A. glutinosa* stand, in fact a valuable and protected seed stand. At the time of analysis the hybrid trees were 20 years old. In characterizing the hybrid trees, the appropriate reference material was provided by leaf proteins of parental species, originating in the case of *A. glutinosa* from individuals of the paternal population and in the case of *A. incana* from the arboretum in Kórnik, since the maternal tree used for hybridization was cut during thinning procedures.

Extraction of antigens

Material used for the studies consisted of leaf proteins of the parental species *Alnus incana* and *Alnus glutinosa* and of five hybrid trees. Fresh leaves were homogenized in an extraction buffer + PVP and quartz sand, mixed at 1:4 ratio, in cooled porcelain mortars. The extraction buffer consisted of Tris 10.89 g, H_3BO_3 16.9 g and EDTA 1.12 g in water. The solution was adjusted to pH 7.4 and to 1000 ml. Immediately before extraction 50 mg of Cleland's reagent was added per 1 g of the fresh leaf mass, suspended in 200 ml of buffer plus, approximately, 250 mg PVP per 1g fresh leaf mass. The homogenate was centrifuged and the clear supernatant was used to obtain antibodies and to study antigenic properties of the proteins. Protein content in the samples was assayed according to Lowry's method.

Antisera

In order to obtain antibodies, rabbits of the New Zeeland White strain were injected six times with an aquacide-concentrated leaf protein extract of the two parental species *A. incana* and *A. glutinosa* and with the protein of one of the hybrids (H1). The weekly injections involved each 1.5 ml (approximately 15 mg) of the protein extract, emulsified with the complete Freund's adjuvant. Serum titre of antibodies after the six injections was 1:32. The animals were bleed and the serum was stored in small portions at —20 °C. The sera were denoted AbAi (*anti-incana*), AbAg (anti-*glutinosa*) and AbH1 (antihybrid H1), respectively.

Serological techniques

Immunodiffusion, simple and rocket immunoelectrophoresis as well as quantitative immunoprecipitation were applied to characterize immunochemically the proteins of the studied trees. Immundiffusion was conducted in 1.5% agarose type A (Pharmacia) in 0.135 M veronal-acetate buffer, pH 6.75, applying 60 μl each of the crude leaf extract and of antiserum. Immunodiffusion analysis was also used to characterize antigens following quantitative immunoprecipitation to reduce and simplify protein spectra of the studied forms. After 48 h of immunodiffusion the plates were washed in 0.9% NaC1 to remove remnants of unprecipitated protein, dried and stained with Coomasie brilliant blue. Simple immunoelectrophoresis was performed using the immunodiffusion buffer diluted 1:1 with distilled water. In chambers of the apparatus the undiluted buffer was used.

The samples pattern on the immunoplates permitted comparison of protein spectra of the parental forms with

*) Adam Mickiewicz University, Department of Genetics, 60-594 Poznań, ul. Dąbrowskiego 165, Poland

**) Institute of Dendrology, Polish Academy of Sciences, 62-035 Kórnik, ul. Parkowa 5, Poland

Table 1. — **Results of quantitative precipitation of proteins of hybrids and parental species with antisera against proteins of** *Alnus incana* (AbAi), *A. glutinosa* (Ab Ag) and hybrid H1 (Ab H1).

Antigens (Ag)	Antibodies (Ab): Ab Ai: µg of precipitate	%	Ab Ag: µg of precipitate	%	Ab H1: µg of precipitate	%	mg proteins/ml of extract	mg proteins g f.w.
A. incana	48.9	100.0	57.4	53.0	108.0	53.0	18.4	73.6
A. glutinosa	21.4	44.0	108.0	100.0	204.0	99.5	35.5	142.0
H 1	36.9	75.5	187.7	173.8	205.0	100.0	20.0	80.0
H 2	109.7	224.0	66.0	61.1	94.3	46.0	20.0	80.0
H 3	80.6	165.0	73.7	68.2	66.0	32.0	21.0	84.0
H 4	116.6	238.5	52.3	48.4	87.4	42.5	45.0	180.0
H 5	139.7	286.0	76.3	70.6	132.0	64.0	21.0	84.0

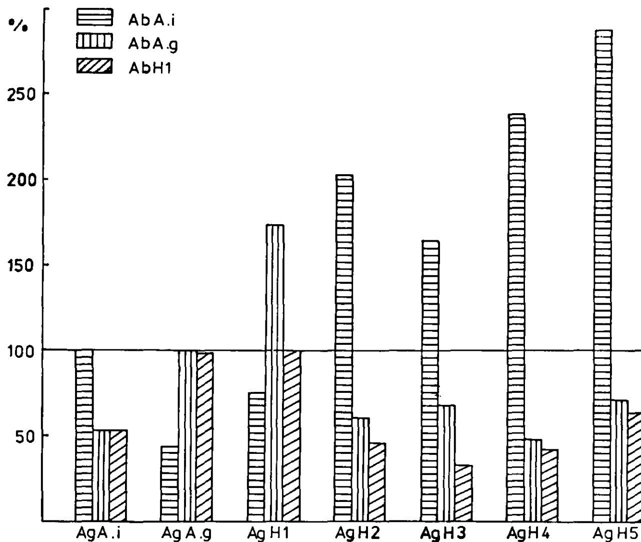

Figure 1. — Results of immunoprecipitation of proteins *Alnus incana* (AgAi), *Alnus glutinosa* (AgAg) and the hybrids (H1—H5) with anisera against *A. incana* (AbAi), *A. glutinosa* (AbAg) and hybrid H1 (AbH1). The results of homological reactions were taken as 100%.

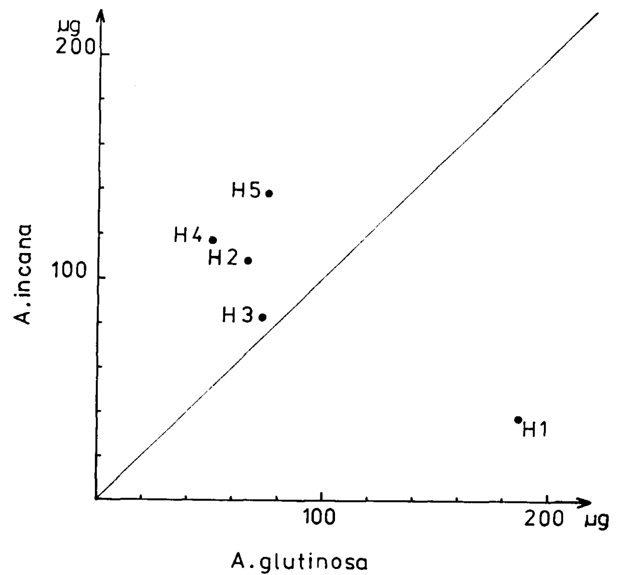

Figure 2. — **Serological similarity of proteins of hybrids to proteins of** ***A. incana*** **and** ***A. glutinosa*****. Based on amount of proteins precipitated with antisera against parental species.**

those of the hybrids. The starting wells were charged with 60 μl of an antigen while the troughs were filled with 240 μl of antiserum. The immunoelectrophoresis was run for 90 min at 180 V and 45 mA per plate, measuring 24 x 9 cm. The rocket immunoelectrophoresis was conducted in the buffer used for simple electrophoresis. Serum concentration in the gel was 100 μl/ml and the amount of antigens used was the same as in immunodiffusion (60 μl). The immunoelectrophoresis was run for 20 h at 50 V and 18 mA per plate, measuring 7.5 cm x 6.0 cm. The plates were washed and stained in the routine manner.

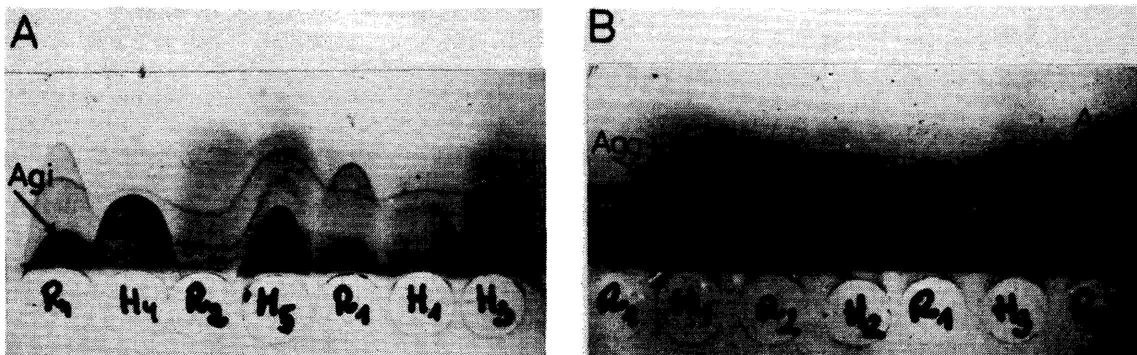

Figure 3. — Rocket immunoelectrophoresis of antigens of *A. glutinosa* (R1), *A. incana* (R2) and hybrids. A — antiserum against proteins of *A. glutinosa*, B. — against *A. incana*. The arrows are showing protein fractions specific for parental forms.

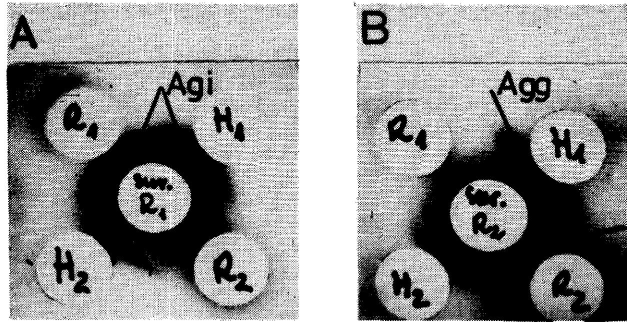

Figure 4. — Double immunodiffusion of proteins of *A. glutinosa* (R1), *A. incana* (R2) and hybrid H1 and H2. In the central wells antisera after partial absorbtion: A — AbAg minus AgAi, B — AbAi minus AgAg. The arrows are showing proteins specific for *A. glutinosa* (A) and *A. incana* (B) present in protein of hybrid form H1.

Quantitative precipitation

Quantitative immunoprecipitation was conducted as described earlier (Prus-Glowacki et al., 1985) using 100 μl of each antiserum and 100 μl of the antigenic extract of the studied forms plus 1.0 ml 0.9% NaC1 as the reaction medium, in triplicate for each antiserum. The amount of precipitate was estimated by Lowry's technique, accepting the result of a homologous reaction (AbAi/AgAi, AbAg/AgAg, AbH1/AgH1) as 100%. Serological relation of the studied hybrids to parental species was calculated as follows:

$$\frac{\text{quantity of precipitate in heterologous reaction}}{\text{quantity of prccipitate im homologous reaction}} \times 100$$

Interpretation of results and numerical analyses

Identification of precipitation lines in immunodiffusion analyses and immunoelectrophoresis, calculation of serological similarity coefficients and of amouts of individual antigenic proteins were performed as described earlier (Prus-Glowacki and Szweykowski, 1979; Prus-Glowacki and Szweykowski, 1980; Prus-Glowacki et al., 1981; Prus-Glowacki, 1983).

Results

Protein content in extracts used for immunochemical analyses was similar in 5 samples and amounted to, approximately, 80 mg per g fresh leaf weight. In the two remaining samples (*A. glutinosa* and one hybrid H4), however the protein content was more or less double that (*Table 1*).

Results of quantitative precipitation in the studied forms with ingividual antisera (anti-*incana*, anti-*glutinosa*, anti-hybrid H1) showed that the first two, precipiated approximately 50% of proteins in cross-reactions, compared to the homologous reactions (i.e. AgAi/AbAi, AgAg/AbAg). On the other hand, hybrid proteins yielded more pronounced reactions with the antisera, even by 286%, compared to the homologous reactions. Amount of protein precipitated with a given antiserum varied with the antigenic similarity of proteins of a given hybrid to the parental form (*Table 1, Figure 1*). Antiserum against hybrid

Table 2. — Results of immunodiffusion analysis of proteins of hybrids *A. incana* x *A. glutinosa* and parental species. Antisera against proteins of *A. incana*. 1.0 — reaction of identity; 0.5 — partial identity; O — lack of precipitin arc.

Samples	Antigens 1	2	3	4	5	6	7	8	9	No of proteins common with A. incana	%	No of specific proteins
A. incana	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0	8	100	-
A. glutinosa	0.5	0	1.0	0	1.0	0	0	0	0	3	37.5	0
H 1	1.0	1.0	0	0	1.0	1.0	1.0	1.0	0	6	75.0	0
H 2	1.0	1.0	0	1.0	0	1.0	1.0	0	1.0	5	62.5	1
H 3	1.0	0	0	1.0	0	0	1.0	0	0	3	37.5	0
H 4	1.0	0	1.0	0	0	1.0	1.0	1.0	0	5	62.5	0
H 5	1.0	0.5	1.0	0	0	1.0	1.0	1.0	0	6	75.0	0

Table 3. — Results of immunodiffusion analysis of leaf proteins of hybrids *A. incana* x *A. glutinosa* and parental species. Antisera against proteins of *A. glutinosa*. The rest of explanation as in *table 2*.

Samples	Antigens 1	2	3	4	5	6	No of proteins common with A. glutinosa	%	No of specific proteins
A. glutinosa	1.0	1.0	1.0	1.0	0	0	4	100	-
A. incana	1.0	1.0	0	0	1.0	1.0	2	50	2
H 1	1.0	1.0	1.0	1.0	0	0	4	100	0
H 2	1.0	1.0	0	0	1.0	1.0	2	50	2
H 3	1.0	1.0	0	0	1.0	0	2	50	1
H 4	1.0	1.0	0	0	1.0	1.0	2	50	2
H 5	1.0	1.0	0	0	1.0	1.0	2	50	2

Table 4. — Results of immunodiffusion analysis of proteins of hybrids and parental species. Antisera against hybrid H1. The rest of explanation as in *table 2*.

Samples	Antigens 1	2	3	4	5	6	7	8	9	No of proteins common with H1	%	No of specific proteins
A. incana	1	1	0	1	1	1	1	0	0	5	83.3	1
A. glutinosa	0.5	0.5	1	0	0	0	0	0	0	3	50.0	0
H 1	1	1	1	1	1	1	0	0	0	6	100.0	-
H 2	1	1	0	1	1	1	1	0	0	5	83.3	1
H 3	0.5	0.5	0	1	1	1	1	1	1	5	83.3	3
H 4	0.5	0.5	0	0	1	1	0	0	0	3	50.0	0
H 5	1	1	0	1	1	1	1	0	0	5	83.3	1

H1 proteins precipiated proteins of parental forms to a variable degree. In the case of *A. glutinosa*, the extent of reaction resembles the homologous reaction while in cases of *A. incana* and hybrid forms intensity of the reaction was lower by, approximately, 50%. Immunochemical similarity of the hybrids to the parental species has been illustrated in *figure 2* presenting the amount of precipiated hybrid proteins in reaction with antisera against proteins of parental forms. As shown, hybrid H1 resembled *A. glutinosa*, H3 was of an intermediate character and the remaining hybrids showed a different degree of similarity to *A. incana*. Immunodiffusion and immunoelectrophoretic analyses (*Figs. 3 and 4*) demonstrated which of the protein fractions were common and which were specific for the studied forms and proved that *A. incana* and *A. glutinosa* exhibited, approximately, half the protein fractions common while the hybrid forms showed different and as a rule fewer fractions than the parental forms. The hybrids showed also specific protein fractions absent in the parental forms. Common and specific protein fractions in the studied trees have been shown in *tables 2, 3, 4* and in *figures 3* and *4*.

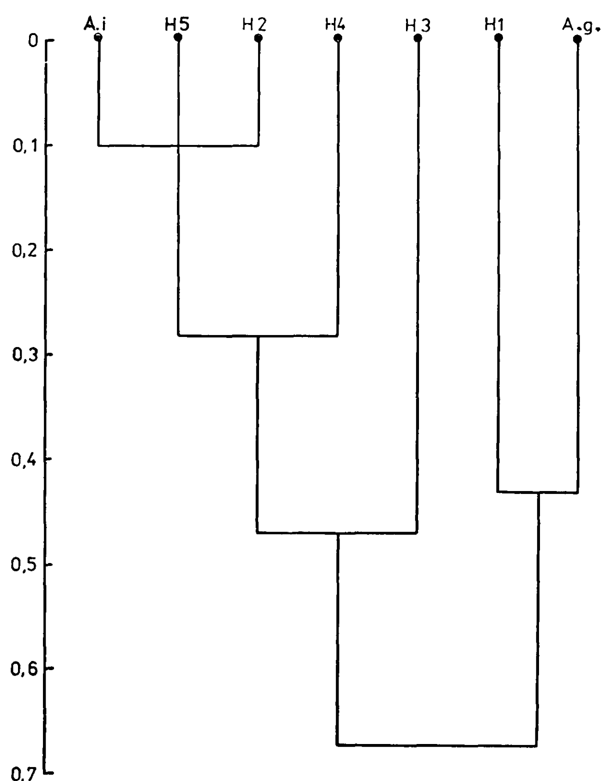

Figure 5. — Dendrogram showing the serological similarity of *A. incana*, *A. glutinosa* and the hybrids. Based on the number of homologous protein fractions.

In all studied individuals the richest spectrum of proteins was noted in reaction to the anti-*incana* serum. Dendrogram constructed on the basis of immunodiffusion analysis, taking into account homology of particular protein fractions in the extract of the studied forms has been presented in *figure 5*. As can be noted in the dendrogram, hybrid H1 was related to *A. glutinosa*, individuals H2 and H5 closely resembled antigenically *A. incana* to which the H4 was also related and H3 was intermediate to greater or smaller extent. Thus, the results confirmed data of quantitative immunoprecipitation analysis.

Discussion and Conclusions

Studies on antigenic proteins of *A. incana* and *A. glutinosa* leaves and leaves of hybrids obtained by pollinating *A. incana* with a pollen mixture from 12 *A. glutinosa* trees indicate specificity of protein spectra of each parental species and a clearly intermediate character of the proteins in the hybrids. Quantitative comparison of *A. incana* and *A. glutinosa* proteins *precipiated* with both antisera directed against proteins of the two parental species or with the antiserum to hybrid H1 proteins has demonstrated that the two alder species comprise approximately 50% proteins which are common for them (*Table 1, Figure 1*). A similar result has been obtained comparing numbers of common and of specific protein fractions distinguished by immunodiffusion and immunoelectrophoresis (*Tables 1* to *4*). Comparison of protein spectra of hybrid individuals and those of parental individuals demonstrated a different degree of similarity of the hybrids to the parental species. Both qualitative analyses (immunoelectrophoresis and immunodiffusion), permitting the demonstration of homology of individual proteins in hybrids and parental species, and quantitative analysis (immunoprecipitation) demonstrate that hybrids H2, H4 and H5 exhibit a matroclinal type of leaf protein inheritance while the protein of hybrid H1 provides an example of patroclinal inheritance. Simultaneously, the latter individual (H1) exhibits expression of genes originating both from the mother (*A. incana*) and from the father (*A. glutinosa*) with presence of both parental pro tein forms and prevalence of *A. glutinosa* proteins (*Fig. 3, 4*). The above notions are confirmed by ANDERSON's hybridization index (ANDERSON, 1949), based on percentage of protein fractions common with parental forms in the

hybrids, and by quantitative immunoprecipitation data (*Table 5*). Apart from proteins inherited from the parental species, the hybrids carry also proteins which are specific for the hybrids (*Table 2, 3, 4*). It remains unclear, however, whether presence of these proteins reflects individual variability of paternal forms (the pollen mixture contained pollens of 12 trees) or the hybridization has resulted in new antigenic properties of the proteins. Presence of proteins of the type, exhibiting novel antigenic properties has been demonstrated by serological techniques in controlled interspecific hybrids of Lolium-Festuca (PRUS-GLOWACKI et al., 1971). Amounts of protein precipitated in hybrid individuals H2, H3, H4 and H5, by *A. incana* serum have been higher by ca 65% to 190% and *A. glutinosa* serum in H1 individual by ca 75% compared to results of homologous reactions (*Fig. 1*). The increased amount of protein might be explained by heterosis as has been earlier demonstrated for morphological traits of hybrids of the two species. The hybrids are higher and their diameter is greater than those of parental trees (MEJNARTOWICZ, 1982). Since the soluble leaf protein, used in this study to produce immune sera, consisted in greater part of ribulose-1,5 -biphosphate carboxylase-oxygenase (Rubisco) (MIZIORKO and LORIMER, 1983), the enxyme which catalyzes CO_2 assimilation in photosynthesis, amount of this protein being increased in the hybrid may increase effectiveness of photosynthesis, inducing hybrid vigor in this way. Thus, both the results pertaining morphological traits (height) and protein traits exhibit correlation in this respect. The present study has shown that serological techniques used to characterize protein traits immunochemically prove most effective in studies on the antigenetic inheritance of hybrid forms. Identifying homology of individual antigens, the technique permit to define precisely the parental "input" into the protein spectrum of the hybrids and to demonstrate heterosis effects, particularly when we deal with controlled hybrids.

Acknowledgements

The authors are indebted to ROMANA NOWAK-BZOWY M. Sc. for technical help.

Literature Cited

ANDERSON, E.: Introgressive hybridization. Eds. Wiley and Sons, New York (1949). —— CHIBA, S.: Studies on the tree improvement by means of artificial hybridization and polyploidy in Alnus and Populus species. Bull. Oji Inst. For Tree Impr. Kuriyama, Hokkaido, 1: 1—165 (1966). —— FER, F. and ŠEDIVÝ, Z.: Přirozeni křiženci olše lepkavé (*Alnus glutinosa* (L. GAERTN.) a olše šedé (*Alnus incana* (L.) MOENCH.) Sborn. Lesn. Fak. Vysoké Školy Zemĕde, Praha, 6: 191—215 (1963). —— MEJNARTOWICZ, L.: Badania zmienności populacji *Alnus glutinosa* (L.) GAERTN. w Polsce. Arboretum Kórnickie 25: 167—180 (1980). —— MEJNARTOWICZ, L.: Badania nad zmiennością rasową olszy czarnej w Polsce. Arboretum Kórnickie 25: 167—180 (1980). —— MEJNARTOWICZ, L.: Morphology and growth of *Alnus incana* x glutinosa F_1 hybrids.

Table 5. — ANDERSON's hybrid indices based on immunodiffusion and quantitative precipitation analysis. The value for reference taxa are: — 100 for *A. glutinosa*, + 100 for *A. incana*.

Hybrids	Immunodiffusion (1)			Quantitative precipitation (2)			Mean value for 1 and 2
	A. incana +	A. glutinosa -	Index (m)	A. incana +	A. glutinosa -	Index (m)	
H 1	75.0	100	-25.0	19.6	100	-80.4	-105.4
H 2	62.5	50	+12.5	100	60.16	+39.84	+ 52.3
H 3	37.5	50	-12.5	100	91.4	+ 8.6	- 3.9
H 4	62.5	50	+12.5	100	44.5	+55.5	+ 68.0
H 5	75.0	50	+25.0	100	54.6	+45.4	+ 70.4

Table 6. — Serological similarity coefficients (SJc) and distances (DJc). The data based on immunodiffusion analyses (homology of precipitin lines). Mean values for three antisera (antiincana (Ab Ai), antiglutinosa (Ab Ag) and antihybrid (Ab H1).

		A.i.	A.g.	H 1	H 2	H 3	H 4	H 5	
D_{Jc}	A.i.		0.220	0.454	0.896	0.581	0.761	0.896	S_{Jc}
	A.g.	0.780		0.571	0.213	0.240	0.249	0.229	
	H 1	0.546	0.429		0.514	0.375	0.467	0.561	
	H 2	0.104	0.787	0.486		0.651	0.625	0.812	
	H 3	0.419	0.760	0.625	0.349		0.461	0.553	
	H 4	0.239	0.751	0.533	0.375	0.539		0.777	
	H 5	0.104	0.771	0.439	0.188	0.447	0.233		

Arboretum Kórnickie 26: 15—29 (1982). —— MEJNARTOWICZ, L.: Influence of emissions from a copper smelter on the content of copper, zinc and magnesium ions and nitrogen and protein in the leaves of alders. Arboretum Kórnickie 31: 205—219 (1986). —— MEJNARTOWICZ, L., BORON, L. and LEWANDOWSKI, A.: The influence of pollution from a copper smelter on the activity of malate dehydrogenase and acid phosphatase in the leaves of alders. Arboretum Kórnickie 31, 221—228 (1986). —— MIZIORKO, H. M. and LORIMER, G. H.: Ribulose 1,5- biphosphate carboxylase oxygenase. Annu. Rev. Biochem. 52: 507—535 (1983). —— PRUS-GLOWACKI, W.: Serological investigation of a hybrid swarm population of *Pinus sylvestris* L. x *Pinus mugo* TURRA and the antigenic differentiation of *Pinus sylvestris* L. in Sweden. In: JENSEN, U. and FAIRBROTHERS, D. E. (Editors): Proteins and nucleic acids in plant systematics. pp 353—361. Springer Verlag (1983). —— PRUS-GLOWACKI, W., SADOWSKI, J., SZWEYKOWSKI, J. and WIATROSZAK, I.: Quantitative and qualitative analysis of needle antigens of *Pinus sylvestris, Pinus mugo, Pinus uliginosa* and *Pinus nigra* and some of individuals from a hybrid swarm population. Genetica Polonica 22, 447—454 (1981). —— PRUS-GLOWACKI, W., SULINOWSKI, S. and NOWACKI, E.: Immunoelectrophoretic studies of Lolio-Festuca Alloploid and its parental species. Biochem. Physiol. Pflanzen. 162, 417—426 (1971). —— PRUS-GLOWACKI, W. and SZWEYKOWSKI, J.: Studies on antigenic differences in needle proteins of *Pinus sylvestris* L., *Pinus mugo* TURRA, *Pinus uliginosa* NEUMANN and *Pinus nigra* ARNOLD. Acta Soc. Bot. Polon. 48 (2), 217—248 (1979). —— PRUS-GLOWACKI, W. and SZWYKOWSKI, J.: Serological characteristics of some putative hybrid individuals from a *Pinus sylvestris* x *Pinus mugo* hybrid swarm population. Acta Soc. Bot. Polon. 49 (1—2), 127—142 (1980). —— PRUS-GLOWACKI, W., SZWEYKOWSKI, J. and NOWAK, R.: Serotaxonomical investigation of the European pine species. Silvae Genetica 34, 162—170 (1985). —— SVOBODA, P.: Lesne dřeviny a jejich porosty. Praha 3: 165—218 (1957). —— VACLAV, E.: Height increment of birch and alder hybrids. Second World Consultation on Forest Tree Breeding. Section II. Breeding for high yielding characters. FAO. Washington 7 to 16 August: 2/11 (1969).

Genetic Control of Growth of Eucalyptus globulus in Portugal

II. Efficiencies of Early Selection

By N. M. G. BORRALHO[1]), P. P. COTTERILL[1]) and P. J. KANOWSKI[2])

(Received 19th March 1991)

Summary

Weighted average genetic parameters from three open-pollinated trials of *Eucalyptus globulus* in central Portugal were used to estimate efficiencies of early phenotypic and combined index selection in improving mature sectional area of stem at end of 8, 13, and 18 year rotations. It was apparent that the relative benefits of early selection increased as plantation rotation increased. However, even under short eight-year rotations, early phenotypic selection on height at four years (when trees were ca. 8 m tall) almost doubled the gains expected in sectional area at eight years, compared with later direct selection. The optimum stage of growth for early selection remained constant at around the time trees reach 8 m tall, regardless of rotation age, or whether selection is based on phenotype or combined indices. The efficiency of early selection is based on phenotype or combined indices. The efficiency of early selection can be increased by strategies which employ preliminary stage-1 selection where grafts of candidate trees are established in breeding arboreta. A final stage-2 selection is carried out when arboreta reach sexual maturity. In this way the testing and breeding phases can be overlapped to some extent and the final phase (stage-2) selection delayed without extending generation interval. Efficiencies of early selection increased substantially with rotation lengths.

Two-stage selection, with an initial index selection at two years and a second at four years, considerably improved the efficiencies of early selection. Efficacy of this procedure, compared with simple one-stage selection, depends largely on the phenotypic correlation between traits at the two stages.

Key words: Early selection, index selection, *Eucalyptus globulus*.

[1]) CELBI, Forest Research Centre, Quinta do Furadouro, Amoreira 2510 Obidos, Portugal.

[2]) Oxford Forestry Institute, South Parks Road, Oxford OX1 3RB, United Kingdom.

Introduction

Shortening the generation intervals in forest tree breeding is essential to maximizing genetic gains per unit time (COTTERILL, 1985). Generation intervals are dependent on the durations of both a test phase and a breeding phase. The testing phase is the time between establishing trees in the field (or some other testing environment) and being able to assess which selections should be used as parents for the next generation. The breeding phase is the time required to cross the newly selected parents and produce in the nursery the new generation of progeny.

The testing phase may be reduced by selecting trees as early as possible. The optimum time of selection depends on changes in heritabilities and additive genetic correlations over time for the species and region (*e. g.* NAMKOONG et al., 1972; LAMBETH, 1980; LAMBETH et al., 1983; KANG, 1985; FOSTER, 1986; MCKEAND, 1988; COTTERILL and DEAN, 1988; DEAN and COTTERILL, 1991). In the case of *Eucalyptus* spp. there appear to be no previous reports on changes in genetic parameters over the plantation rotation, despite breeding programs being established in many parts of the world.

Further reductions in the generation interval may be achieved by two-stage selection with the testing and breeding phases overlapping to some extent (DEAN and COTTERILL, 1991). The breeding phase may also be minimized by accelerating flowering of the selected parents.

This paper examines efficiencies of early selection in improving sectional area of stem at the end of plantation rotation for *Eucalyptus globulus* LABILL. in central Portugal. These estimates of efficiencies are based on pooled estimates of genetic parameters for height and sectional area taken from Part I of this study (BORRALHO *et al.*, 1992). The implications of early selection combining phenotypic and family information, and two-stage selection, are also investigated.