the orchard was reduced in density. The multilocus model yielded a similar trend but the difference was not significant. The outcrossing estimates are so close to 1 that the differences would have little practical significance, even if they were statistically significant. The possibility that factors in orchard management may cause increased levels of outcrossing is supported by the fact that average outcrossing estimates in the background stands of loblolly were lower than those found in these seed orchards. As more studies of this type are done in seed orchards, the effects of number of clones, density, design, species, and pollen production inside and outside the orchards are likely to be further elucidated. The one example mentioned above of decreased outcrossing with increasing proximity of ramets of the same clone suggests that present practices of separating ramets, of the same clone in seed orchards are effective. At the high levels of outcrossing found in this study, the presence of self-fertilized progeny is not a problem in the crops from these seed orchards. However, selfing may still be a problem, however, due to its effect on the production of filled seed.

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Serotaxonomical Investigation of the European Pine Species

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Summary

Studies on serological similarity of European subgenus *Pinus* pine species have disclosed two groups of species. The first group includes four serologically very similar taxa i.e. *Pinus sylvestris, P. mugo, P. uliginosa* and *P. uncinata*. With it *P. nigra* and *P. leucodermis* are linked, but exhibiting significant serological specificity from the *sylvestris-mugo-uliginosa-unicinata* group. The second group of serologically similar species includes *P. brutia, P. eldarica* and *P. pinaster*. The three species are joined loosely by *P. halepensis* and more loosely by *P. canariensis* and *P. pinea*. Two species from subgenus *Strobus, P. cembra* and *P. peuce* are serologically very different from the studied species of subgenus *Pinus*.

Key words: Subgenus *Pinus*, serological diversity, hybridization, taxonomy, proteins.

Zusammenfassung

Serologischen Untersuchungen nach, weisen die europäischen Arten der Untergattung *Pinus* zwei verschiedene Gruppen auf; die erste von ihnen enthält vier serologisch sehr ähnliche Taxa: *Pinus sylvestris, P. uncinata, P. uliginosa* und *P. mugo*. Mit dieser Gruppe sind auch *P. nigra* und *P. leucodermis* eng verwandt. Diese sind aber serologisch von den vier oben erwähnten Arten ziemlich verschieden. Die zweite Gruppe mit serologisch ähnlichen Arten schließt *P. brutia, P. eldarica* und *P. pinaster* ein. *Pinus halepensis* zeigt eine gewisse Ähnlichkeit mit diesen Arten, *P. pinea* und *P. canariensis* können als gesondert stehende Arten der zweiten Gruppe betrachtet werden.

Die zwei europäischen Arten der Untergattung *Strobus, P. cembra* und *P. peuce*, sind von den oben genannten Arten serologisch sehr verschieden.

1. Introduction

Genus *Pinus*, the most numerous one in the *Pinaceae* family, presents a number of interesting taxonomical problems. The genus includes over 100 species and speciation processes continue to take place particularly intensely in Mexican and Central American mountains and less obviously so in South-East Asia. Hybridization irregularities in the genus are of interest from the taxonomic point of view. Many pine species even belonging to distant taxonomic groups cross easily with each other forming a num-

ber of intermediate forms while other species belonging to the same taxonomic group have strong barriers preventing hybridization (Mirov 1967). Existence of intermediate forms and irregular hybridization present serious problems for the systematics of the genus. Great intraspecific variability in species with wide ranges represents another problem for taxonomists. These species form many races and varieties among which some authors distinguish taxons of subspecies or even species rank. The three phenomena, i.e. speciation, irregular hybridization and considerable intraspecific variability result in the systematics of the genus *Pinus* being unclear on several points and requireing further studies.

Hitherto the systematics of pine species has been determined on the basis of morphological and anatomical features, crossability, biochemical traits and also karyotype analysis. The oldest and most complete classification has been presented by Shaw (1914). He based his system on the morphological traits of cones and seeds and on anatomical characters such as resin duct structure, structure of parenchyma, position and number of resin ducts in the needles. A somewhat altered classification of the genus has been proposed by Pilger (1926). It was based on the number of needles in shoots, morphology of seed wings and on the numbers of resin ducts in the needles. Each of the two systems has its antagonists and protagonists. A classification based on genetic principles, i. e. on crossability of pines belonging to various groups, has been suggested by Duffield (1952). The system has been criticized by Gaussen (1960) who has pointed to the phenomenon of irregular crossing in pine and therfore to the low suitability of the trait for such classification. Considering leaf anatomy, position of resin ducts and size of pollen grains Gaussen has suggested an alternative classification of the genus. It is controversial even if it has introduced some new aspects to the problem. Critchfield and Little (1966) have used in their pine systematics, criteria similar to those of Duffield indicating that crossabilities may be useful for a taxonomic revision of some of the systematic groups. Saylor (1964, 1972, 1983) presented interesting taxonomical studies based on karyotype analyses of numerous species of pines. Attempts of using biochemical traits in systematics of genus Pinus have been noted since the early sixties. Substances which have proven useful for such studies involved low molecular compounds such as polyphenols, alkaloids and terpenoids (Mirov 1967). Majority of these taxonomical studies performed till now made use of the low molecular compounds. Use of proteins as taxonomic markers in systematics of the genus has been minimal. First attempts of applying protein electrophoresis for taxonomical studies of a few *Pinus* species and their hybrids were undertaken by Bingham *et al.* (1964). Some further papers have indicated suitability of electrophoretic methods for phylogenetic studies (Pei-Show Juo and Stotzky 1970, 1973, Hamaker and Snyder 1973) for studies on introgression and hybrids between various pine species (Prus-Glowacki and Szweykowski 1983, Florence and Hicks 1980) as well as for studies on subspecies (Wheeler and Guries 1982, Bonnet-Masimbert and Bikay-Bikay 1978, Nikolić and Tućić 1983). Serological methods have been used in taxonomical studies of the genus *Pinus* in a somewhat broader range. Hagman (1967) examined pollen antigenic proteins in a few pine species. The paper demonstrated differences in immunodiffusion patterns between Haplo- and Diploxylon species. He continued the studies (Hagman 1977) comparing pollen antigens of 27 pine species belonging to various taxonomical groups. The paper demonstrated both similarities in antigenic proteins of various *Pinus* species as well as intraspecific differentiation. Saito (1968) compared seed antigens of 16 pine species. His results allowed identification of species groups serologically similar to each other to a variable extent. The study of Prager *et al.* (1976) discussed evolution of antigenic proteins of *Pinaceae* seeds and examined antigenic distances between 11 pine species. Petricević *et al.* (1977) and Djurbabic *et al.* (1977) compared pollen antigens of *P. sylvestris, P. nigra* and *P. densiflora* showing incompatibility between the species. Few papers concern hybrid swarms and serological affinities of species considered as probable parents of the hybrid individuals (Prus-Glowacki *et al.* 1978, 1981, Prus-Glowacki and Szweykowski 1979, 1980). Serological similarity of pollen protein was shown for several pine species by Kormutak (1984).

The above review indicates that electrophoretic, enzymatic and serologic protein traits have only been used in a restricted manner as taxonomic markers in studies on the genus *Pinus*. Nevertheless proteins being the so called biological semantids of the first order may supply numerous valuable informations for the systematics and phylogeny of the genus (*Smith* 1976). Serological methods seem particularly promising in this respect, allowing the examination of the degree of similarity of homologous proteins in individual species. The present investigation is aimed at the examination of serological similarity of 84 species of the genus *Pinus,* subgenus *Strobus* being represented by 28 species and subgenus *Pinus* by 56 species. Some species were represented by up to a dozen seed samples originating from various populations and in such cases intraspecific differentiation of antigens was also examined. The results of intraspecific serological diversity will be published later. The present report is devoted to the presentation of methods and of the serological similarity of European pine species. Later antigenic differentiation of proteins in Asiatic, North American and Central American species will be examined.

2. Material and Methods

2.1. *Antigens*

Studies were conducted on seed proteins of 14 European taxa of genus *Pinus*. The species and place of seed collection are presented in *Table 1*. Systematic position of the pines according to various authors is presented in *Table 2*. The systematic classification of genus *Pinus* as well as the nomenclature used in this study were those of Critchfield and Little (1966). For protein extraction the seed coat was removed and the seeds were pulverized and delipidated for 6—7 h in Soxhlet apparatus using petroleum ether (fraction 60—70° C). The antigens for serological analysis were extracted as described previously (Prus-Glowacki *et al.* 1978). Antigens for immunization were prepared as described above for comparative studies, except that no 2-mercapto-ethanol was used.

2.2. *Antisera*

Proteins of six pine species, 2 belonging to subgenus *Strobus* and 4 to subgenus *Pinus (P. cembra, P. strobus, P. sylvestris, P. thunbergiana, P. ponderosa* and *P. banksiana)* were used for production of antisera. In comparative studies antisera were used containing a full range of antibodies and sera subjected to partial absorption. To sera against proteins of subgenus *Pinus (P. sylvestris, P. thunbergiana, P. ponderosa* and *P. banksiana)* a protein extract of *P. koraiensis* was added representing subgenus *Strobus* while

Table 1. — List of european pine species used in serological investigations x — seed samples used for interspecies comparisons.

SPECIES	PLACE OF COLLECTION	SUPPLIER
Pinus pinea L. /PIN/	a. no data	1 Portugal, Hortus Botanicus Coimbra
	b. no data	2 France, Jardin Botanique de Bordeaux
	xc. Karanci, Turkey	3 INRA, Laboratoire d'Amelioration des Conifers, Bordeaux, France
	d. no data	4 Italy, Hortus Botanicus Sienensis, Siena
Pinus pinaster Ait. /PIR/	a. no data	1
	b. no data	4
	xc. Medoc, Grayan, France	3
Pinus halepensis Mill. /HAL/	a. Pashin, Pakistan elev. 1500 m, 30°N, 67°E	5 Canada Forestry Service, Chalk River
	b. Turkey	3
	xc. Italy	6 Faculty of Forestry, University Zagreb Yugoslavia
Pinus canariensis Smith /CAN/	xa. Canary Islands	3
Pinus brutia Tenore /BRU/	xa. Bucak, Pamucak, Turkey 37°30'N, 30°40'E	3
	b. Turkey	6
Pinus eldarica Medw. /ELD/	xa. Georgia, USSR	3
Pinus nigra Arnold /NIG/	a. var austriaca, Romania, elev. 250 m, 45°20'N, 24°25'E	5
	xb. Austria	5
	c. var banatica, Romania, elev. 725 m, 44°50'N, 22°30'E	5
	d. var laricio calabrica, Domaine des Barre France	3
	e. var corsica, no data	7 SPRL Pepinieres Georges Jas. Grand Halleux, Belgium
	f. Vrhovine, Mt, SR Hrvatska, elev. 850-950 m, Yugoslavia	6
Pinus leucodermis Ant. /LEU/	a. Mt Pirin, Bandenza, elev. 2000 m, Bulgaria	8 Institut of Botany, Bulgarian Academy of Sciences, Sofia
	xb. Pec, A.P. Kosovo, Metohija, Yugoslavia	6
Pinus mugo Turra /MUG/	xa. Tatra Mts, Hala Gąsienicowa, elev. 1400 m, Poland	9 Department of Genetics, Poznań University, Poland
Pinus uliginosa Neuman /ULG/	xa. Batorowskie Peat Bog. Sudety Mts, elev. 750 m, 50°50N, 16°30'E, Poland	9
Pinus uncinata Ramond /UNC/	xa. Mt Confleut, France	3
Pinus sylvestris L. /SYL/	a. Jonavas, Latvia, 55°15'N, 24°20'E elev. 110 m, USSR	5
	b. Belgium	7
	c. Umea, 64°20'E, Sweden	10 Dept. of Forest Genetics and Plant Physiology, SUAS, Umea, Sweden
	d. Granada, 37°04'N, 3°28'W	10
	e. var mongolica, 46°N, 127°E, China elev. 700 m	5
	f. var mongolica, Heilung-Kiang Prov. 46°N, 125°50'E, China	5
	g. Altyre, 57°N, 4°W, Scotland	10
	xh. Piekielna Góra, Sudety Mts, elev. 700 m, 50°45'N, 16°50'E, Poland	9
	xi. Promno near Poznań, elev. 100 m, 52°50'N, 17°20'E, Poland	9
	j. Bavarian Alps, West Germany	11 Institut für Frostgenetik und Frostpflanzenzuchtung, Grosshansdorf, BRD
	k. Schwarzwald, West Germany	11
	l. Further Odenwald-Zentwald, West Germ.	11
Pinus peuce Griseb. /PEU/	xa. Begora Cesma, Pelister, elev. 1200-1300 m, Macedonia, Yugoslavia	6
Pinus cembra L. /CEM/	xa. Buttier elev. 1557 m, Italy	12
	b. A. Pianasse, elev. 1921 m, Italy	12
	c. Dolina Białej Wody, Tatra Mts elev. 1240 m, Czechoslovakia	13 Institut of Dendrology, Kórnik, Poland
	d. Werfen, Alps, elev. 1600 m 13°E, 47°30'N, Austria	13
	e. Zillertal, Tirol, elev. 2000 m, 12°E, 47°N, Austria	13
	f. Stgiermark, elev. 1900 m, 14°E, 47°15'N, Austria	13

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antisera against *Strobus* subspecies were absorbed with *P. sylvestris* proteins. The absorption aimed at removing from the anti-*Pinus* sera of antibodies reacting also with subgenus *Strobus* and from anti-*Strobus* sera of antibodies reacting also with subgenus *Pinus*. The absorption may be presented as follows.

$$\frac{AbTHU}{AgKOR},\ \frac{AbSYL}{AgKOR},\ \frac{AbPON}{AgKOR},\ \frac{AbBAN}{AgKOR},\ \frac{AbCEM}{AgSYL},\ \frac{AbSTR^*}{AgSYL}$$

*) PON - *P. ponderosa*, BAN - *P. banksiana*, CEM - *P. cembra*, STR - *P. strobus*, THU - *P. thunbergiana*, SYL - *P. sylvestris*, KOR - *P. koraiensis*, Ab - antibodies, Ag - antigens.

The absorption was performed as described previously (PRUS-GLOWACKI, SZWEYKOWSKI *1979).*

2.3. Serological methods

In comparative studies double immunodiffusion according to OUCHTERLONY (1962) and quantitative precipitation were applied.

2.3.1. Double immunodiffusion

Immunodiffusion was performed as described in our previous publications (PRUS-GLOWACKI *et al.* 1978, PRUS-GLOWACKI, SZWEYKOWSKI 1979). In interspecific comparisons, each species was compared with a standard protein of the species against which antiserum was obtained.

2.3.2. Quantitative precipitation

Quantitative precipitation was performed to examine the amount of serologically similar proteins of the studied pines as described previously (PRUS-GLOWACKI, SZWEYKOWSKI 1979). For precipitation, antisera to proteins of *P. cembra,* subgenus *Strobus* (AbCEM) and of *P. thunbergiana,* subgenus *Pinus* (AbTHU) were used. Proportions of the reagents were established from an analysis of the equivalence curve according to BOYDEN (1958) obtained for the antisera and homologous antigens. The obtained results were presented as percent of the precipitate formed in a homologous reaction:

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

2.4. Interpretation of results

In the obtained immunodiffusion and immunoelectrophoretic plates, numbers of precipitation lines were estimated and the degree of identity of the observed antigens with the homologous ones (standard antigens) was established, according to the types of immunodiffusion reactions described by OUCHTERLONY (1962). The data were listed in tables using the following code:
0 — lack of a given antigen in a given sample, 2 — antigen identical to a standard antigen, 1 — antigen partially identical to a standard antigen.

2.5. Numerical analysis

The obtained data served for the calculation of Euclidean distances between examined taxons on the basis of average results for 6 antisera and for the construction of a dendrite. Also, cluster analysis was performed for the studied objects using the nearest neighbourhood method and a dendrogram was constructed. Data for absorbed sera served to calculate coefficients of similarity according to the formula of JACCARD ($S_{Jac.}$) (SNEATH and SOKAL 1973)

$$S_{Jac.} = \frac{a}{a + b + c}$$

where a is the number of characters (antigens) present in both (OTK 1 and OTK 2) operational taxonomic units (species) and b and c are the numbers of characters present in OTK 1 but not OTK 2 and vice versa. The obtained coefficients of similarity were used for the construction of dendrite and dendrogram.

3. Results

3.1. Serological similarity of the studied species

3.1.1. Nonabsorbed sera

Figure 1 (A and B) presents an example of immunodiffusion plates with reaction of extracts of the studied pine species with antisera AbSYL and AbTHU (antisylvestris and antithunbergiana). Im some cases the obtained pattern was difficult to interpret due to the high number of precipiation systems resulting in overlapping bands. List of

Table 2. — Systematic positions of investigated european pine species in major classification systems of genus Pinus.

Species	Shaw /1914/			Pilger /1926/		Duffield /1952/		Gaussen /1960/		Critchfield and Little /1966/		
	Subgenus	Subsection	Group	Subgenus	Section	Subgenus	Group	Subgenus	Section	Subgenus	Section	Subsection
CEM	Haploxylon	Cembra	Cembrae	Haploxylon	Cembra	———	———	Cembrapinus	Armandoides	Strobus	Strobus	Cembrae
PEU	Haploxylon	Cembra	Strobi	Haploxylon	Strobus	———	———	Cembrapinus	Strobоides	Strobus	Strobus	Strobi
CAN	Diploxylon	Parapinaster	Longifoliae	Diploxylon	Sula	———	———	Eupinus	Halepensoides	Pinus	Ternatae	Canariensis
PIN	Diploxylon	Paraolnaster	Pinae	Diploxylon	Pinae	———	———	Cembrapinus	Parryanoides	Pinus	Ternatae	Pinae
SYL	Diploxylon	Pinaster	Lariciones	Diploxylon	Eupitys	Diploxylon	Lariciones	Eupinus	Khasyosylvestroides	Pinus	Ternatae	Sylvestres
MUG	Diploxylon	Pinaster	Lariciones	Diploxylon	Eupitys	Diploxylon	Lariciones	Eupinus	Khasyosylvestroides	Pinus	Ternatae	Sylvestres
ULG	Diploxylon	Pinaster	Lariciones	Diploxylon	Eupitys	Diploxylon	Lariciones	Eupinus	Khasyosylvestroides	Pinus	Ternatae	Sylvestres
UNC	Diploxylon	Pinaster	Lariciones	Diploxylon	Eupitys	Diploxylon	Lariciones	Eupinus	Khasyosylvestroides	Pinus	Ternatae	Sylvestres
NIG	Diploxylon	Pinaster	Lariciones	Diploxylon	Eupitys	Diploxylon	Lariciones	Eupinus	Khasyosylvestroides	Pinus	Ternatae	Sylvestres
LEU	Diploxylon	Pinaster	Lariciones	Diploxylon	Eupitys	Diploxylon	Lariciones	Eupinus	Halepensoides	Pinus	Ternatae	Sylvestres
HAL	Diploxylon	Pinaster	Insignes	Diploxylon	Banksia	Diploxylon	Lariciones	Eupinus	Halepensoides	Pinus	Ternatae	Sylvestres
BRU	Diploxylon	Pinaster	Insignes	———	———	Diploxylon	Lariciones	Eupinus	Halepensoides	Pinus	Ternatae	Sylvestres
ELD	Diploxylon	Pinaster	Insignes	———	———	Diploxylon	Lariciones	Eupinus	Halepensoides	———	———	———
PIR	Diploxylon	Pinaster	Insignes	Diploxylon	Eupitys	Diploxylon	Lariciones	Eupinus	Taedoponderosoides	Pinus	Ternatae	Sylvestres

Table 3. — Results of immunodiffusion analysis of seeds proteins of european pine species with the use of six antisera. Denotation of species as in *Table 1.*

SPECIES	ANTISERA ANTIGENS AbSYL 123456789101112	AbTHU 12345678910111213	AbPON 12345678910111213	AbBAN 123456789101112	AbCEM 12345678910	AbSTR 12345678910111213
SYL	222220000 0 0 0	022220000 0 0 0 0	200220000 0 0 0 0	202200000 0 0 0	020002200 0	000022000 2 0 0 0
UNC	012220000 0 0 0	012200000 0 0 0 2	000112200 0 0 0 0	202222000 0 0 2	002002200 0	020022000 0 0 0 0
ULG	212002000 0 0 0	020200000 0 0 0 2	002222000 0 0 0 0	201220000 0 2 0	002002200 0	020022000 0 0 0 0
MUG	222100000 0 0 0	012220020 0 0 0 0	200222000 0 0 0 0	202220000 0 0 0	002002200 0	000022000 2 0 0 0
NIG	222120000 0 0 0	000220002 0 0 0 0	202222000 0 0 0 0	202222000 0 0 0	002002200 0	000022000 0 2 0 0
LEU	002120000 0 2 0	010220000 2 0 0 0	202222020 0 0 0 0	202220000 0 0 0	002012200 0	020222000 0 0 0 0
BRU	002220000 2 2 0	012202000 0 0 0 0	002222000 2 0 0 0	220220000 0 0 0	020000000 0	000022000 0 0 2 0
ELD	102220000 0 0 0	000220000 0 0 0 0	002022200 2 0 0 0	200222000 0 0 0	002002200 0	010022000 0 0 2 0
PIR	102200200 0 0 0	000022000 0 2 0 0	000222000 0 0 0 0	202222000 0 0 0	202000020 0	010022020 0 0 0 0
HAL	002020000 2 2 0	010222000 0 0 0 0	000210020 0 0 0 0	001222220 0 0 0	002002200 0	010020200 0 0 0 0
CAN	212220000 0 0 0	000220000 0 0 0 0	002222000 0 2 0 0	020220000 2 0 0	002002200 0	010022000 0 0 0 2
PIN	202100220 0 0 0	000222000 0 2 0 0	022220002 0 0 0 0	020220000 0 0 0	102000022 0	000022022 0 0 0 0
PEU	201100000 0 0 2	100210200 0 0 0 0	222200000 0 0 0 2	022201002 0 0 0	212010000 2	120222000 0 0 0 0
CEM	201100000 0 0 2	200210100 0 0 0 0	222200000 0 0 2 2	022210000 0 0 0	222220000 0	102222000 0 0 0 0

the distinguished precipitation bands and their identification with homologous antigens is presented in *Table 3.* The data were used for calculating mean Euclidean distances between the studied pine species. *Figure 2* presents the dendrite and dengrogram based on mean Euclidean disances for the six antisera. In the dendrite *(Fig. 2 A)* studied species formed no evident clusters. The longest distance on the dendrite is noted between *P. pinea* (PIN) and the pair of species of the *Strobus* subgenus, *P. peuce* (PEU) and *P. cembra* (CEM). The rest of studied species are connected by more or less the same distances.

In the dendrogram *(Fig. 2 B),* result of the cluster analysis the species position generally resembles that of the dendrite, but an evident group of six species draws attention. It includes *P. leucodermis, P. sylvestris, P. nigra, P. mugo* as well as *P. uncinata* and *P. uliginosa. P. eldarica* and *P. canariensis* are linked to the group. The remaining species form no evident clusters. The greatest

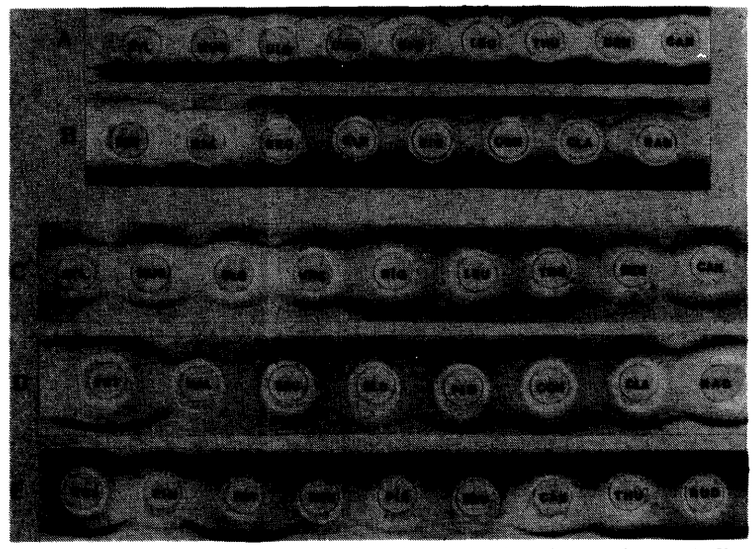

Fig. 1. — Example of immunodiffusion plates of antigenic proteins of studied pine species. A and B — upper part of plates antiserum against proteins of *P. sylvestris,* lower part of plates against proteins of *P. thunbergiana.* C, D and E — plates with the use of antisera absorbed with proteins of *P. sylvestris.* Upper parts of the plates antiserum against *P. cembra* proteins, lower part against *P. strobus.* Particular species denoted as follow: SYL — *P. sylvestris,* MUG — *P. mugo,* ULG — *P. uliginosa,* UNC — *P. uncinata,* NIG — *P. nigra,* LEU — *P. leucodermis,* THU — *P. thunbergiana,* DEN — *P. densiflora,* CAN — *P. canariensis,* PST — *P. pseudostrobus,* HAL — *P. halepensis,* BRU — *P. brutia,* ELD — *P. eldarica,* PIR — *P. pinaster,* CON — *P. contorta,* CLA — *P. clausa.* RAD — *P. radiata,* PIN — *P. pinea,* ROX — *P. roxburghii,* HWA — *P. hwangshanensis.*

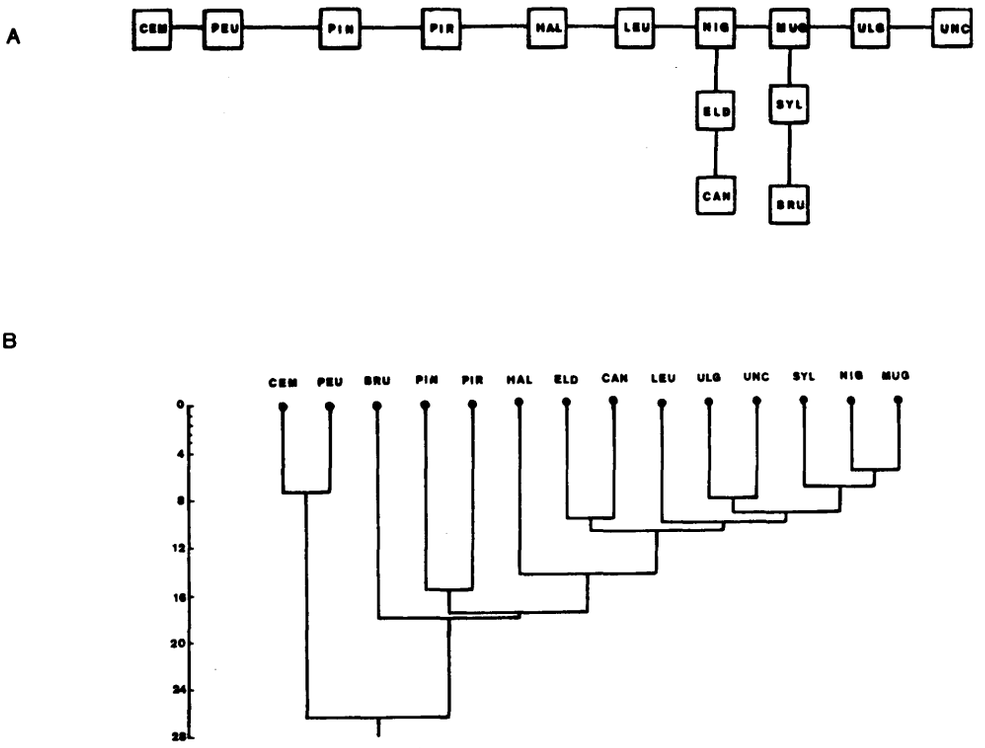

Fig. 2. — Dendrite /A/ based on Euclidean distances and dendrogram /B/ constructed on the basis of the nearest neighbourhood method showing serological similarity of studied pines. Mean data for all six antisera. Denotation of species as in *Fig. 1.*

distance in the dendrogram separates a pair of species i.e. *P. cembra* and *P. peuce* (both of subgenus *Strobus)* from the remaining studied pine species.

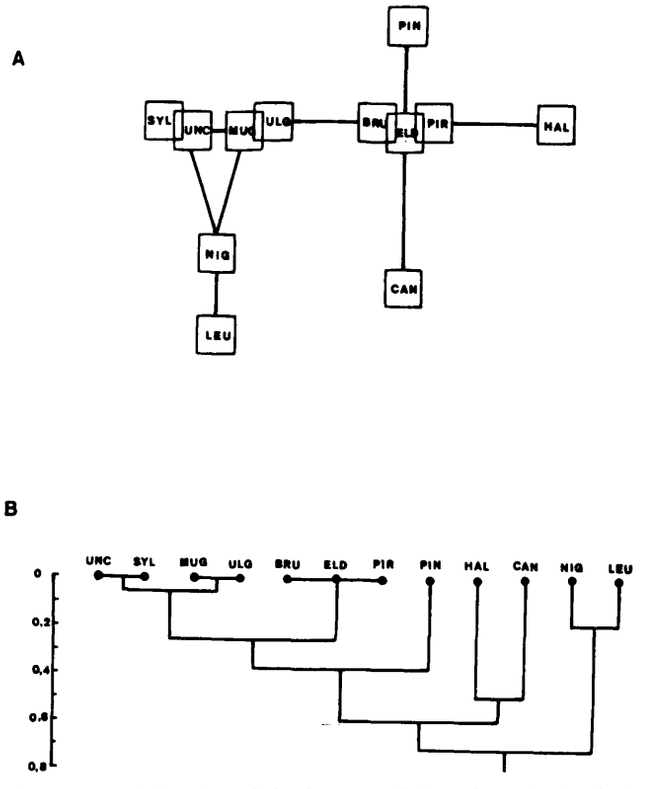

Fig. 3. — Dendrite /A/ and dendrogram /B/ based on simple similarity coefficients according to JACCARD showing serological similarity of the studied pines. Mean data for the absorbed antisera. The denotation of species as in *Fig. 1.*

3.1.2. Absorbed sera

As mentioned above, overlapping of precipitation bands in immunodiffusion analysis with antisera of a full spectrum of antibodies has made it necessary to reduce the number of obtained precipitation systems to simplify the immunodiffusion patterns. Examples of immunodiffusion plates with absorbed anti-*P. cembra* and anti-*P. strobus* sera are presented in *Fig. 1 C, D, E.* Presence of identical, partially identical or unique antigens have been summed up in *Table 4.* The data have been used to calculate coefficients of similarity according to JACCARD. The coefficients of similarity have been used to draw a dendrite and the dendrogram *(Fig. 3),* visualizing serotaxonomic relations between the studied species. The compared species form two clearly distinct groups. The first group includes serologically closely related species *P. sylvestris, P. mugo, P. uliginosa* and *P. uncinata. P. nigra* and *P. leucodermis* are linked to the group and show reciprocal serological similarity to each other. The other group is formed by *P. brutia, P. eldarica* and *P. pinaster.* Out of the latter three species, *P. brutia* and *P. eldarica* are serologically very similar. These three taxons are separated by significant distances from *P. halepensis* and *P. pinea* and particularly from *P. canariensis.* The dendrogram drawn on the basis of coefficients of similarity shows species positions similar to those of the dendrite. Separate position in relation to the remaining species has been noted in the case of the species pairs *P. nigra* with *P. leucodermis* and *P. halepensis* with *P. canariensis.*

3.1.3. Quantitative immunoprecipitation

Results of quantitative immunoprecipitation obtained in reactions of anti-*P. cembra* and anti-*P. thunbergiana* sera with proteins of individual species are presented in *Fig. 4.* Using anti-*P. thunbergiana* serum, the lowest values have

Table 4. — Results of immunodiffusion analysis with absorbed antisera. The intensity of reactions was coded 0 to 6; 0 — lack of precipitation band, 6 — very intensive band, p — partial identity reactions. Denotation of species as in *Table 1*

Species /Antigens/	Absorbed antisera AbPON / AgKOR	AbTHU / AgKOR	AbBAN / AgKOR	AbSYL / AgKOR	AbCEM / AgSYL	AbSTR / AgSYL	Number precipit. lines
SYL	3	6 6	6	6	003 1	03 1	9
UNC	3	4 5	4	5	003 1	03 1	9
ULG	2	6 6	5	5	003 2p	03 1	9
MUG	2	5 3	3	5	003 2p	03 1	9
NIG	6	4 2	2	3	623 0	43p1	11
LEU	6	4 2	5	4	630 0	43p1p	10
BRU	4	3 0	6	2	003 1	02 0	7
ELD	4	3 0	5	2	003 1	02 0	7
PIR	3	2 0	1	1	001 1p	03 0	7
HAL	1p	1p0	1	1	001p1p	01p0	7
CAN	0	0 0	0	1p	004p2	03 0	4
PIN	1	2 0	0	0	002 1	02 0	5
PEU	0	0 0	0	0	6	6	2
CEM	0	0 0	0	0	6	6	2

been obtained for *P. pinea, P. peuce* and *P. cembra,* 30 to 40% below the homologous reaction. For the remaining species the amount of precipitate ranges between 83.5% and 109.9%, as compared to the homologous reaction. The anti-*P. cembra* serum has yielded the greatest amount of precipitate with *P. nigra* proteins and then in the homologous reaction and with proteins of *P. peuce* and *P. pinaster ca* 91%. The studied species of subgenus Pinus form two groups. The greater of them includes six species, i. e. *P. sylvestris, P. uliginosa, P. uncinata, P. mugo, P. leucodermis* and *P. canariensis.* The other group is formed by *P. brutia, P. eldarica. P. pinaster* and *P. halepensis. P. nigra* and *P. pinea* are positioned beyond the two groups. Species belonging to subgenus *Strobus* are clearly separated from the remaining species.

4. Discussion and Conclusions

The performed studies on serological relations between European species of pines from subgenus *Pinus* have disclosed two groups, each of which shows close serological similarity within the group *(Fig. 3, Fig. 4).* The first group includes four serologically very similar taxons, i.e. *P. syl-*

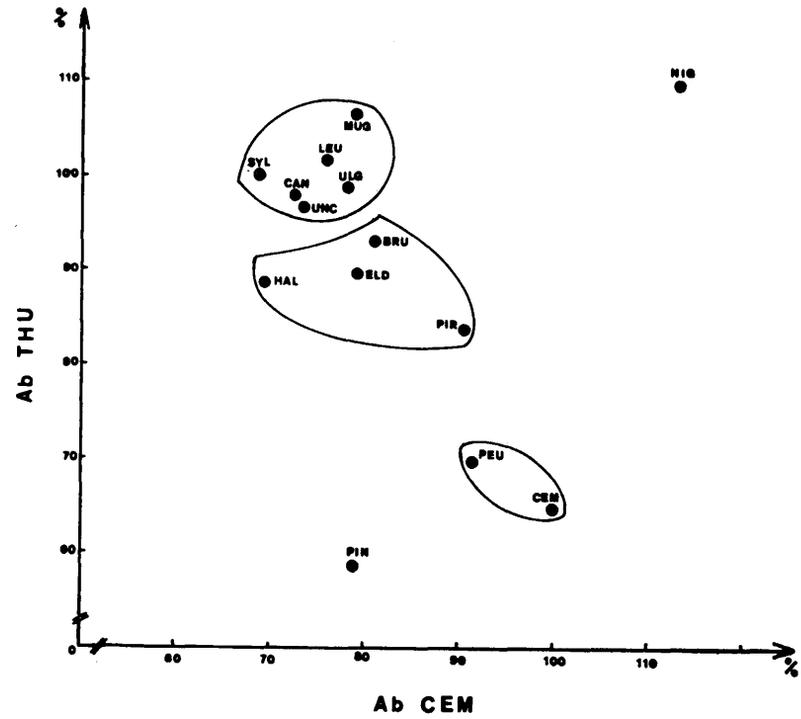

Fig. 4. — Diagram showing results of quantitative precipitation of proteins of studied species with antisera against proteins of *Pinus thunbergiana* /AbTHU/ and proteins of *P. cembra* /AbCEM/. The denoation of species as in *Fig. 1.*

vestris, P. mugo, P. uliginosa and *P. unicinata.* THIELGES (1969) has demonstrated extensive chemical similarity of polyphenolic compounds originating from *P. sylvestris* and *P. mugo.* The species can cross in nature and the demonstrated serological similarity may reflect their affinities. *P. uliginosa* and *P. uncinata* taxons used to be included with *P. mugo* in the *P. montana* complex and their systematic position has been reflected in the serological similarity. This group of serologically close taxons is linked to *P. nigra* and *P. leucodermis.* The two species exhibit significant serological specificity as compared to the group, and are clearly distinct from each other. They exhibit antigens, present also in the Asiatic pine species — *P. thunbergiana,* which are not encountered in the other European pine species. The significant antigenic divergence of *P. nigra* from *P. sylvestris, P. mugo* and *P. uliginosa* has also been demonstrated in studies on needle antigens of the pines (PRUS-GLOWACKI, SZWEYKOWSKI 1979, PRUS-GLOWACKI *et al.* 1981). Studies of PETRICEVIC *et al.* (1977) on pollen antigens of *P. nigra, P. sylvestris* and *P. densiflora* have demonstrated a relatively loose serological similarity of *P. nigra* and *P. sylvestris.* Also, studies of THIELGES (1972 a) on hybrids of *P. nigra, P. mugo, P. sylvestris, P. thunbergiana and P. densiflora* have shown only a low genetic affinity of *P. nigra* to *P. sylvestris* and *P. mugo.* Even if *P. nigra* and *P. Ieucodermis* are serologically close to each other, they still exhibit a significant antigenic specificity.

MIROV (1967, pp. 540—568) has demonstrated the distinct chemical character of *P. leucodermis* as compared to *P. nigra,* even if the former used to be classified by some authors as a variety of *P. nigra.* He has noted that *P. nigra* oleoresin exhibits a much higher limonen content than that of *P. leucodermis.* THIELGES (1969) studying taxonomic relations between *P. nigra* and *P. heldreichi (P. leucodermis)* has found that the two taxons are loosely related as far as polyphenol compounds are concerned.

The other group a species showing close serological similarity includes *P. brutia, P. eldarica* and *P. pinaster.* These three species are linked to *P. halepensis, P. pinea* and *P. canariensis.* In the former group *P. brutia* and *P. eldarica* are serologically most similar.

P. pinaster has shown serological similarity to the two taxons while *P. halepensis,* regarded as closely related to *P. brutia,* has been shown to be serologically distant from the species. According to MIROV (1967), *P. eldarica* and *P. pityusa* (the latter has not been compared serologically in this study) can be regarded as varieties of *P. brutia* due to their content of delta-3-carene. *P. halepensis* does not contain the compound while it is present in *P. sylvestris.* Studies of THIELGES (1969) have demonstrated that *P. halepensis* and *P. brutia* differ extensively in their chromatographic patterns of polyphenol compounds. On the other hand, *P. brutia* resembles in that respect *P. sylvestris, P. mugo* and *P. nigra.* Our results to a great extent correspond to these findings *(Fig. 3* and *Fig. 4).* Taxonomic position of *P. pinea* the only representative of SHOWS (1914) group *Pinea* and GAUSSEN's (1960) subgenus *Cembrapinus* remains unclear. In our studies the species is linked to the *brutia-eldarica-pinaster* group. MIROV (1967) indicates that it features a specific spectrum of terpenes but contains also caryophylene, present also in *P. pinaster.* Chemotaxonomic studies of THIELGES (1969) also point to its greatest chemical similarity with the *Sylvestres* group, to *P. brutia,* nevertheless clarification of origin and systematic position of the pine requires further studies.

The last species, *P. canariensis,* shows only a slight serological similarity to the remaining species of subgenus *Pinus.* Serologically, it is similar to the *brutia-eldarica-*

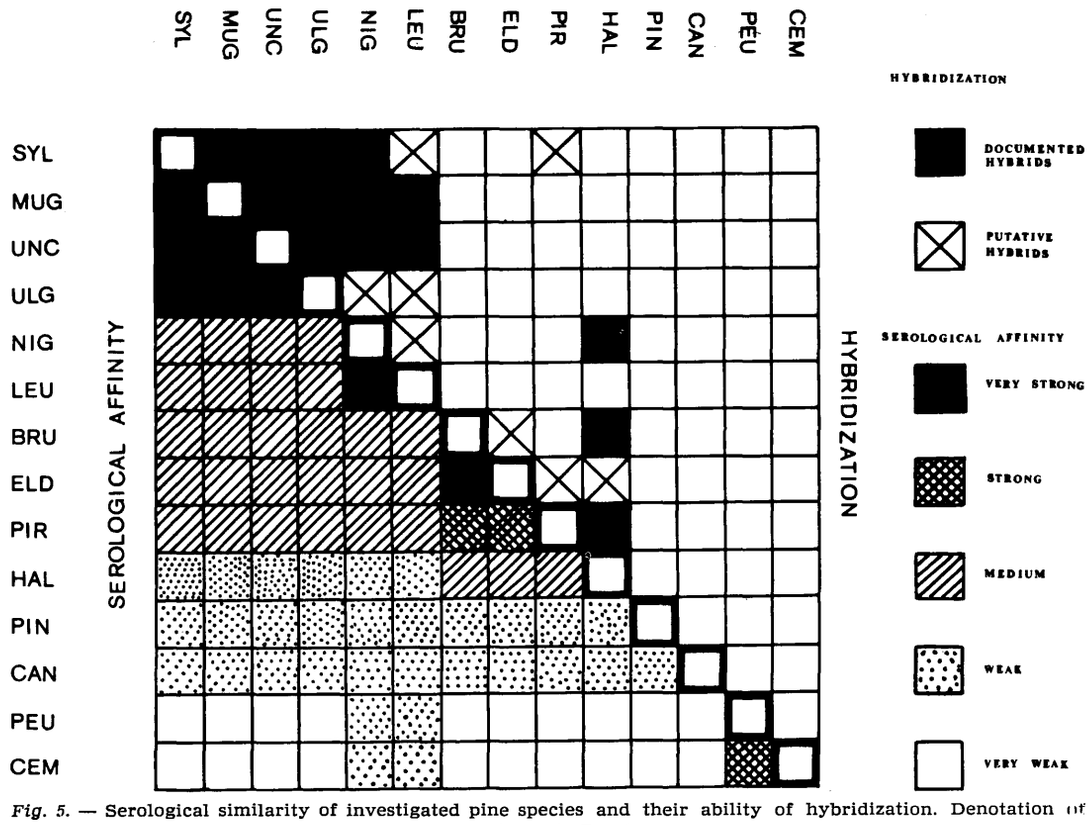

Fig. 5. — Serological similarity of investigated pine species and their ability of hybridization. Denotation of species as in *Fig. 1.*

pinaster group. This endemic pine belongs to the *Longifoliae* group of SHOW (1914) and the taxon most closely related to it — *P. roxburghii* — grows at the foot of the Himalayas. In comparative studies the two species exhibit significant antigenic similarity. Positioning of *P. canariensis* in section *Halepensoides* by GAUSSEN (1960) seems groundless in the light of the low serological similarity of the species to the remaining species of the group. For the same reason as well as for its similarity to *P. nigra, P. leucodermis* should not be included in the group.

As far as the two European species of subgenus *Strobus* are concerned, i.e. *P. cembra* and *P. peuce,* serologically they differ very much from the studied species of subgenus *Pinus* as well as, but to a smaller extent, between each other. A more detailed discussion of their serological relationships would require a background of other species of the subgenus.

The serological studies performed show how far the antigenic traits of proteins, i.e. their serological similarities, reflect actual relationships between the studied pine species. *Fig. 5* sums up results of successful artificial hybridizations and documented natural hybrids among European pine species (DUFFIELD 1952, MIROV 1967, GIERTYCH 1970, STASZKIEWICZ 1970, PRUS-GLOWACKI, SZWEYKOWSKI 1980, 1983, WRIGHT and GABRIEL 1958, THIELGES 1972 b, PETRICEVIC *et al.* 1977, VIDAKOVIĆ 1977, HAGMAN 1975, GARRET 1979). If crossing ability can be accepted as a measure of relationship a clear correlation can be noted between the ability to hybridize and the serological similarity.

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