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An interesting chemical polymorphism in *Pinus sylvestris* L.

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Abstract

Intra- and interpopulational polymorphism in the production of phenolic compounds is described in Polish populations of *Pinus sylvestris* L. Two mutually exclusive forms of pine trees are present in changing proportions in all populations studied. This allows three groups of populations to be distinguished. The character of this differentiation is discussed.

INTRODUCTION

Variation in Scots pine (*Pinus sylvestris* L.), one of the most important European forest trees, has been studied intensively (Prawdin 1964, Przybylski et al. 1976, Staszkiewicz 1970), but the results, at least as far as the racial differentiation of this tree is concerned, are still debatable (Krzakowa 1979, Krzakowa and Szweykowski 1969). Some authors mention clines (Langlet 1959, Przybylski et al. 1976) others see more or less distinct races (Wright and Bull 1963, Staszkiewicz 1968, Przybylski 1966, Prawdin .1964). Studies were carried out concerning morphological characters (Staszkiewicz 1968), physiological ones (Langlet 1959, Przybylski 1970), isozymes (Krzakowa 1979, Krzakowa and Szweykowski 1979, Mejnartowicz 1979), terpenes (Mirov 1967) and phenolics (Thielges 1972, Börtitz 1963). We report here on an interesting intra- and interpopulational polymorphism of phenolic compounds.

MATERIAL

Needles were collected in 12 natural populations (Table 1) during the winter 1977 (populations 1-8) and in the late autumn 1976 (populations

Table 1

Populations sampled for phenolic compounds studies

Population number	Locality	District	n (number of trees studied)
1	Janów Lubelski	Tarnobrzeg	28
2	Spała	Piotrków	30
3	Miłomłyn	Olsztyn	30
4	Ruciane	Suwałki	30
5	Supraśl	Białystok	30
6	Rychtal	Opole	30
7	Bolewice	Poznań	30
8	Gubin	Zielona Góra	30
9	Jarząbek	Wałbrzych	30
10	Szczeliniec	Wałbrzych	30
11	Piekielna Góra	Wałbrzych	30
12	Lasówka	Wałbrzych	30

9-12). From each population 30 trees were randomly sampled. The only exception was the population no. 1 (Janów Lubelski) where needles from 28 trees were taken.

METHODS

FLAVONOID EXTRACTION AND SEPARATION

Needles cut in small pieces were dried at +37°C for 72 h. Dry needles (2.5 g) were shaken with 12.5 ml of ethyl ether for 1 h and filtered. The procedure was repeated 5 times. The remaining needles were dried for 24 h at 20°C and shaken with 12.5 ml of n-butanol for 1 h. This procedure was also repeated five times. The n-butanol fractions containing phenolic substances were then evaporated to dryness in a rotary evaporator (Unipam, Poland) and redissolved in 1.3 ml of n-butanol; 0.3 ml of this extract was applied on Whatman 3 MM paper (28 × 33 cm) and the compounds were separated by two-dimensional, ascending chromatography. Needles from each tree were processed separately. The solvents used were: a) BAW-n-butanol:acetic acid:distilled water (4:1:5 v/v); b) 2 ml of formic acid: 20 g of sodium formate : 200 ml distilled water. For each tree two chromatograms were made. They were examined in UV light with and without ammonia vapour.

STATISTICAL TREATMENT

To check whether neighbouring populations can be viewed as interdependent (in a statistical sense), Moran's statistic "I" was computed as a measure of autocorrelation (Sokal and Oden 1978—the following discussion is based mainly on this paper). For computation of this statistic, the stations of sampled populations were connected on the map according to the Gabriel criterium, i.e. two stations A and B were connected if their geographical distance satisfied the condition $d^2_{AB} < d^2_{AC} + d^2_{BC}$, where C represents any other station, The Gabriel-connected graph obtained in this way was used for preparation of an adjacency matrix (Table 2). Then, Moran's coefficient was computed according to the formula:

$$I = \frac{n \sum_{ij} w_{ij} z_i z_j}{W \sum_{i=1}^{n} z_i^2},$$

where *n* stands for number of populations studied, w_{ij} for weight given to joins between stations of populations *i* and *j*. These weights were set arbitrarily equal to one when the two stations in question were connected directly, and 0 when they were not. For the rationale of this procedure see below; z_i and z_j represent deviations from the general mean ($z_i = x_i - \bar{x}$), in our case the deviations from the mean frequency of the trees of type I, i.e. from the value 0.534; *W* denotes the sum of all weights ($W = \Sigma w_{ij}$) given to joins between population sites. In our case, *W* equals the sum of all ones in the adjacency matrix (Table 2).

Table 2
Adjacency matrix

Population number (i)	x_i	z_i	Population number (i)											
			1	2	3	4	5	6	7	8	9	10	11	12
1	0.61	+0.076	–	1	0	0	0	0	0	0	0	0	0	0
2	0.53	−0.004	1	–	1	0	0	1	0	0	0	0	0	0
3	0.33	−0.204	0	1	–	1	0	0	0	0	0	0	0	0
4	0.20	−0.334	0	0	1	–	1	0	0	0	0	0	0	0
5	0.37	−0.164	0	0	0	1	–	0	0	0	0	0	0	0
6	0.53	−0.004	0	1	0	0	0	–	1	0	1	0	0	0
7	0.47	−0.064	0	0	0	0	0	1	–	1	0	0	0	0
8	0.17	−0.364	0	0	0	0	0	0	1	–	0	0	0	0
9	0.75	+0.216	0	0	0	0	0	1	0	0	–	1	1	0
10	0.80	+0.266	0	0	0	0	0	0	0	0	1	–	0	0
11	0.90	+0.366	0	0	0	0	0	0	0	0	1	0	–	1
12	0.75	+0.216	0	0	0	0	0	0	0	0	0	0	1	–

x_i — frequency of type I plants

z_i — deviation from the general mean

PROBLEM OF WEIGHTS GIVEN TO THE JOINS BETWEEN PARTICULAR POPULATION SITES

The geographical distances between sites varied in our case from 5 km (between populations 9 and 10) to 243 km (between populations 2 and 3)

—the mean distance equalled 117 km. We decided to treat these distances as equal basing on the following premises. The frequency of trees showing a particular phenolic pattern depends on several factors mainly connected with the ecology and history (including human influence) of particular sites. However, the gene flow, depending on the geographical distance between sites, is equally important. According to recent estimates (Stern and Roche 1974), the possibility of pollination with pine pollen is restricted to the closest neighbourhood. Populations over 100 km apart are thus practically completely isolated. All our populations, except those growing in the mountains (nos. 9-12), lie at least 108 km apart. The distance between four pine stands in Kotlina Kłodzka Valley (nos, 9 through 12) are much shorter (from 5 to 16 km). These populations are, however, small islands in spruce or beech forests and, according to our estimates, they also can be treated as completely isolated. Thus, the influence of gene flow may be neglected in our case and this means that the genetic structure of our populations can be treated as independent of the geographical distance.

Moran's statistic "I" tells us whether the neighbouring populations are interdependent, i.e. whether knowing the characteristics of one particular population one can predict the characteristics of its neighbours. This statistic can also be used to check whether there exists a pattern in the geographical distribution of populations, and how large are the relatively homogenous patches. For this purpose, a "corellogram" has been prepared. We computed, namely, Moran's statistics for pairs of populations two (II), three (III), four (IV) and five (V) joins apart (e.g. I_{II} between populations 1 and 3, 1 and 6, 2 and 4, and so on; I_{III} between populations 1 and 4, 1 and 7, 1 and 9, 2 and 5 and so on—Fig. 2, 3a). Then the values of the compouted I's were plotted on the diagram, correlógram, showing the dependence of the autocorrelation coefficient values on the distance between population sites measured as the number of joins in the Gabriel-connected graph.

Finally, ordination and clustering of populations according to their taxonomic distances were performed. The distances used were calculated according to the formula:

$$d_{ij} = |x_i - x_j|,$$

where x_i and x_j—frequencies of type I trees in populations i and j respectively.

RESULTS

The general variation of phenolic substances spectra will be dealt with elsewhere. Here, we describe an interesting polymorphism concerning probably only one compound produced in two sharply different amounts. Thus, two categories of trees were found among the 358 tested: plants showing

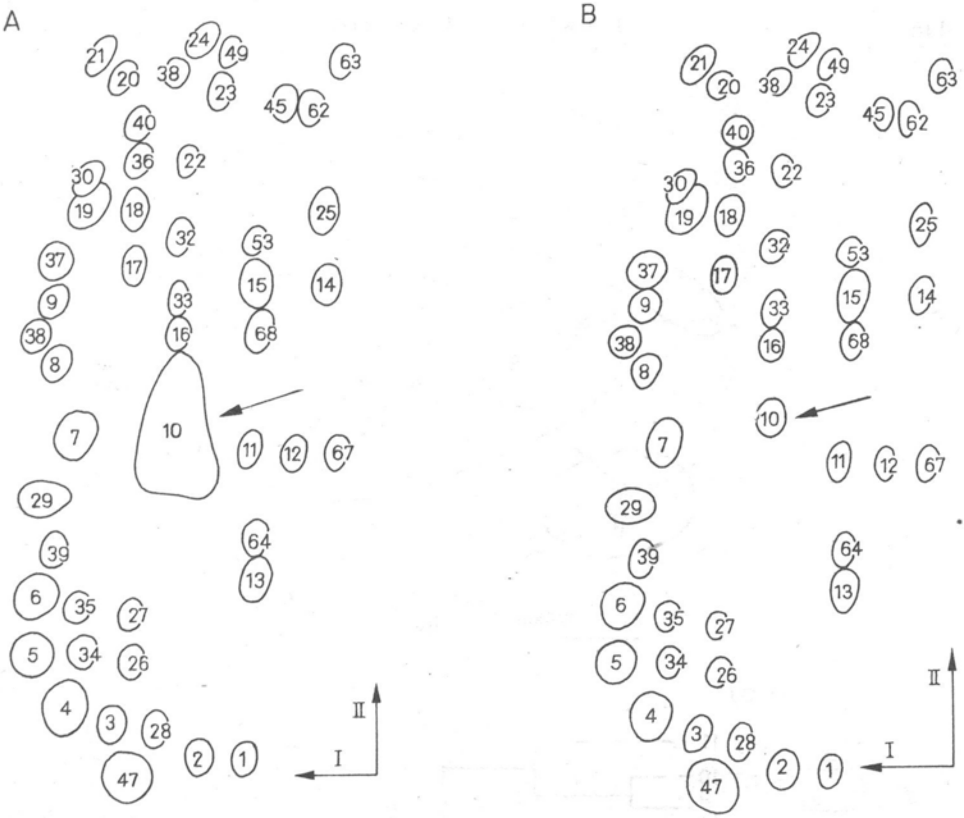


Fig. 1. Diagrammatic representation of chromatographic patterns revealed in Polish Scots pine. A—type II, B—type I. From unpublished data of M. Krzakowa and L. Urbaniak

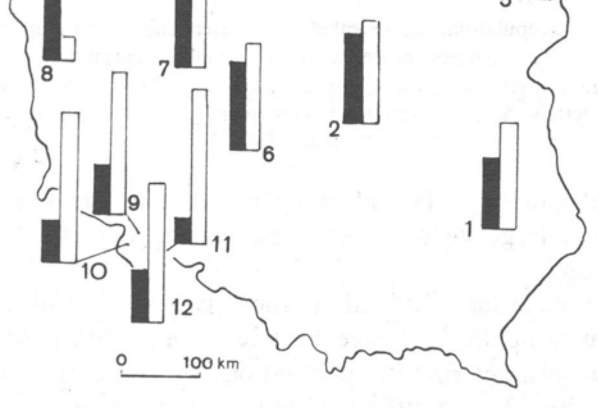
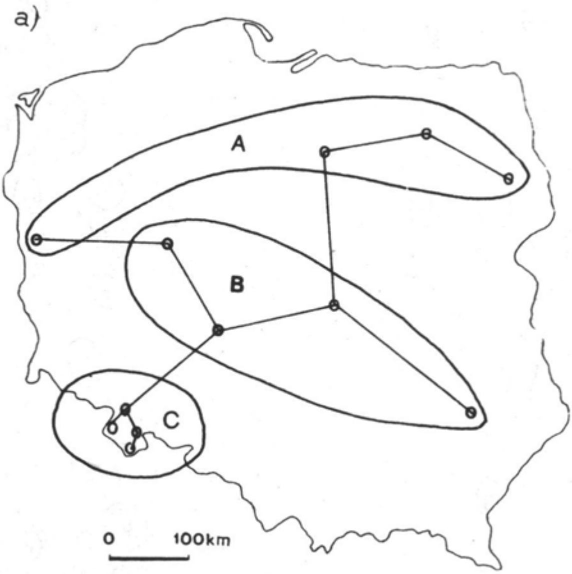


Fig. 2. Frequencies of type I (white bars) and type II (black bars) trees in populations studied



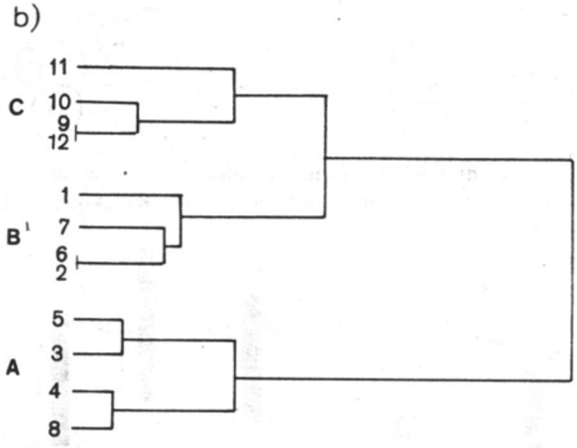
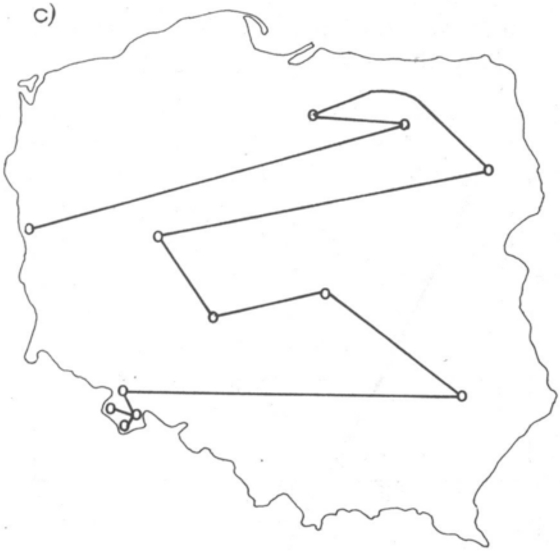


Fig. 3a. Groups of populations as revealed by cluster analysis. Lines connecting stations (circles) represent joins in Gabriel-graph

Fig. 3b. Dendrogram based on results of cluster analysis. Arabic numerals stand for population numbers, capital letters A, B, C represent particular groups of populations shown on the map (3a)

a small yellow spot (type I) and those showing in the same place on the chromatogram a large yellow spot (type II, see Fig. 1). No intermediate forms were found.

Particular populations differed in these two spot frequencies. As these two forms are mutually exclusive (a tree bilongs either to type I or to type II) we will characterize the populations by frequencies of type I only. When mapped (Fig. 2), the differentiation seems to have a clear geographical character: the frequencies of type I trees were low in the northern part



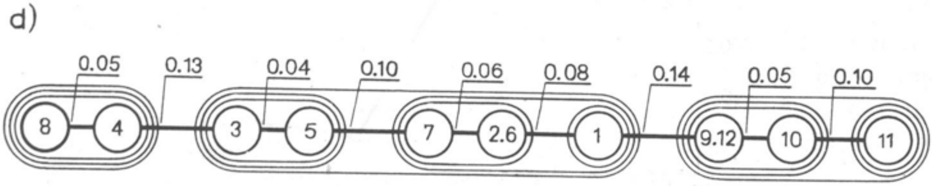


Fig. 3c. Ordination of population sites according to shortest taxonomic distances (see also Fig. 3d)

Fig. 3d. Minimum spanning tree based on shortest taxonomic distances between populations. Lengths of lines connecting the population symbols (circles with numerals standing for population numbers) are proportional to the real taxonomic distances

of the country and high in the mountains. Intermediate values were observed in the populations from the Central Polish Lowland, except in the most western one (np. 8 from Gubin) which was similar in this respect to the north-eastern populations.

The autocorrelation coefficient compouted as Moran's statistic based on the Gabriel-connected graph (Fig. 3a) was rather high, positive, and significantly different from zero:

$$I = +0.6359, \text{ (critical value } I_{005} = +0.4520).$$

It means that the neighbouring (i.e. directly connected) populations tend to be similar. This similarity extends also to the second neighbour population (i.e. population two joins or two graph edges apart). However, the situation changes dramatically if third neighbour populations (i.e. three joins apart) were compared: the autocorrelation coefficient becomes negative in this case, indicating that such populations are, on the average, dissimilar as far as their frequencies of type I trees are concerned. The same situation exists in respect to the fourth and fifth neighbours. The changes in geographical pattern are illustrated by the correlogram (Fig. 4).

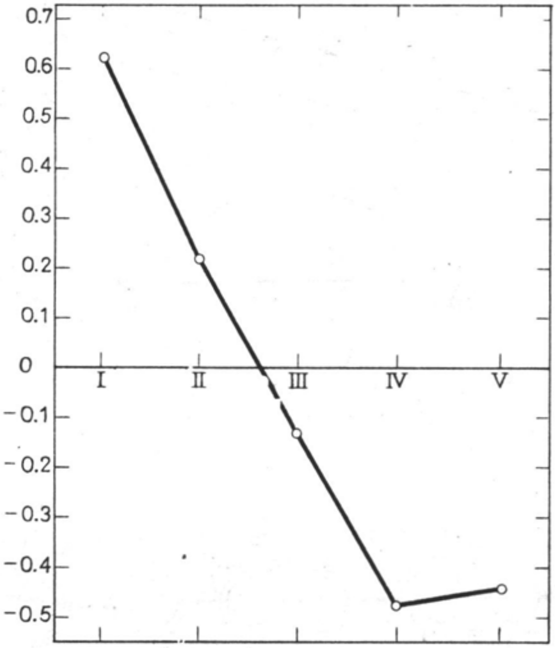


Fig. 4. Corellogram showing the dependence of autocorrelation coefficients (Morans's I) on the geographical distance measured in numbers of joins in the Gabriel-connected graph (see Fig. 3a). Vertical axis—values of I, horizontal axis—number of joins

Results of ordination (minimum spanning tree—Fig. 3c,d) and of clustering (dendrogram, Fig. 3a,b) show exactly the pattern discovered. Three north-eastern populations together with the most western one (no. 8 from Gubin) form the first group. The second one comprises also four populations from the southern and western parts of the Central Polish Lowland. And the last four populations from Kotlina Kłodzka Valley (Central Sudetes) cluster together forming the third group. The mountain populations are more closely related to the Central Lowland ones than both are to the members of the first group.

DISCUSSION

The variation in trees is usually studied in two different ways: either (i) it is done in what is called provenances, i.e. plants grown in one place from seed harvested in various natural populations or (ii)—on field material, i.e. on tress growing in natural conditions. The first method allows the modifying influence of differing conditions to be eliminated. Thus, the differences observed in this case have most probably a genetical basis. However, the resulting picture of variation is not necessarily the same as is realized in nature: natural populations are composed of plants that are survivors from a vast amount of seed and seedlings produced and their genetic structure is shaped by a long lasting selection process occuring under the growing conditions of the sites occupied. Plants grown from seeds in provenance experiments grow in conditions where selection is relaxed and, moreover, factors causing elimination of maladapted plants might be quite different from those acting on natural sites. Thus, the genetic structure of natural papulations and that of their provenances might not be the same. The second method allows studying variation directly in nature, i.e. in adult populations that are the result of selection operating *in situ*. In this case, however, the amount of variation caused by environmental factors (i.e. non-heritable variation) cannot be easily estimated.

The great advantage of studying phenolic compounds is that their occurence is but little dependent on growing conditions (McClure and Alston 1964, 1966, Alston 1967, Thielges 1972—data for *Pinus sylvestris*), at least as far as the qualitative aspect is concerned. Our material was collected during a short time interval in the winter (except the mountain populations which were sampled four months earlier). The needles used came from adult, healthy trees. Thus, we are positive about the fact that the chromatographic differences are not caused by environmental influence.

The phenolic compounds polymorphism described above seems to be intersting from several points of view. Firstly, it has a striking intrapopulational aspect: two mutually exclusive forms of pines, i.e. type I (small amount of compound no. 10) and type II (large amount of, probably, the same compound) grow together in each population studied. Secondly, their frequencies differ, and this differentiation shows a rather distinct geographical pattern: type I becomes rare southwards, while trees of type II dominate in the southern populations. As the differences between these two types are quite sharp and trees of both types occur in the same ecological conditions, there is little doubt that two genetically different forms are at hand.

According to Dobzhansky (1970), the concept of genetic races is based on discrepancies in the gene or genotype frequencies. Thus, the problem arises whether the described differentiation of Scots pine populations in Poland can be treated in this way. The solution depends on the geographical character of the observed differentiation. If the variation in pine type frequencies is discontinuous then the race concept is relevant here. If, however, the variation is of clinal character rather, no races can be distinguished. Our material is too scanty to solve this question. There are, however, some arguments speaking in favour of the first possibility. Namely, it seems that the phenolic type frequencies are not proportional to the geographical distances between populations. E.g. very similar populations nos. 4 and 8, showing 20% and 17% of type I trees, respectively, are ca. 490 km apart, whereas populations nos.

7 and 8 with the respective frequencies 47% and 17% belong to two defferent groups, in spite of the fact that their geographical distance is only 108 km. Neither does the close similarity of the population in Gubin (no. 8, the most western one) to the three populations from North-Eastern Poland (nos. 3, 4 and 5) fit the clinal variation concept: according to the latitude, the situation of this population is similar to the Central Polish group (populations nos. 1, 2, 6 and 7) whereas according to its type I frequency it belongs to the northern group, in fact it shows the lowest frequency of trees of this type.

The second problem to be discussed in connection with the described polymorphism is its biological meaning. Phenolic compounds were once considered as substances playing a minor role in shaping the adaptive value of plants (Alston 1967). However, our ideas changed as the role of phenolics in plant resistance to pathogenic fungi and plant-feeding animals (mostly insects—cf. Lunderstädt 1976, for references) was discovered. As genetic polymorphism is well known as an adaptive strategy in diminishing the intensity of predation (see e.g. discussion on the case of *Tortrix viridana* feeding on *Quercus robur* in Stern and Roche 1974, pp. 80-83) this aspect of our discovery is worth stressing. We cannot say anything on the possible link of substance no. 10 polymorphism with a fungus or insect predation. For this, more specialised studies are required. We want to stress, however, that some observations on geographic variation in the susceptibility of *Pinus sylvestris* to pathogenic fungi and phytophagous insects are already known: decline of resistance to *Phacidium infestans* and *Scleroderris lagerbergii* (pathogenic fungi) from north to south (Björkmann 1971); resistance to the pine midge (*Thecodiplosis brachyntera* (Schwägr.), an insect) increases from south-west to north-east (Skuhravy and Hochmut 1969); moreover, Polish north-eastern populations of Scots pine are resistant to the fungus *Lophodermium pinastri* (Przybylski et al. 1976); other examples can also be cited. The profound influence of plant-feeding insects and of their selectivity in attacking trees of a definite chemical constitution on the genetical differentiation are best shown by Sturgeon (1979) in the case of bark beetle predation on the ponderosa pine (*Pinus ponderosa*). The racial differentiation of this pine is a result, at least in part, of the bark beetle activity (Sturgeon 1979). Thus, the phenolic polymorphism described in our paper is certainly worth further, more thorough, study.

Acknowledgments

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REFERENCES

Alston R. E., 1967. Biochemical Systematics. Evol. Biol. 1: 197-305.

Björkman E., 1971. Resistensprövning mot parasitsvampar hos skogsträd i Sverige. Sveriges Skogsv. Förb. Tidskr. 69: 499-510.

Börtitz S., 1963. Identification of *Salicaceae* and other forest trees by paper chromatographic separation of their fluorescent constituents. Bull. Acad. Polon. Sci. Ser. Sci. Biol. 11: 549-554.

Dobzhansky T., 1970. Genetic of Evolutionary Process. Columbia University Press, New York-London.

Krzakowa M., 1979. Enzymatyczna zmienność międzypopulacyjna sosny zwyczajnej (Interpopulational differentiation of Scots pine populations revealed by isozyme electrophoresis—in Polish). Wyd. Naukowe UAM, Poznań.

Krzakowa M., Szweykowski J., 1979. Variation of 6-PGD in the populations of Polish Scots Pine (*Pinus sylvestris*). In: Proc. Conf. on Biological Genetics of Forest Trees 1978. D. Rudin (ed.). Umeå, Sweden, pp. 86-98.

Langlet O., 1959. A cline or not a cline—a question of Scots Pine. Silvae Genetica 8: 1-36.

Lunderstädt J., 1976. Isolation and Analysis of Plant Phenolics from Foliage in Relation to Species Characterization and to Resistance Against Insects and Pathogens. In: Modern Methods in Forest Genetics. J. P. Miksche (ed.). Springer, New York, pp. 158-164.

McClure J. W., Alston R. E., 1964 Patterns of selected chemical components of *Spirodela oligorhiza* formed under various conditions of axenic culture. Nature (London) 201: 311-313.

McClure J. W., Alston R. E., 1966. Chemotaxonomic Study of *Lemnaceae*. Amer. J. Bot. 53: 849-860.

Mejnartowicz L., 1979. Genetic variation is some isoenzyme loci in Scots Pine (*Pinus silvestris* L.) populations. Arboretum Kórnickie 24: 91-104.

Mirov N. T., 1967. The Genus *Pinus*. The Roland Press, New York.

Prawdin L. F., 1964. Sosna obyknoviennaya (Scots pine—in Russian). Nauka, Moskwa.

Przybylski T., 1966. Les races locales du Pin Sylvestre (*Pinus silvestris* L.) en Pologne, leur valeur génétique et leur origine. Šumarski List 90: 96-104.

Przybylski T., 1970. Zmienność sosny zwyczajnej (*Pinus silvestris* L.) polskich proweniencji. (Variation in Polish provenances of *Pinus sylvestris* L.—in Polish). Zakł. Dendr. i Arboretum Kórnickie PAN, Kórnik.

Przybylski T., Giertych M., Białobok M., 1976. Genetics of Scots Pine (*Pinus sylvestris* L.) Ann. Forest. Acad. Sci. Art. Slavorum Merid. Zagreb 7: 59-105.

Skuhravy U., Hochmut R., 1969. Befallsdichte der Kiefernnadelgallmücke *Thecodiplosis brachyntera* (Schwägr.) an *Pinus silvestris* L. verschiedener Provenienz. Anz. Schädlingsk. 42: 165-169.

Sokal R. R., Oden N. L., 1978. Spatial autocorrelation in biology. 1. Methodology. Biol. J. Linn. Soc. 10: 199-228.

Staszkiewicz J., 1968. Badania nad sosną zwyczajną z Europy południowo-wschodniej i Kaukazu oraz jej stosunkiem do sosny z innych obszarów Europy oparte na zmienności szyszek. (Studies on cone variation in Scots pine from S. E. Europe and Caucasus and on its relationship to the same species from other parts of Europe—in Polish). Fragm. Flor. Geobot. 14: 259-315.

Staszkiewicz J., 1970. Systematyka i zmienność. (Systematics and Variation—in Polish). In: Sosna zwyczajna, *Pinus silvestris* L. S. Białobok (ed.). PWN, Warszawa, pp. 55-77.

Stern K., Roche L., 1974. Genetics of Forest Ecosystems. Springer, Berlin-Heidelberg-New York.

Sturgeon K. B., 1979. Monoterpene Variation in Ponderosa Pine Xylem Resin Related to Western Pine Beetle Predation. Evolution 33: 803-814.

Thielges B. A., 1972. Intraspecific Variation in Foliage Polyphenols of *Pinus* (Subsection *Sylvestres*). Silvae Genetica 21: 114-119.

Wright J. W., Bull W. J., 1963. Geographic Variation in Scotch Pine. Silvae Genetica 12: 1-25.

Interesujący plimorfizm chemiczny u sosny

Streszczenie

Opisano wewnątrz- i międzypopulacyjny polimorfizm w 12 polskich populacjach sosny zwyczajnej. Istnieją dwie wykluczające się formy tego drzewa we wszystkich badanych populacjach. Na podstawie różnic w częstości jednej z form wyróżniono trzy grupy geograficzne. Przeprowadzono dyskusję nad charakterem i znaczeniem odkrytego polimorfizmu.