ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 50, nr 3: 481—492
1981

Geographic and climatic differentiation of electrophoretic forms of esterase, glutamate dehydrogenase and peroxidase in Scots pine tissues

BARBARA KIELISZEWSKA-ROKICKA

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

(Received: January 5, 1980)

Abstract

The enzymatic systems have been investigated in individual trees derived from 17 distant localities in Europe and Asia. Esterase and glutamate dehydrogenase polymorphism was determined in extracts from mixture of 50 macrogametophytes, peroxidase polymorphism was investigated in needle extracts from 3-month-old seedlings.

While testing by disc electrophoresis needle extracts of Scots pine deriving from specimens of different geographic ranges it was found that those from the South displayed more isoperoxidases than those from the North. Most pronounced differences in number and relative activity of esterase and glutamate dehydrogenase isozymes were found in the specimens originating from isolated stands. It is suggested that hight temperatures and dry summer periods could influence the number and activity of the isozymes.

INTRODUCTION

The geographic distribution of Scots pine (*Pinus silvestris* L.) is of a continuous type with outlier locations along its border; it comprises a huge area from the Seas of Okhotsk and Japan up to the Atlantic Ocean (Szafer 1949). This species is characterized by a marked variation in its physiological and morphological features. A great number of races of Scots pine have been distinguished so far, however there is still no uniform intraspecific system. Recently, numerous biochemical studies have been performed in order to determine the genetic distance between populations of Scots pine on the basis of analysis of enzymatic polymorphism (Mejnartowicz 1979). Stimultaneously great attention was paid to the relations between the

frequency of occurrence of some electrophoretic forms of enzymes and climatic conditions of particular localities (Mitton et al. 1977, Yang et al. 1977, Bergmann 1975, 1978, Rudin and Ekberg 1978).

MATERIAL AND METHODS

Seed samples were collected from 17 single trees of Scots pine origination from distant localities in Europe and Asia (Fig. 1). Esterase and glutamate dehydrogenase isoenzymes were analysed in extracts obtained from a mixture of 50 macrogametophytes, peroxidase isoenzymes were determined in extracts from needles of 3-month-old seedlings grown in a greenhouse (16-h photoperiod) watered with Laiho (1970) nutrient solution.

ISOLATION OF ENZYMES

Seeds were allowed to swell for 3 days on wet paper at 5°C. Macrogametophytes were homogenized in 0.05 M Tris-glycine buffer pH 8.3 containing 20% sucrose and 0.2% ascorbic acid (4 µl of buffer was used per 1 mg of fresh weight). The homogenate was then centrifuged for 30 min at 14000×g and the supernatant used for electrophoresis. Needles were homogenized in cold 80% acetone, proteins were then precipitated, filtered under vacuum, rinsed with anhydrous acetone and dried. Proteins were eluted from the acetone powder with Tris-glicyne buffer pH 8.3 and centrifuged for 30 min at 14000×g. The supernatant was used for electrophoresis.

ELECTROPHORESIS

Enzymatic extracts were separated on polyacrylamide gels using disc electrophoresis according to Davis (1964). Proteins were determined according to Potty (1969). Separation was conducted for 3 hours at 5°C, using 20 V per one gel column 5 mm in diameter.

STAINING

Esterase was stained with alpha-naphtyl acetate as a substrate (Sahulka and Beneš 1969), glutamate dehydrogenase using glutamic acid as a substrate (Hadačova and Sahulka 1973) and peroxidase with benzidine as a substrate (Safonov et al. 1969).

The absorbance of the bands was recorded in Vitatron MPS densitometer with an integrator counter.

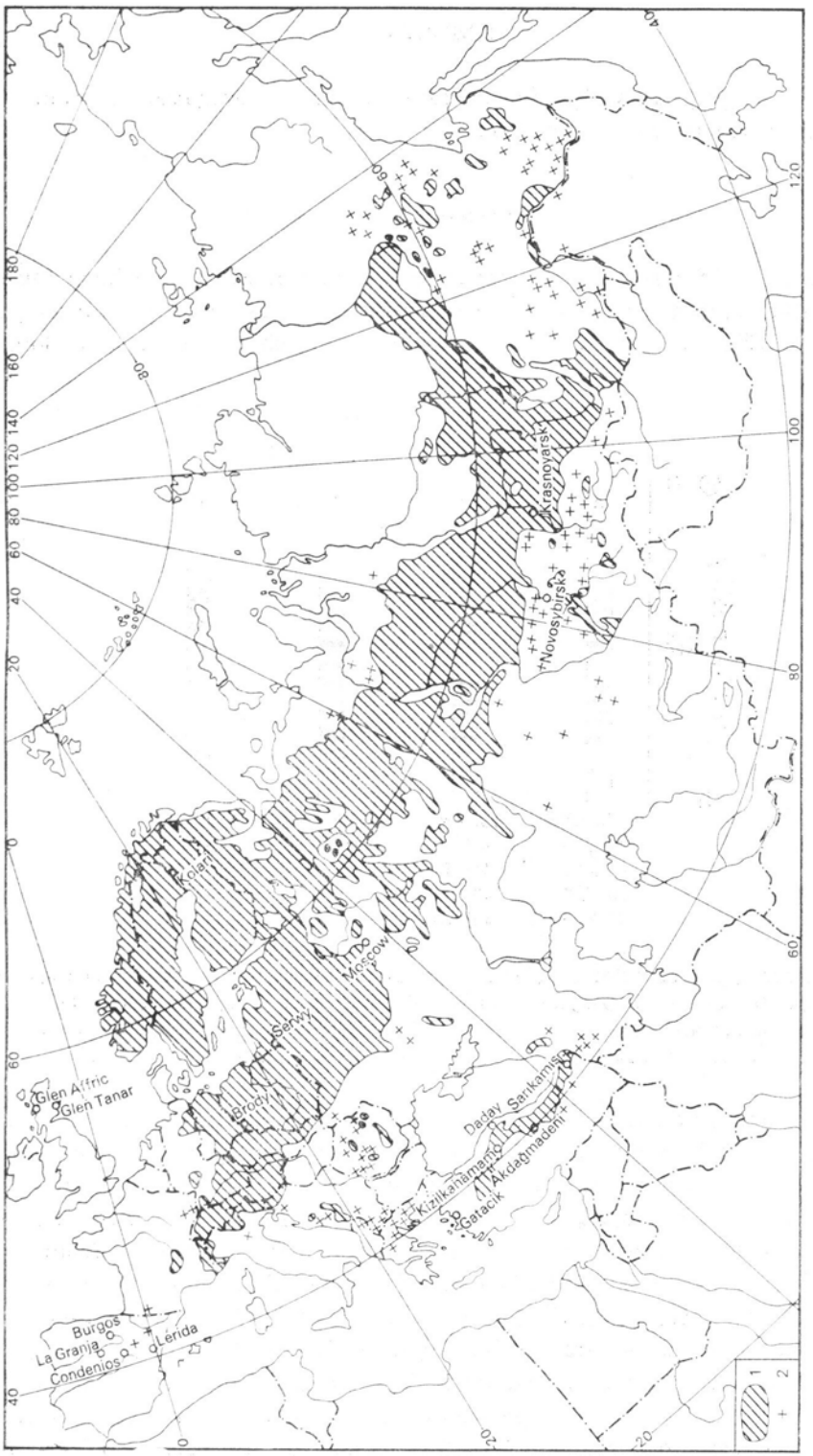

Fig. 1. Distribution of Scots pine (*Pinus silvestris* L.) in Europe and Asia
Locations of the investigated trees are indicated as spots. 1 — *Pinus silvestris*; 2 — isolated occurrance

RESULTS

Some geographic and climatic data of the investigated locations of Scots pine are presented in Table 1.

ESTERASE (EST)

A total of 16 different electrophoretic forms of EST have been found in 17 investigated Scots pine trees (from 12 to 14 different forms per one tree) (Fig. 2). Trees originating from Polish, Turkish and Soviet

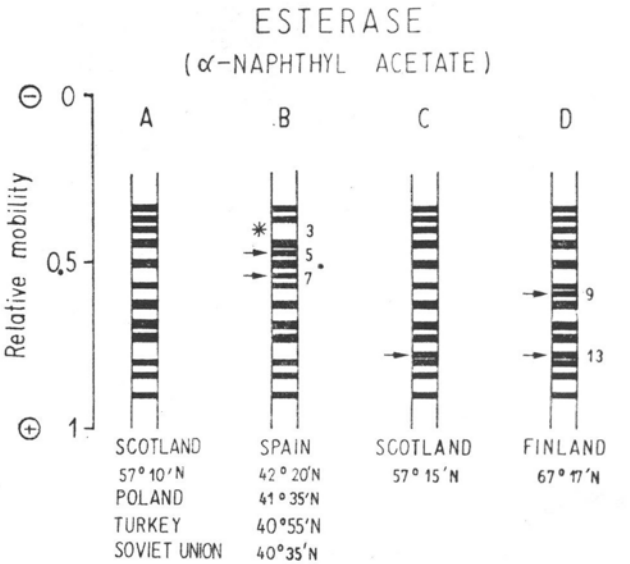

Fig. 2. Four types of esterase electropherograms (A, B, C, D) from macrogametophytes of 17 investigated individuals and their geographic latitude
Additional bands are indicated by arrows. The lack of a given band in a comparison with the 12-banded isozymic pattern is indicated by asterisks

localities and from the Scottish locality Glen Tanar possessed 12 bands of EST. 13 EST bands were found in macrogametophytes from Spain i.e. there were 2 additional bands EST-5 (Rf 0.46) and EST-7 (Rf 0.53). On the other hand, band EST-3 (Rf 0.40) was absent in these samples. Individuals from the Scottish locality Glen Affric differed from the 12-banded trees by an additional anodal band EST-13 (Rf 0.78) while the most northerly located tree (Finland-Kolari 67°17′) possessed two additional bands of esterase EST-9 (Rf 0.59) and EST-13 (Rf 0.78) in comparison with the 12-banded individuals. Fig. 3 presents densitometer tracings of EST activity in particular electrophoretic fractions. Worth noting are the proportions of EST-4 band to the band EST-6. The

Table 1

Geographic coordinates and some climatic data of the investigated localities of Scots pine. Data on temperature and precipitation were compiled after Walter and Lieth (1960)

Country	Locality	Latitude	Longitude	Altitude (m)	Temperature (°C)			Precipitation (mm)		Type of pattern*		
					mean annual	mean of July	absolute minimum	annual sum-total	mean of July	EST	PO	GDH
Finland	Kolari	67°17′N	23°51′E	155	−2.1	13.0	−40.0	365	65	D	B	B
Scotland	Glen Affric	57°15′N	5°00′W	57	8.3	13.0	−13.9	2008	105	C	B	B
Scotland	Glen Tanar	57°10′N	2°55′W	278	6.5	13.0	−20.0	844	78	A	B	B
USSR	Krasnoyarsk	56°00′N	92°50′E	155	0.6	19.0	−48.9	308	65	A	B	B
USSR	Moscow	55°50′N	37°20′E	167	3.2	17.0	−40.8	538	70	A	A	B
USSR	Novosybirsk	55°00′N	83°00′E	107	−0.3	18.0		376	60	A	B	B
Poland	Serwy	53°50′N	23°15′E	130	6.2	17.0	−30.0	524	80	A	A	B
Poland	Brody	51°45′N	14°45′E	74	8.5	18.0	−29.4	589	80	A	A	B
Spain	Burgos	42°20′N	3°40′W	860	9.8	17.0	−14.0	453	18	B	C	A
Spain	Lerida	41°35′N	0°35′E	150	14.0	23.0	−10.4	339	18	B	C	A
Spain	La Granja	40°55′N	4°10′W	1005	11.0	22.0		549	20	B	D	A
Spain	Condenios	40°35′N	3°10′W	695	12.8	22.0	−12.0	386	12	B	D	A
Turkey	Daday	41°30′N	27°10′E	800	9.8	20.0	−26.9	450	30	A	F	B
Turkey	Sarikamis	40°20′N	42°35′E	2300	4.1	18.0	−37.0	530	62	A	E	B
Turkey	Kizilkahaman	40°30′N	33°20′E	728	10.1	20.0	−34.0	523	20	A	E	B
Turkey	Akdagmadeni	39°40′N	35°50′N	1920	8.6	19.0	−23.7	528	10	A	E	B
Turkey	Gatacik	38°10′N	32°35′E	1500	10.7	22.0	−26.3	362	10	A	E	B

* See Figs. 2, 4 and 5.

relation between EST-4 and EST-6 activities was expressed as the rate of absorption of the coloured product of the enzymatic reaction and was higher for trees of north and middle european provenances than for trees originating from southern parts of the Scots pine distribution range.

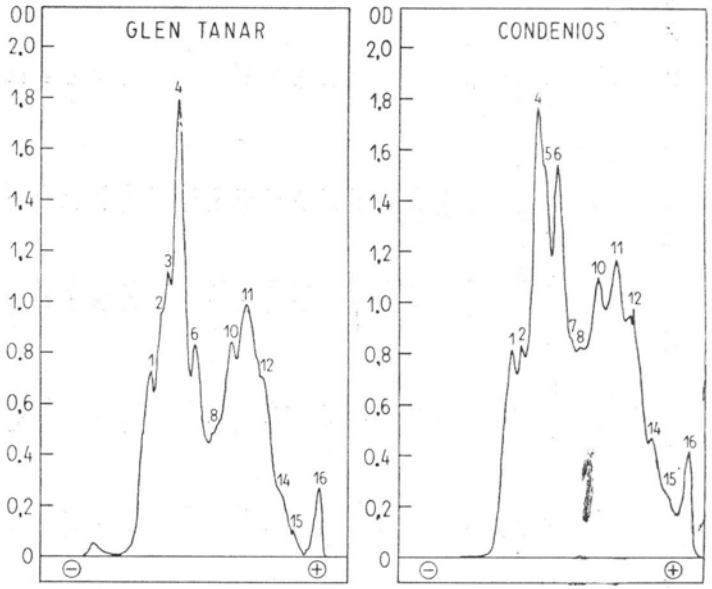

Fig. 3. Scans of disc electropherograms of esterase from two individual trees: Glen Tanar (Scotland) and Condenios (Spain)

GLUTAMATE DEHYDROGENASE (GDH)

Two fractions of GDH were found in all investigated macrogametophytes after separation on polyacrylamide gels (Fig. 4). In trees from Spain, the more anodal fraction (GDH-2) had an approximately 20 times lower activity than the more cathodal fraction (GDH-1) while trees from all remaining localities indicated similar activity of these two fractions.

PEROXIDASE (PO)

From 10 to 14 PO bands were determined in needle extracts from 3-month-old Scots pine seedlings (Fig. 5). Trees located in the southern parts of Scots pine range possessed more electrophoretic forms of PO than those from northern localities. Band PO-2 was present in trees

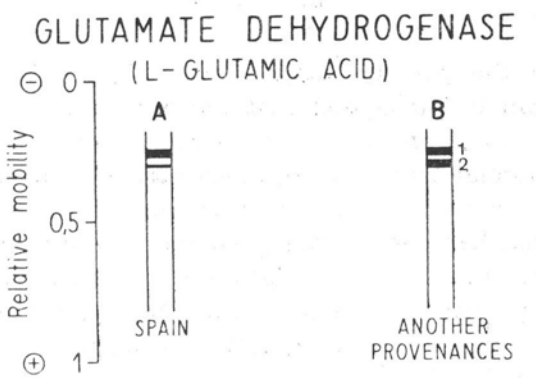

Fig. 4. Two types of glutamate dehydrogenase electropherograms from Scots pine macrogametophytes

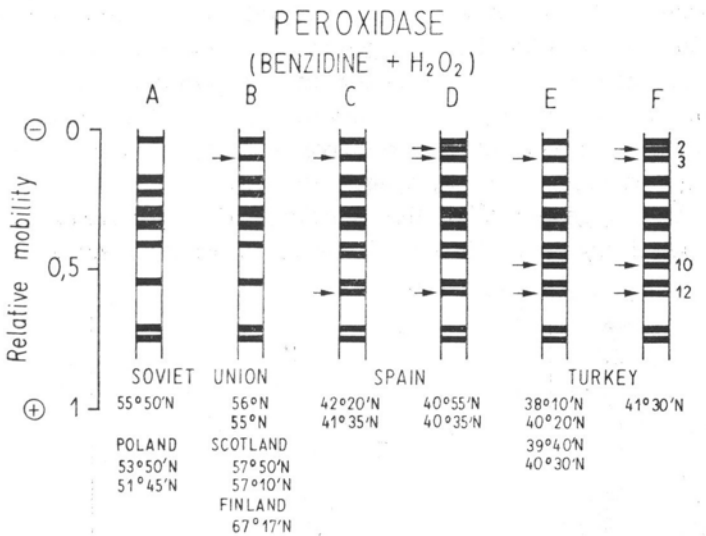

Fig. 5. Six types of peroxidase electropherograms (A, B, C, D, E, F) from needles of 3-month-old seedlings representing progeny of the 17 investigated trees Geographic latitude of the investigated trees is presented below the electropherograms. Additional bands in comparison with isoenzymatic patterns consisting of the lowest number of bands, are indicated by arrows

from the two Spanish localities (La Granja and Condenios) and one Turkish locality Daday. Band PO-10 occurred only in trees originating from Turkey. Band PO-12 occurred only in trees from Turkey and Spain. On the other hand, band PO-3 was absent in trees from the Polish localities Serwy and Brody as well as from the Russian locality Moscow.

DISCUSSION

According to the present analysis of EST, GDH and PO electropherograms it can be postulated that the occurrence of certain electrophoretic forms of these enzymes was not related to geographic longitude, altitude, mean annual temperature, mean annual sum-total of precipitation in mm and absolute minimum of temperature. On the other hand, a clear correlation has been found between the number of PO electrophoretic fractions and geographic latitude as well as summer drought. Trees originating from dry areas (Spain, Turkey) possessed 2 to 4 PO bands more than trees originating from northern areas where drought does not occurr (Fig. 5).

In most of the investigated trees from the northern part of Scots pine range the acitivity of the EST-6 band was 1.5 to 2 times lower than that of the EST-4 band. On the other hand, the above bands had almost equal activity in trees originating from areas where summer drought occurs. Fig. 6 presents the correlation between the proportion of EST-4 to EST-6 activities and geographic latitude. It is possible that high temperature and low humidity in the summer can result in an increase of the frequency of occurrence of the EST-6 band. This is reflected in an increase of the relative activity of the EST-6 band in the investigated mixture of macrogametophytes.

It can be suggested that the splitting of a previously continuous range followed by formation of isolated localities resulted in genetic

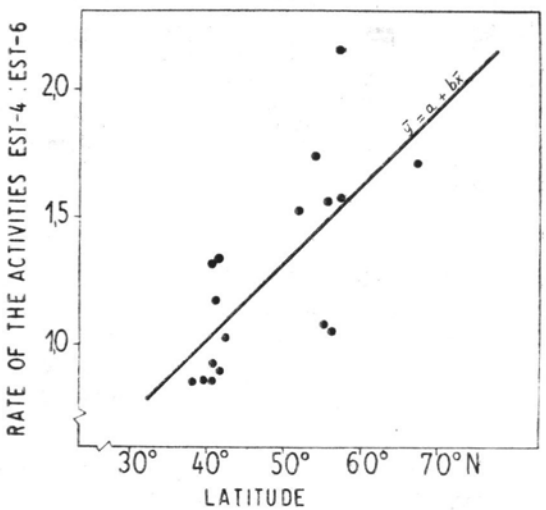

Fig. 6. Correlation between the proportion of two esterase band activities (EST-4 to EST-6) in macrogametophytes and geographic latitude of the investigated trees

The enzymatic activity is expressed as the absorbance of the bands at 430 nm

fixation of certain changes in populations of Scots pine. Taking into account the occurrence of EST electrophoretic forms, the Scots pine originating from isolated Spanish localities differed most significantly from the others in the presence of bands EST-5 and EST-7 and in the absence of the EST-3 band. On the other hand, band EST-13 was present only in the isolated Scottish locality (Fig. 2).

Only in the case of Spanish localities did the GDH-2 band have a 20-times lower relative activity than band GDH-1, which suggests that the former is much less frequent in trees from these localities (Fig. 4).

Tigerstedt (1974) indicated the correlation between latitude and the number of heterozygotes in 9 populations of Norway spruce (*Picea abies*). Marginal populations of this species possessed more leucine aminopeptidase (LAP) heterozygotes than central ones while the reverse was true for esterase. Mejnartowicz (1979) indicated that variation of 19 populations of Scots pine with regard to the frequency of occurrence of LAP and acid phosphatase (APH) patterns was higher along the geographical longitude than latitude. Yang et al. (1977) studied geographic variation of some enzymatic systems in young seedlings of Douglas fir derived from 9 natural populations in United States and Canada. The above authors indicated that certain environmental factors like soil type and stress of humidity in summer may have a marked selective effect on the proportions of EST-B and glutamate-oxalate-transaminase-B (GOT-B) heterozygotes. Bergmann (1975, 1978) indicated the correlation between a cold climate and the frequency of occurrence of alleles coding for the single band of acid phosphatase APH-B_1/B_2. Alleles coding for the double pattern APH-B_3/B_4 occurred more frequently in zones of moderate climate. The same author suggested that one or more temperature parameters can have some selective effect on the enzymatic patterns. Rudin and Ekberg (1978) take into account that temperature may influence the frequency of recombination since at higher temperatures the frequency of crossing-over is higher than at lower ones.

In the twenties and thirties of this century extensive morphological studies of Scots pine were initiated and then continued by numerous workers. Wright and Bull (1963) according to the provenance studies carried out on 3-year-old seedlings, distinguished 14 geographic ecotypes of Scots pine from Europe and Siberian part of Asia, differing in morphological and physiological characters. Information on variation of Scots pine was then completed by provenance studies carried out by Patlaj (1965) who distinguished 15 races of this species growing in USSR.

Scots pine individuals investigated in the present study represent several geographic ecotypes (Table 2). Electrophoretic patterns of EST,

Table 2

Variation of isoenzymatic patterns of esterase (EST), peroxidase (PO) and glutamate dehydrogenase (GDH) in trees originating from 17 localities in Europe and Asia. The presence or absence of a given electrophoretic fraction are indicated by (+) and (−) respectively

Locality	E-co-ty-pe	Group of ecotypes	EST					PO					GDH	
			3	5	7	9	13	2	3	9	10	12	1	2
Kolari	A	Scandina-vian-Baltic--Siberian	+	−	−	+	+	−	+	−	−	−	+	+
Glen Affric	L	West-Euro-pean	+	−	−	−	+	−	+	−	−	−	+	+
Glen Tanar	L	West-Euro-pean	+	−	−	−	−	−	+	−	−	−	+	+
Krasnoyarsk	E	Scandina-vian-Baltic--Siberian	+	−	−	−	−	−	+	−	−	−	+	+
Novosybirsk	E	Scandina-vian-Baltic--Siberian	+	−	−	−	−	−	+	−	−	−	+	+
Moscow	VI	according to Patlaj 1955	+	−	−	−	−	−	−	−	−	−	+	+
Serwy	F	Middle-Eu-ropean	+	−	−	−	−	−	−	−	−	−	+	+
Brody	G	Middle-Eu-ropean	+	−	−	−	−	−	−	−	−	−	+	+
Burgos	N	West-Euro-pean	−	+	+	−	−	−	+	−	−	+	+	+
Lerida	N	West-Euro-pean	−	+	+	−	−	−	+	−	−	+	+	+
La Granja	N	West-Euro-pean	−	+	+	−	−	+	+	−	−	+	+	+
Condenios	N	West-Euro-pean	−	+	+	−	−	+	+	−	−	+	+	+
Daday	K	South-East--European	+	−	−	−	−	+	+	+	+	+	+	+
Sarikamis	K	South-East--European	+	−	−	−	−	−	+	+	+	+	+	+
Kizilkaha-man	K	South-East--European	+	−	−	−	−	−	+	+	+	+	+	+
Akdagma-deni	K	South-East--European	+	−	−	−	−	−	+	+	+	+	+	+
Gatacik	K	South-East--European	+	−	−	−	−	−	+	+	+	+	+	+

GDH and PO are mainly in accordance with the ecotypic division of Scots pine distribution presented by Wright and Bull (1963). A comparison of isoenzyme patterns of these enzymes calls for a special treatment of trees from Spain distinguished as N-ecotype (West-European group). Enzymatic systems of EST and PO from these individuals differed significantly from the other trees studied here. Furthermore, isoenzymatic patterns of PO support the existence of differences within this ecotype. Svoboda (1953) divided Spanish Scots pines into 2 races: Pyreneian and Iberian. In the present study, Scots pines of the Iberian race (La Granja and Condenios) possessed band PO-2 while it was absent in pines of Pyreneian race (Burgos, Lerida). Pines originating from Kolari (Finland), Krasnoyarsk and Novosybirsk (USSR) belonging to the group of Scandinavian-Baltic-Siberian ecotypes had identicial systems of PO and GDH, however, the pine from Kolari (ecotype A) differed from the Russian pines (ecotype E) in the EST system. Individuals from Serwy and Brody (Poland) representing ecotypes F and G respectively possessed the same EST, GDH and PO systems as an individual from Moscow (race VI according to Patlaj 1965).

Results presented here support suggestions of the other authors that among various climatic parameters, high temperature in summer be related to climatic conditions of a given geographic area. It is possible that among various climatic parameters, high temperature in summer and drought may have the most pronounced effect upon selection of certain forms of enzymes. Moreover, geographic isolation also had a significant effect on the intraspecific variation of Scots pine.

Acknowledgments

The author is much indebted to Prof. Mirosław Tomaszewski for valuable advices and for review of the manuscript; and to Mrs Danuta Ratajczak for technical assistance.

REFERENCES

Bergmann F., 1975. Adaptative acid phosphatase polymorphism in conifer seeds. Silvae Genetica 24: 175-177.

Bergmann F., 1978. The allelic distribution at an acid phosphatase locus in Norway spruce (*Picea abies*) along similar climatic gradients. Theor. Appl. Genet. 52: 57-64.

Davis B. J., 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. New York Acad. Sci. 121: 404-427.

Hadačova V., Sahulka J., 1973. The regulation of glutamate dehydrogenase isozymes by nitrite in isolated pea roots. Biol. Plant. 15: 346-349.

Laiho O., 1970. *Paxillus involutus* as a mycorrhizal symbiont of forest trees. Acta Forest Suec. 106: 1-72.

Mejnartowicz L., 1979. Genetic variation in some isoenzyme loci in Scots pine (*Pinus silvestris*) populations. Arboretum Kórnickie 24.
Mitton J. B., Linhard Y. B., Hamrick J. L., Beckman J. S., 1977. Observation on the genetic structure and mating system of Ponderosa pine in the Colorado front range. Theor. Appl. Genet. 51: 5-13.
Patlaj I. N., 1965. Vliyane geograficheskogo proiskhozhdenia semian na rost i ustoychivost sosny v kulturakh severnoy levoberezhnoy chasti USSR. Ukrainskaya Selskokhozaystvennaya Akademia Kiev (Avtoreferat).
Potty V. H., 1969. Determination of protein in the presence of phenols and pectin. Anal. Bioch. 29: 535-539.
Rudin D., Ekberg L., 1978. Linkage studies in *Pinus silvestris* L. — using makrogametophyte allozymes. Silvae Genet. 27: 1-12.
Safonov W. I., Safonova M. P., Narbut A. N., 1969. Studies on the soybean seed proteins by means of disc electrophoresis. Biol. Plant. 11: 183-189.
Sahulka J., Beneš K., 1969. Fractions of non-specific esterase in root tip of *Vicia faba* L. revealed by disc electrophoresis in acrylamide gel. Biol. Plant. 11: 23-33.
Svoboda P., 1953. Lesni dřeviny a jejich porosty. I. 411. Praha. Státni Zem. Nakl.
Szafer W., 1949. Zarys ogólnej geografii roślin. Czytelnik, Warszawa. pp. 230-234.
Tigerstedt P. M. A., 1974. Genetic structure of *Picea abies* populations as determined by the isozyme approach. Proc. IUFRO Joint Meet. Work. Parties on Pop. and Ecolog. Genetics, Breed Theory and Progeny Test. Stockholm 1974.
Walter H., Lieth H., 1960. Klimadiagramm-Weltatlas. Gustav Fischer Verlag, Jena.
Wright J. W., Bull W. I., 1963. Geographic variation in Scotch pine. Silvae Genet. 12: 1-25.
Yang J. Ch., Ching T. M., Ching K. K., 1977. Isoenzyme variation of Coastal Douglas-fir. I. A study of geographical variation in three enzyme systems. Silvae Genet. 26: 10-18.

Geograficzno-klimatyczne zróżnicowanie form elektroforetycznych esterazy, dehydrogenazy glutaminianowej i peroksydazy w tkankach sosny zwyczajnej

Streszczenie

Stosując elektroforezę w żelu akrylamidowym analizowano trzy systemy enzymatyczne w tkankach osobników sosny zwyczajnej pochodzących z 17 odległych od siebie stanowisk w Europie i Azji. Esterazę i dehydrogenazę glutaminianową badano w ekstraktach z 50 makrogametofitów połączonych razem, a peroksydazę w ekstraktach igieł 3 miesięcznych siewek. Osobniki sosny rosnące na południu zasięgu geograficznego, gdzie występują okresy suszy, mają w igłach więcej pasm elektroforetycznych peroksydazy niż osobniki ze stanowisk północnych. Najistotniejsze różnice w ilości i względnej aktywności pasm esterazy i dehydrogenazy glutaminianowej stwierdzono u osobników ze stanowisk izolowanych. Wydaje się, że wysoka temperatura i brak wilgoci w lecie mogą wpływać na zmniejszenie częstości występowania niektórych form elektroforetycznych enzymów i ujawnianie się innych form w tkankach sosny zwyczajnej.