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Geographic Variation in European Black Pine

By CHEN HUI LEE[1])

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Introduction

European black pine (***Pinus** nigra* ARNOLD) is a widely distributed species in southern Europe. It has been planted extensively in other parts of the world and in many areas is one of the most important timber trees. In the United States it has been used mostly as an ornamental but it also has a good potential for timber production.

Provenance stwdies in other wldespread species has shown a great amount of genetic variability associated with the geographic origin of the seed. European black pine appears to be no exception. During the past century taxonomists have described many varieties and forms. A few unreplicated European provenance studies have also showed striking differences among trees grown from seed collected in different parts of the range.

My stwdy was untertaken as part of a long range project for the improvement of black pine planted in north central United States. The total project includes replicated provenance-test plantations in several states. My work was concentrated on one of the most complete of these, at the W. K. Kellogg Forest in southeasbern Michigan. This plantation furnishes information applicable to American conditions. In addition, being replicatad, it offers solutions to some problems unsolved in the earlier work. My primary objective was to determine the genetic variability pattern in a variety of morphological, growth, and physiological characteristics, and to relate that pattern to features in the original habitat of the various seedlots. A secondary objective was to provide the basic information necessary for improvement of the species by breeding.

Distribution of European Black Pine

My main source of information was a German researcher RÖHRIG's (1957) excellent work published in Silvae Genetica. Other sources included MACDONALD *et al.* (1957), SEXTON (1947) and FRITZ HALLER'S (1951) World Forest Atlas, SALVADOR (1927) and BIEL (1944). Valuable unpublished distribution data were obtained from Prof. LUIS CEBALLOS of Ciudad University, Madrid; Dr. PIERRE BOUVAREL of National Institute of Agronomy Research, Nancy; Prof. RICCARDO MORANDINI of Silvicultural Experiment Station, Firenze; Prof. MAX SCHREIBER of Hochschule für Bodenkultur, Institut für Waldbau, Wien; and the Turkish Forest Service.

European black pine has a large natural range in central and southern Europe and possibly northern Africa. It has a 13-degree latitudinal range from 35° to 48° N. and a 48-degree longitudinal range from 6° W. in Spain to 42° E. in Turkey (***Fig.*** 1). The African stands were not sampled in the present study.

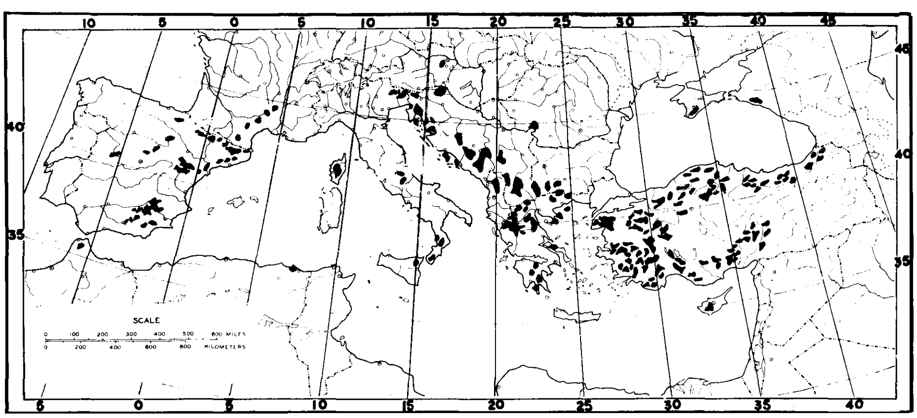

Fig. 1. — Natural distribution of European black pine.

Black pine is predominantly a mountain tree but can also be found at sea level along the shores of the Adriatic sea. In Spain, Corsica, and Italy black pine is limited to highland areas between elevations of 2,600 to 5,000 feet above sea level. On the French mainland it is found between elevations of 800 and 2,600 feet. In Austria and Yugoslavia, it is distributed bebween 1,000 and 3,000 feet; in southern Greece and Turkey it occurs up to 4,300 feet; in southern Taurus Mountains of Turkey it occurs higher than 6,000 feet.

The majority of the natural range is characterized by a Mediterranean type of climate – dry and holt summers with cool, moist winters. A few black pine stands are found at

[1]) Assistant Professor of Conservation, Wisconsin State University, Stevens Point, Wisconsin. This work is part of a thesis submitted in partial fulfillment of the Ph. D. degree from Michigan State University. The work was supported in part by funds from the U. S. Department of Agriculture as part of regional tree improvement project NC-51 titled, "Forest Tree Improvement through Selection and Breeding".

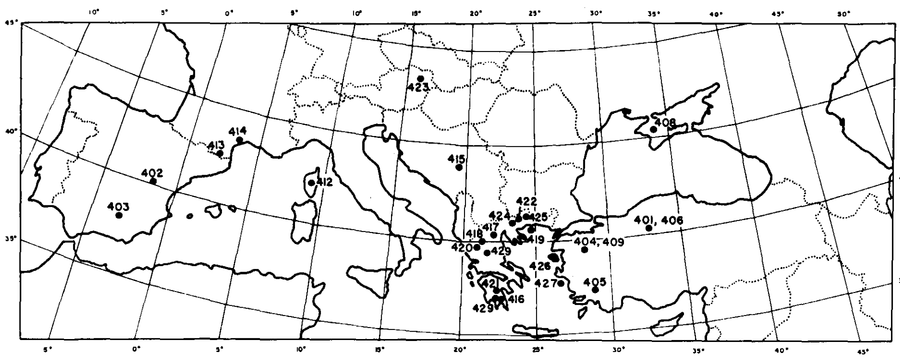

Fig. 2. — Distribution of the black pine native stands from which seed was collected for the provenance study.

high altitudes where winter frost and snow frequently occur. Annual precipitation in the areas varies from 16 to 60 inches or more.

Black pine grows on dry, usually calcareous soils. The soils are usually shallow and the slopes moderate to steep. It is sometimes able to grow in cracks in rocks. In Corsica where it is found on deep soils it grows most rapidly.

The natural stands are very scattered. In many cases the stands are less than a mile across. Pure stands occur under the most favorable growth conditions. Mixed stands are most common at the species' lower and higher distribution limits.

European black pine has been extensively planted outside its natural range. Promising results have been reported from New Zealand, Great Britain, France, Belgium, Argentina, and the United States.

Material and Methods

In the summer of 1958 black pine seed was requested from several research foresters in southern Europe. Twenty-seven seedlots *(Fig. 2)* were received from that request, each including seed from ten average trees situated within a radius of one mile. The seeds were stored dry at 35° F. until sowing.

On May 13, 1959 the seeds were sown in a 4-replicated, randomized complete block design in the research nursery at E. Lansing, Michigan. Each plot consisted of one 4-foot row. The rows were 6 inches apart and were thinned to a density of 20 seedlings per square foot. A fifth set of plots was broadcast sown to provide stock for permanent outplanting. The trees received conventional nursery care characteristic of commercial practice in central Michigan. Possibly due to greater soil fertility and lower seedbed density, they were approximately 25 percent taller than average commercial stock when placed in their permanent locations.

The study plantation (MSFGP 5-61) was planted with 2—0 seedlings on March 23, 1961. It is located in Compartment 26-D, Kellogg Forest, one mile north of Augusta, Kalamazoo County, Michigan and was planted on an 8 × 8 foot spacing. A randomized complete block design with 10 replicates and 4-tree plots was used.

The plantation site had not been used since 1948 and had a heavy sod of blue grass. This was furrowed before planting. The soil is Oshtemo loamy sand to sandy loam. The site is rolling with eastern and southern aspects. The slopes vary from 0 to 25 percent. Woody vegetation was killed with 2, 4, 5 - T during 1958—59. On May 4, 1964 Mr. W. Lemmien, resident forester of the Kellogg forest, applied a directed spray of simazine and amino-triazole to 2-foot strips containing the trees. The spray was applied at the rate of 4 pounds simazine plus two gallons of amino-triazole per acre treated.

The northernmost two trees in each plot were fertilized with 45-0-0 urea pellets at the rate of two ounces per tree (85 pounds per acre) on April 10 and April 28, 1964. This was applied to the surface of a 16-inch diameter circle around each tree. The other two trees in the plot remained unfertilized. The plantation is now considered as following a split plot design with fertilizer as the main factor, and provenance as the subfactor.

Growth and survival have been satisfactory in most cases. Only in trees from Corsica and southern France has there been appreciable winter kill and mortality.

Total height was measured in late 1963, 1964 and 1965 to the nearest half-inch. Branch angle (uppermost whorl) was measured to the nearest 5 degrees and the amount of winter damage was measured in April 1964. Mortality was counted annually.

Sampling for the study of needle anatomy was conducted on September 15, 1964. One fascicle was collected from the south side of the leader of each tree and immediately fixed in a 1-1-18 mixture of formaline-acetic acid-alcohol. A preliminary study which showed no significant differences between needles of the same fascicle indicated that this sampling procedure was sufficient. Later, 16 anatomical characters were studied on freehand section stained with Safranin 0. Paraffin sections cut at 20 microns thick and stained by the Safranin 0 and Fast Green FCF staining schedule (Johansen, 1940) were used to obtain photomicrographs.

I collected 70 foliage samples in mid-November 1964 following Dr. D. P. White's recommendation. Each sample contained needles from trees of a single seedlot and fertilizer treatment. In most cases a sample was from all 10 replicates; for 8 samples there were separate samples for the first five or second five replicates. The foliage samples were quickly brought back from the field and oven-dried immediately at 149° F. (65° C.) for 48 hours. When dried they were ground in a Wiley mill and made to pass a 20-mesh screen. The samples were analyzed spectrographically for 10 elements. Nitrogen was determined by the micro-Kjeldhal method and potassium by the flame photometry. All analyses were made at the Plant Nutrition Laboratory, Department of Horticulture, Michigan State University.[2])

[2]) The coefficients of variability due to instrumental error for the spectrographically determined elements have been calculated by Dr. A. L. Kenworthy as follows: P — 2.6%, Ca — 5.3%, Mg — 4.9%, Na — 21.2%, Mn — 5.3%, Fe — 8.4%, Cu — 14.3%, B — 7.4%, Zn — 40.0%, Al — 8.6%.

The ground needle tissue was scored independently for color by Dr. J. W. WRIGHT and myself, using 17 color grades. The coefficient of variability due to eye (instrumental error) was 39%. The MUNSELL equivalents of color grade 1 and 17 were 5.0 Y 6/6 and 5.0 Y 6/8 respectively.

The study plantation contained 540 subplots of which 36 (= 6.7 percent) were missing. Substitute values were calculated by computing the average for the surviving plots of the same provenance and treatment.

After substituting the missing plot values and reducing the degrees of freedom accordingly each set of field or anatomical measurements was subjected to analysis of variance for which the degrees of freedom were as follows: blocks — 9, fertilizer — 1, error A (block × fertilizer) — 9, provenance — 26, fertilizer × provenance — 26, error B (block × provenance plus fertilizer × prov. × block) — 432, total — 503.

For the chemical study the degrees of freedom were 1, 26, and 26 for fertilizer, provenance and error respectively. Such analyses could show the significance of main effects but not of fertilizer × provenance interaction. To do that, data from the four bulked samples (fertilized or unfertilized from replicates 1 to 5 or 6 to 10 respectively) from each of 8 selected provenances were subjected to special analyses of variance with degrees of freedom of 1, 1, 1, 7, 7, and 14 for block, fertilizer, error A, provenance, fertilizer × provenance, and error B respectively. Then the error terms derived from these special analyses were used to estimate significance of the provenance differences and provenance × fertilizer interaction for the entire 27 provenances.

Most statistical analyses were carried out with an electronic computer, CDC 3600.

Results

The study plantation had the highest survival of any of the NC-51 European black pine plantations established in 1961. At the end of the first growing season average mortality was 23 percent. Much of that was replaced at the start of the 1962 growing season. The number of dead or missing trees was 10 percent at the end of the 1962 growing season, increasing to 11.5 percent in 1963. It changed little after that.

Approximately ⅓ of the total mortality occurred in only four seedlots — three of Corsican origin and one from Greece which had suffered severe root pruning at the time of lifting. The heavy mortality of the Corsical material was probably related to the winter damage suffered by the tops of these trees while in the nursery. The roots appeared undamaged and untransplanted seedlings continued to grow, but evidently the trees' entire physiology was upset.

Growth has been average for the species in Michigan, but somewhat slower than recorded for Scotch, red, and other commonly planted pines. Despite the hilly site, within-plot variability compared favorably with that found in other productive provenance tests planted at Kellogg Forest.

Several differences in general appearance were sufficiently marked that a casual observer could quickly learn to distinguish some origins. Curved needles, slow growth, and winter damage were characteristic of the three Corsican seedlots. Seedlot No. 414 from France had unusually broad crowns. Moderately fast growing seedlot No. 423 from Austria bore its needles at nearly right angles to the twigs, so that the needles did not hide the white, resinous buds. In contrast, the Corsican and Crimean trees bore needles at an acute angle, hiding the buds from lateral view. The Spanish trees had longer and more slender needles than most other origins, and also had slender, orange twigs.

Table 1. — Growth characteristics of *Pinus nigra* from different provenances.

Country	Number of seedlots	Survival 1965 (1)	Height 1965 (2)	Branch angle (3)	Trees with winter injury 1963-64 (4)
	Number	Percent	cm.	Degrees	Percent
Corsica	3	82	63*	53*	66*
Spain	2	95	86	62	8*
France	3	97	84	57	24*
Austria	1	95	79	64	0
Yugoslavia	1	100	91	64	0
Greece (mean)	13	93	83	62	1
(range)		(50—100)	(72—116)	(55—67)	(0—3)
Turkey	3	97	85	62	1
Crimea	1	100	98	67	0

* Differs significantly (5 percent level) from the mean for the Greek population.

Growth Differences

The *growth rate* differences are summarized in *Table 1.* The slow-growing seedlots from Corsica were the only ones which could be definitely shown to be significantly different from the several Greek seedlots in the study.[3])

The growth performance of Corsican trees was, however, reported to be superior to most other black pine seed sources under European condition (DELEVOY, 1949, 1950; GATHY, 1957). The yield was even greater than for Scotch pine (MIEGROET and JANSSENS, 1956; ROBINSON, 1945). The discrepancy between European and Michigan data is possibly that Corsican trees have been planted in warmer parts of European countries.

The Austrian seedlot No. 423 was slowest growing among non-Corsican origins. This is of practical importance because most plantations in the United States are believed to be of Austrian origin. Evidently considerable improvement can be expected by planting other types.

A slight geographic pattern was evident among the thirteen seedlots from Greece. Three seedlots from the Peloponnesus Peninsula at the southern extremity of the country were below average in growth rate, with 1965 heights of 72, 76, 80 centimeters. Three seedlots from northeastern Greece (Macedonia) were above average, with 1965 heights of 94, 96, 116 centimeters.

The *branch angle,* although rarely studied, is an important character in forestry. The three Corsican seedlots were characterized by much more acute branches and were the only ones which showed significant difference from the Greek material. Trees from the rest of the range were characteristically flat branched.

Differences in *winter injury* have been demonstrated in most species with a wide natural range. Take black pine for example, Corsican trees were most heavily damaged (*Table 1*). In general, all of the three western provenances were susceptible to winter injury in Michigan. Greek and Turkish trees were only slightly injured. Those from Austria, Yu-

[3]) Because several countries were represented by one or a few seedlots, it was impossible to make one single analysis of variance to show the relative importance of between and within country variation. However, several seedlots were represented from Greece. By calculating the standard deviation for the Greek population, it was possible to test whether one of a few seedlots from another country differed significantly from that population. Hence such comparisons are made for this and other characters.

goslavia and the Crimea were resistant to the subzero climate in Michigan.

There was a similar trend in winter injury between the 2 and 3 year data (WRIGHT and BULL, 1962) and the 6 year data. Those authors were able to recognize the Corsican and non-Corsican ecotypes by this character. Interestingly all three island-inhabiting seedlots from Greece (Thasos, Lesbos and Samos Islands) suffered slight winter injury (3 percent for each). Those results suggested that the Corsican and southwestern French seed sources should be discouraged for Michigan planting.

Differences in Leaf Anatomy

DOI and MORIKAWA (1929) considered leaf anatomy important and presented a key to pine species. VIDAKOVIĆ (1957) so used this tool for identifying black pine from different parts of Yugoslavia. When the specimens are too young to bear cones needle characters are frequently used to verify pine hybridity. MERGEN (1958), VIDAKOVIĆ (1958) and KENG and LITTLE (1961) adopted this approach.

Some rarely occurring features were found in leaf cross section. Three fibrovascular bundles were observed in single leaves from three seedlots from Greece and one from Spain. One needle from a tree of MSFG 418 (Greece) was completely devoid of resin canals.

There were significant (1 percent level) between-seedlot differences in *needle length (Table 2)*. Spanish trees had by far the longest needles, and were easily recognizable by casual observers from this characteristic. The Corsican and French trees, also from Western Europe, had needles above average in length. So, too, did Crimean trees from eastern Europe.

Average needle lengths were similar for the trees from Austria, Yugoslavia, Greece and Turkey. However, within Greece there was a discernible pattern — needles were longer than average in the island-inhabiting seedlots and shorter than average in the seedlots from the Peloponnesus Peninsula.

For the species as a whole there was an apparently inverse relationship between needle length and growth rate. Among the 13 Greek seedlots there was no such relationship (rank correlation coefficient – 0.20).

According to REHDER (1927) the average needle lengths for wild-grown European trees are 80—100 mm. for Austria and Crimea, 100—140 mm. for Corsica and up to 160 mm.

Table 2. — Needle characteristics of *Pinus nigra* from different provenances.

Country	Length	Width	Color	Resin Canals No.	Resin Canals Position	Serrations per 2mm.	Rows of Stomata Ventral	Rows of Stomata Dorsal
	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
	Mm.	Mm.		No.		No.	No.	No.
Corsica	103*	1.35	blue-green*	4.4*	ext-medial*	8.7*	7.5	11.3
Spain	122*	1.34	medium	4.3	ext-medial*	6.9	7.3	11.8
France	109*	1.31	blue-green*	3.9	ext-medial*	7.6	7.5	11.7
Austria	88	1.39	green	4.3	medial	7.1	7.7	12.0
Yugoslavia	87	1.28	green	3.5	medial	7.1	7.4	11.2
Greece (mean)	83	1.31	green to	3.4	medial	7.5	7.4	11.4
(range)	74-101	1.25-1.45	medium	2.7-4.3		6.7-8.0	6.6-8.7	10.4-13.2
Turkey	87	1.36	green	3.1	medial	7.8	7.7	11.8
Crimea	102*	1.40	blue-green*	3.1	medial	7.5	8.0	12.4

Country	Hypoderm layers Ventral	Hypoderm layers Corner	Hypoderm layers Dorsal	Sclerenchyma layers	Distance between fibrovas. bundles	Endoderm Length	Endoderm Width	Endoderm Length/width ratio
	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)
	Number				Microns			Number
Corsica	1.0*	1.7*	1.1*	.5*	80	746	383	1.9
Spain	1.1	2.0*	1.2*	.9	79	769	384	2.0
France	1.0*	1.8*	1.3*	.8*	74	759	391	1.9
Austria	1.3	2.1	2.0	.8*	81	838	400	2.1*
Yugoslavia	1.2	2.2	1.8	.9	74	746	367	2.0
Greece (mean)	1.3	2.3	2.0	1.0	76	766	392	2.0
(range)	1.1-1.6	2.1-2.5	1.8-2.2	.9-1.0	62-88	720-857	366-444	1.9-2.0
Turkey	1.3	2.4	2.1	1.0	76	806	404	2.0
Crimea	1.4	2.4	2.2	1.0	78	846	441	1.9

*Differs significantly (5 percent level) from the mean of all Greek seedlots.

for Spain and France. Those are longer but in the same rank as for the Kellogg trees.

Needle width was independent of length. In general there was not much variation, most needles being about 1.3 mm.

Among the 13 Greek seedlots there was a significant (5 percent level) but possibly meaningless growth rate — needle width relation (rank correlation coefficient = 0.65).

The *color* of the *ground foliage* samples from Corsican, French and Crimean trees were slightly (but significantly) more bluish than the dark green needles typical of most of the species. The Spanish trees were intermediate, as were a few Greek seedlots. It was difficult to see any pattern within the Greek seedlots.

The variation of foliage color has been extensively studied in Scotch pine. Widely tested throughout the United States, Scandinavian and Siberian varieties are yellowest, south European ones are greenest in autumn (WRIGHT *et al.*, 1966). They recognized four different color grades for Scotch pine in the Mediterranean area (Italy, Austria-Yugoslavia, Greece-Turkey, Spain in increasing order); I observed three grades for black pine. The most striking discrepancy was that the Austrian-Yugoslavian seedlots differed from the Greek-Turkish ones in Scotch pine, whereas in black pine those four seed origins had the same color grade (green). STEINBECK (1965) stated that color difference in the fresh foliage is equally distinct as in the ground dry foliage.

The *number* of *resin canals* per needle varied from 0 to 8. The range in seedlot means was 2.7 to 4.4. The highest numbers were found in trees from western Europe and Austria, and the lowest from Turkey, Crimea and from the Peloponnesus Peninsula in southern Greece. SCHWARZ (1936) found the same east-west trend. The goegraphic varition of number of resin canals was also observed in eastern white pine. MERGEN (1963) found that northern seedlots had more resin canals than southern ones.

The numbers are far less than reported by VIDAKOVIĆ (1957) for wide-grown Yugoslavian trees. He reported a range from 7.4 to 11.1 in the mean resin canals. This character changes possibly with age. Mean number of resin canals in Austrian trees on the Michigan State University campus was 6.2 in five 20-year-old specimens; 10.3 in five 40-year-old ones. The complete absence of resin canals was reported as characteristic of lodgepole pine in the coastal and Mendocino Plain populations (ENGELMANN, 1886; MCMILLAN, 1956; CRITCHFIELD, 1957). One needle from a tree of Greek seedlot 418 was devoid of resin canals. The cause of this rare character is not known.

Position of resin canals is important in pine identification. DOI and MORIKAWA (1929) and HARLOW (1931) described black pine as having medial canals. This characteristic is typical of most of the seedlots, however, those from western range had canals slightly toward external position which was statistically significant from the average Greek seedlots.

Number of serrations per two millimeters gives an indication of needle smoothness. The range in seedlot means was 6.7 to 8.7 Corsican trees being greatest (coarsest needle edge) of all. The rest of the seedlots had needles similarly serrated as did the Greek ones. MERGEN (1963) observed that eastern white pine from southern latitudes was less serrated than that from northern ones.

Number of stomata is relatively independent of the environment. MERGEN (1963) demonstrated more stomata in eastern white pine from southern latitudes than from northern ones. A study by MERGEN (1958) on slash pine from 12 geographic locations indicated that seedlings from eastern origins had more stomata per unit length than did those from western ones. This morphological character has been further used to verify interspecific hybridity. MERGEN (1959) examined the number of stomata in 4- to 6-year-old hybrid seedlings of several combinations. It was intermediate between the two parents .

In general, there was the similar east-west trend in the number of rows of stomata with European black pine. Seedlot means varied 6.6 to 8.7 rows in ventral stomata and 10.4 to 13.2 rows in dorsal ones. The highest numbers in ventral and dorsal stomata were found in trees from Crimea and from Samos Island in Greece; the lowest in ventral stomata from Spain and Thasos Island in Greece, and the lowest in dorsal stomata from Corsica, Yugoslavia and Thasos Island, Greece. A slight pattern was evident in the Greek material. The four seedlots from the Greek mainland were average or below average with ventral stomata of 7.4, 7.1, 7.4, 7.4 rows and with dorsal stomata of 11.3, 10.9, 11.0, 11.4 rows.

A correlation analysis using seedlot means as items showed the relationship between needle width and number of rows of stomata to be strong and positive ($r = 0.746$ for dorsal and $r = 0.750$ for ventral rows of stomata).

Hypodermal layers per needle varied 1 to 3 observed at the three locations of a needle cross section — on ventral and dorsal surfaces and at the corner. This agrees with the DOI and MORIKAWA's description (1929).

The between-seedlot differences were significant at the 1 percent level. The three western provenances had a below-average number of hypodermal layers observed in all three locations of the needles. The rest of the seedlots fell within the range of the Greek material. There were more layers of hypodermal cells at the corners than on either surface. In the latter more layers were present on the dorsal surface than on the ventral one. This pattern was common in all seedlots.

Sclerenchymatous cells were absent or present; if present, they form one or more irregular layers on the phloem side of the fibrovascular bundles in black pine leaves. Number of layers of sclerenchyma was below average for the Corsican, French and Austrian seedlots, Corsican trees had the fewest. This character is relatively uniform for the rest of the provenances and for all Greek seedlots.

Distance between fibrovascular bundles was relatively constant for the entire range.

Average endodermal lengths were similar for the entire range. However, within Greece there was a discernible pattern — endoderms were shorter than average in the three seedlots from the Peloponnesus Peninsula.

Average endodermal widths were not variable among seed origins.

Endodermal length/width ratio of the single Austrian seedlot was the largest of all (2.10); the three island-inhabiting seedlots from Greece had a low ratio (1.91, 1.95, 1.93). No pattern was visible for the rest of the range, most endoderms being elliptical in shape and with the length/width ratio of 2.0. They were composed of equal sized cells with uniformly thickened walls in this pine species.

Differences in Foliar Mineral Elements

Several tree species show differences in inherent ability to absorb mineral nutrient ions from the soil medium. YOUNGBERG (1950) found that the nutrient uptake capacity of Norway spruce from central Wisconsin was not depressed by extremely low fertility of parent soils.

The work on Scotch pine by GERHOLD (1959) and STEINBECK (1965) was of particular interest because this species has

Table 3. — Foliar mineral element concentration of *Pinus nigra* from different provenances.

Country	Nitrogen	Potassium	Phosphorus	Sodium	Calcium	Magnesium	Manganese	Iron	Copper
	(21)	(22)	(23)	(24)	(25)	(26)	(27)	(28)	(29)
	Percent	Percent	Percent	Ppm.	Percent	Percent	Ppm.	Ppm.	Ppm.
Corsica	1.31	.57	.15	51	.33	.08*	288	44	8
Spain	1.44	.62	.17	89	.39*	.12	296	63	11
France	1.49	.62	.17	59	.35	.11	316	49	10
Austria	1.52	.65	.17	44	.35	.15*	305	53	8
Yugoslavia	1.52	.61	.17	87	.34	.12	265	44	11
Greece (mean)	1.43	.61	.16	73	.31	.11	315	57	10
(range)	1.30-1.55	.55-.66	.15-.18	43-127	.27-.38	.09-.14	229-407	41-67	7-18
Turkey	1.40	.63	.16	86	.33	.11	283	51	11
Crimea	1.48	.63	.16	52	.34	.14	260	47	11

Country	Boron	Zinc	Aluminum	K/P	Na/K
	(30)	(31)	(32)	(33)	(34)
	Ppm.	Ppm.	Ppm.	Number	Number
Corsica	13*	27	339	3.77	.009
Spain	19	45*	337	3.73	.014
France	15*	35	307	3.59	.010
Austria	20	35	350	3.75	.007*
Yugoslavia	19	36	324	3.52	.014
Greece (mean)	20	30	316	3.74	.012
(range)	17-23	23-44	192-412	3.41-4.02	.008-.021
Turkey	18	39	321	4.04*	.014
Crimea	15*	49*	320	3.86	.008

***Differs significantly (5 percent level) from the mean of all Greek seedlots.**

an overlapping range with European black pine in southern Europe. Both workers indicated a difference in the foliar mineral composition among seed sources.

The coefficients of variability (= C.V.) for the 12 mineral elements were calculated by dividing the standard deviation by the plot mean and then multiplying by 100. The smaller the C.V., the more dependable the data.[4])

There was a close relationship between the C.V.'s of black pine and Scotch pine, although the coefficients were slightly higher for black pine. The correlation coefficient of the C.V. between black pine and Scotch pine at Russ, Newaygo, and Higgins Lake plantations was 0.904, 0.823 and 0.863 respectively. The three values were all statistically significant (1 percent level) with 10 degrees of freedom. In other words, elements which were most variable in Scotch pine were also variable in black pine. The strong correlation suggests that the error term derived from these two species may be applicable to others.

Table 3 shows foliar mineral composition of the species from different seed sources. Not listed in this table are the four ratios which are relatively constant among seed sources. Their range in seedlot means was 8.61 to 9.08 for N/P, 2.23 to 2.51 for N/K, 2.43 to 4.09 for Ca/Mg and 4.71 to 6.57 for Mn/Fe. The optimum N/K ratio for Japanese larch (Leyton, 1957) was 2.4, being within the range of black pine. Throughout the chemical study, copper was the only element showing a significant fertilizer × provenance interaction (1 percent level).

Nitrogen is the key nutrient for plant growth. Its requirement varies greatly between species. However, very little is known of within species variation. This study showed that there was little variation in foliar nitrogen within this pine species.

The three island-inhabiting seedlots and the three from the eastern part of the Greek mainland had nitrogen content below those from the rest of the 13 seedlots.

The range between the most extreme seedlots for black pine was 1.28 to 1.60 percent; that for Scotch pine from Russ Forest was 1.68 to 2.22 percent (Steinbeck, 1965).

Among the eight provenances there was no growth rate-foliar nitrogen relation (rank correlation coefficient = 0.32). Much slower growing Austrian trees had rather high nitrogen content.

Potassium content is fairly constant among different species. The between-seedlot range for black pine was 0.55 to 0.66 percent; the corresponding range for Scotch pine

[4]) The C.V. is showed in the order of black pine at Kellogg, Scotch pine at Russ, Newaygo and Higgins Lake for the 12 mineral elements as follows: N — 6.2, 6.1, 4.6, 4.7; K — 7.4, 7.2, 7.7, 7.3; P — 6.4, 7.4, 6.2, 7.2; Na — 41.7, 36.7, 38.8, 26.0; Ca — 12.8, 15.4, 18.6, 8.7; Mg — 19.5, 18.9, 32.2, 8.6; Mn — 18.9, 21.4, 17.6, 15.2; Fe — 16.9, 16.5, 15.9, 18.3; Cu — 28.2, 17.5, 22.9, 27.1; B — 19.5, 12.9, 11.2, 13.5; Zn — 26.9, 18.5, 25.8, 15.7; Al — 22.4, 15.1, 10.6, 13.5%.

(Russ Forest) from the STEINBECK's finding was similar (0.44 to 0.62 percent). Also revealed was that there was not much variation in the potassium content within a single species which has a wide natural distribution such as black pine.

There was a slight pattern to the Greek material. The three seedlots from the eastern part of the mainland had low potassium contents of 0.56, 0.59, 0.55 percent.

The foliar nitrogen-potassium relation was not significant (rank correlation coefficient = 0.61).

The content of foliar *phosphorus* is nearly identical among seed sources. The between-seedlot range was 0.15 to 0.18 percent for black pine; that reported for Scotch pine was 0.19 to 0.27 percent. There was no significant foliar nitrogen-phosphorus relation in black pine (rank correlation coefficient = 0.38).

The *sodium* content of black pine ranged 36 to 127 ppm. which overlapped with that of Scotch pine (18 to 134 ppm.). Some pattern was evident in the Greek population. The three seedlots each from the Peloponnesus Peninsula (69, 51, 69 ppm.) and from the eastern part of the mainland (43, 48, 58 ppm.) were characteristic of low sodium content.

Calcium presents a slight pattern to the geographic variation. Spanish seedlots had higher calcium content than did other seedlots whereas not much difference was found between European black pine (0.27 to 0.41 percent) and Scotch pine from Russ Forest (0.27 to 0.52 percent).

The *magnesium* content differred among geographic races. It was lowest in the slowest growing Corsican trees; highest in the next slowest growing Austrian ones. Within the Greek population, the three seedlots from the Peloponnesus Peninsula were low in the magnesium level (0.09, 0.10, 0.11 percent). The range for black pine (0.08 to 0.15 percent) was nearly identical to that for Scotch pine (0.04 to 0.11 percent).

It was difficult to find a pattern in foliar *manganese* for the entire range. However, the differences were moderate between the two pine species. It was 229 to 407 ppm. in black pine, the range being lower than that reported for Scotch pine (548 to 1,202 ppm.).

Foliar *iron* content was uniform throughout the range of black pine. It ranged 41 to 68 ppm.; the corresponding range for Scotch pine was higher (72 to 143 ppm.).

The *copper* level of most seedlots fell within the range of the Greek population. The three seedlots from the Peloponnesus Peninsula were considerably below average in this trace element (6.5, 10.3, 7.7 ppm.).

The two pine species had a similar range in the copper content: 6.5 to 17.5 ppm. for black pine and 6.0 to 13.7 ppm. for Scotch pine.

Boron was lowest in the slowest growing Corsican seedlots. French and Crimean trees were also low in this trace element. Within the Greek material, three seedlots from the Peloponnesus Peninsula had less foliar boron (16.7, 18.0, 18.4 ppm.) than did most others. There was also a moderate variation in foliar boron between species. It ranged 11.7 to 23.0 ppm. for European black pine; 23.6 to 52.4 ppm. for Scotch pine.

Zinc in the foliage of black pine ranged from 23 to 50 ppm.; 36 to 99 ppm. for Scotch pine.

Both Crimean and Spanish seedlots had more foliar zinc than did trees from the rest of the range. The three seedlots from the Peloponnesus Peninsula (23.4, 22.5, 26.0 ppm.) were characterized by below-average zinc content.

There was no geographic variation pattern so far the level of foliar *aluminium* was concerned. It ranged 192 to 429 ppm. for black pine, being much lower than the range for Scotch pine (725 to 1,329 ppm.).

The potassium-phosphorus ratio was found higher in Turkish seedlots than in the rest of the range. Some pattern was also recognizable within the Greek population: the three island-inhabiting seedlots (3.80, 3.78, 3.77) and the three from the Peloponnesus Peninsula (3.92, 3.75, 3.98) were above average in the K/P ratio. Its range for black pine was 3.39 (a French seedlot) to 4.22 (a Turkish seedlot).

The single Austrian seedlot had smaller *sodium-potassium ratio* than did other seedlots. Also, the three seedlots from the Peloponnesus Peninsula (0.010, 0.008, 0.011) and the three from the eastern part of Greek mainland (0.008, 0.008, 0.011) were characteristic of low in the Na/K ratio.

Application of the Provenance Test Results

The Corsican seedlots formed a separate group because of the slowest growth rate, acutest branch angle, most winter burn, highest number of resin canals, highest number of leaf serrations, lowest foliar N, P, Mg and Boron. Curly leaves were found only on the Corsican trees.

Moderate winter injury and long and soft needles were characteristic of the Spanish and French seedlots. Spanish trees differ from French ones in their yellowish brown branchlets, longer needles, higher Na, Ca and Zn.

The Austrian seedlot was separable from other winter-hardy seedlots by its slow growth rate.

The Crimean seedlot differred from the Austrian one in its faster growth rate, longer needles, more bluish foliage color, fewer resin canals, less elliptical in the endodermal shape, lower boron and higher Zn content.

The Yugoslavian-Greek-Turkish seedlots differred from the Austrian one in their faster growing, more sclerenchyma layers, lower Mg content, higher K/P and Na/K ratios. They were separable from the Crimean seedlot by their shorter needles and much greenish foliage color.

The five groups tentatively considered equivalent to previously described varieties are: Corsican (var. *poiretiana*), Spanish-French (var. *pyrenaica* and var. *cebennensis*), Austrian (var. *austriaca*), Yugoslavian-Greek-Turkish ('Balkan') and Crimean (var. *caramanica*). The breakdown was based on combined growth, anatomical and nutrient data. Of previous taxonomic classifications of the species, REHDER's (1949) coincided most closely with the results of provenance study. The major discrepancies are as follows. REHDER defined var. *cebennensis* as including both Spanish and French mainland populations, I considered these as separate because of the differences in amount of winter injury and needle length. Under var. *austriaca* REHDER included material extending from Austria to Balkan Peninsula. The provenance test showed that Austrian material differred from the Balkan.

No materials from Italy or Sicily were included in my study. I think it best to include them under, var. *poiretiana*. SCHOUW (cited in ELWES and HENRY, 1907) compared the specimens from the botanical garden at Naples with the large Corsican tree growing in the Jardin des Plantes at Paris and convinced himself of the "absolute" identity of the Corsican tree and trees in Sicily, Calabria, Sila and Aspromonte. Also, REHDER grouped Corsican, Italian and Sicilian trees together under this variety. STEVENS (1934) studied cone types, needle characters, bark and branching habit and considered the Italian trees to have gradations from the typical Austrian pine to the typical Corsican pine.

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Summary

European black pine has a 13-degree latitudinal range from 35° to 48° N., and a 48-degree longitudinal range from 6° W. to 42° E. The majority of the natural range is under influence of the Mediterranean type of climate. Some black pine stands are found at high altitudes where winter frost and snow frequently occur. The stands are sporadic and rarely continuous.

Black pine seed, collected from 27 native stands throughout the species range, was sown in the Michigan State University Bogue Nursery in the spring of 1959. Each seedlot consisted of seed from about 10 average trees per stand. In 1961 two-year old stock was used to establish the permanent test plantation at the Kellogg Forest, Augusta, Michigan following a randomized complete block design with 10 replications. Each replicate has 27 four-tree plots. In the spring of 1964, two ounces of the 45-percent urea pellets were applied to the northernmost two trees in each plot and the southernmost two trees were left unfertilized. The plantation follows a split plot design with fertilizer treatment as the main factor, and provenance as the subfactor. The study was designed to determine genetic variation pattern and to clarify taxonomic status in this species.

Three growth, 19 anatomical and 18 chemical sets of data were studied.

Western provenances were susceptible to winter burn in lower Michigan, Corsican trees being the most heavily injured. Austrian trees were winter hardy, however, they were of considerably slow growth. Seed for planting purposes in the United States should be obtained from Balkan Peninsula and from the Crimean S.S.R. No flowering was observed.

Most characters were useful in the study of geographic variation pattern. Needle length, foliage color, number of resin canals, number of serrations, hypodermal layers were of special importance. It was difficult to establish a relationship between the three major nutrients (N, P, K) and growth rate. On the other hand, the strong correlation between the coefficients of variability of black pine and Scotch pine for the 12 mineral elements suggested that the error term derived from these two species might be applicable to others.

Based on a combined judgement of growth, anatomical and chemical data it was possible to divide black pine into five distinct groups or races. The features which are most characteristic for each race and the correct varietal name were suggested and discussed. Var. *poiretiana* had most winter burn and characteristic curly leaves. Var. *pyrenaica* and var. *cebennensis* had moderate winter injury and long and soft needles. Var. *pyrenaica* differred from var. *cebennensis* in their yellow branchlets and longer needles. Var. *austriaca* was slowest growing among winter-hardy seedlots. Var. *caramanica* and var. 'Balkan' were winter hardy, however, var. *caramanica* had longer needles and much bluish foliage color than var. 'Balkan'.

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