Genetic variation in morphological and anatomical needle characteristics in the Black pine of Peloponnesos

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Summary

In a black pine *(Pinus nigra)* seed orchard, including 52 clones selected in the natural forest of Peloponnesos, Greece, ten morphological and anatomical needle characteristics (needle length, needle width, needle thickness, sheath thickness, number of serrations per cm of needle length, number of rows of stomata in the dorsal and ventral faces, number of stomata per cm of needle length in the dorsal and ventral faces and number of resin canals) were studied. The results showed that:

Significant differences exist between clones in needle length, needle thickness, needle width and sheath width; needles of clones from the southern part of Peloponnesos (xeric) were shorter than needles from the northern part. Differences between clones in the number of serrations per cm needle length, rows and number of stomata in both faces, as well as in the number of resin canals were also significant. Resin canals varied among clones from 4.0 up to 12.7 (overall mean 8.4).

Correlation coefficients among the characteristics were variable; thicker needles had more rows of stomata on both faces ($r = 0.60$ for dorsal face and $r = 0.65$ for ventral face) while needle length was found uncorrelated to the number of rows of stomata and negatively related to the number of stomata per cm row length as well as the number of serrations.

Broad sense heritabilities for the number of stomata per cm needle length in dorsal and ventral faces, serration number and number of resin canals were quite high with values 0.78, 0.74, 0.66 and 0.77 respectively, indicating strong genetic control.

Key words: Variance, stomata, serration, resin canals, dorsal face, ventral face, heritability.

Zusammenfassung

In einer *Pinus nigra*-Samenplantage mit 52 verschiedenen Klonen aus Naturwäldern des Peloponnes (Griechenland), sind 10 morphologische und anatomische Nadelmerkmale untersucht worden, und zwar: Länge, Breite und Dicke der Nadeln, Dicke der Scheide, Anzahl der Zähne pro cm Nadellänge, Anzahl der Stomata-Reihen an der dorsalen und ebenen Seite, Anzahl der Stomata pro cm innerhalb der Reihe an der dorsalen und ebenen Nadelseite und Anzahl der Harzkanäle. Die Untersuchungen haben gezeigt:

Es bestehen signifikante Klonunterschiede in der Länge, der Breite und der Dicke der Nadeln, sowie in der Dicke der Scheide. Die Nadeln der Klone aus dem südlichen Teil des Peloponnes (trockenerer Teil) sind kürzer als die Nadeln der nördlichen Gebiete. Signifikante Unterschiede zwischen den verschiedenen Klonen gibt es auch in der Anzahl der Zähne pro cm Nadellänge, der Reihenanzahl der Stomata und ihrer Anzahl an den beiden Nadelseiten, sowie in der Anzahl der Harzkanäle. Die Anzahl der Harzkanäle schwankt innerhalb der Klone zwischen 4,0 und 12,7 (Durchschnitt 8,4).

Die phänotypischen Korrelationskoeffizienten zwischen den verschiedenen Merkmalen waren variabel. Die dicksten Nadeln zeigten mehrere Reihen von Stomata an beiden Seiten ($r = 0{,}60$ für die dorsale und $r = 0{,}65$ für die ebene Seite). Zwischen der Nadellänge und der Anzahl der Stomata-Reihen konnte keine Korrelation festgestellt werden. Eine negative Korrelation besteht zwischen der gesamten Nadellänge und der Anzahl der Stomata pro cm Nadellänge, sowie der Anzahl der Zähne.

Die Heritabilitäten im weiteren Sinne für die Anzahl der Stomata pro cm Nadellänge an der dorsalen und ebenen Seite, die Anzahl der Zähne und der Harzkanäle waren beträchtlich hoch, mit jeweils 0,78; 0,74; 0,66 und 0,77. Diese Resultate zeigen, daß die genannten Merkmale einer starken genetischen Kontrolle unterliegen.

Introduction

Black pine *(Pinus nigra)* is a widespread species. Its natural distribution has been described by many authors, and a lot of information is given by VIDACOVIĆ (1974) in his monograph. In Greece it covers a discontinuous area extending from the southermost part of the Peloponnesos peninsula up to the northern border of the country (including the islands of Euboia, Thasos, Lesvos and Samos) forming many isolated populations. Altitudinally it grows from an elevation of 100 m. (area of Dadia, Thraki) up to 1800 m. (Taygetos mountain) (DEBAZAC, 1970) but mainly from 500—800 up to 1200—1600 meters (BASSIOTIS, 1967). Silviculturally it is considered one of the most important coniferous species and is extensively used in reforestation programs throughout the country. In order to satisfy the demand for large quantities of seed, clonal seed orchards have been recently established (MATZIRIS, 1978; PALMBERG, 1982). The present study refers to the clonal seed orchard of Koumani, Peloponnesos which includes 52 clones selected in the natural stands of Peloponnesos and a total number of 2 700 grafts. This orchard (established in 1978) was sampled with the primary objective of investigating genetic variability in a variety of morphological and anatomical needle characteristics and to determine the magnitude of interrelationship that may exist among these characteristics.

Material and Methods

Material

In the fall of 1978 a black pine clonal seed orchard was established which includes 2 700 grafts (at 6 × 6 meters spacing) from 52 clones selected in the natural stands of Peloponnesos. Details of the establishment are given elsewhere (MATZIRIS, 1978). At the end of the third year (December 1981) one-year-old needle samples were collected from the middle section of the longest branch on the Southern side of the uppermost whorl. The sampled block was in a homogeneous part of the seed orchard (48 × 312 meters) on which each one of the 52 clones was represented by eight ramets; the total number of grafts sampled was 52 × 8 = 416.

Measurements

The characteristics measured on five needles of each ramet were:

1. Needle length
2. Needle width
3. Needle thickness
4. Sheath thickness

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5. Number of serrations per cm of needle length
6. Number of rows of stomata per needle on the dorsal face
7. Number of rows of stomata per needle on the ventral face
8. Number of stomata per cm of row length in the dorsal face
9. Number of stomata per cm of row length in the ventral face
10. Number of resin canals in the middle of the needle.

Serrations, stomata per cm and rows of stomata were counted in midsection; incomplete rows were discarded. Free-hand cross sections were made to determine the number or resin canals.

Analyses of variance for each characteristic were made on the mean values of the five needles of each ramet using established procedures. Variance components were estimated by equating mean squares to the corresponding expectations and solving the resultant equations. The variance between clones (σ^2_C) was interpreted as:

$$\sigma^2_C = \text{Cov}(X, Y) = \sigma^2_G + \sigma^2_{CL}$$

Where: X, Y are rametes of the same clone

σ^2_G is the total genetic variance within the population represented by the physiographic area of the clones (Peloponnesos).

σ^2_{CL} is the cloning effect variance due to vegetative propagation (Shelbourne, 1969).

According to Wilcox (1974) this represents covariance among ramets and among other sub-samples of the same clone due to common physiological age and other non-genetic similarities carried from the ortet by cloning. Because the cloning variance cannot be separated from the between-clone variance, part of the clonal variation may be due to variation in ortet age. Because the age differences of the selected ortets were small it is assumed that the cloning effect variance can be ignored.

The variation between ramets within clones (σ^2_w) is a measure of the environmental variance. Differences among root-stock may be confounded with environmental differences. However, the analyses of variance showed that σ^2_w accounts for only a small proportion of the total variance and, therefore the root-stock contribution to the σ^2_w has been considered negligible and was not taken into consideration.

Results and Discussion

Needle Dimensions

The mean values of needle length per clone, needle width, needle thickness and sheath thickness are given in *Table 1*.

The overall mean of the needle length of the 52 clones was 129 mm, with a range between clone means from 93 to 173 mm. The differences between clones were statistically significant for all needle characteristics *(Table 2)*. The

Table 1. — Mean values of ten needle characteristics in 52 black pine clones grown in the seed orchard of Koumani, Peloponnesos.

Clone No	Needle Length mm	Needle Width mm	Needle thick mm	Sheath thick mm	Rows of stomata dorsal No	Rows of stomata Ventral No	stomata/cm dorsal No	stomata/cm Ventral No	Serrations/cm No	Resin canals No
1	145	1.70	1.10	2.10	13.6	8.3	112	119	33.3	11.7
2	159	1.90	1.10	2.10	15.5	10.2	113	112	25.0	8.3
3	142	1.90	1.10	2.10	18.3	11.2	111	122	36.6	12.7
4	121	1.60	1.00	1.90	12.9	8.1	120	123	30.9	5.8
5	155	1.80	1.10	2.20	16.3	11.4	109	112	35.9	11.3
6	123	1.50	0.90	1.90	11.8	7.6	120	120	30.1	6.3
7	118	1.70	1.00	2.10	16.5	9.2	122	119	32.7	9.9
8	128	1.70	0.90	1.90	14.2	10.3	122	128	35.1	8.3
9	113	1.60	0.90	1.80	12.5	8.1	128	129	37.0	4.0
10	173	1.80	1.10	2.30	15.5	9.3	104	118	28.4	8.8
11	129	1.70	1.10	2.00	16.9	10.1	122	126	32.7	7.7
12	141	1.70	1.10	2.10	15.2	9.0	106	118	27.0	9.2
13	128	1.80	1.10	2.10	18.1	10.8	114	122	32.9	10.0
14	132	1.70	1.00	1.90	14.2	9.3	113	122	32.9	7.9
15	141	1.60	1.00	2.00	14.3	8.5	128	132	36.6	8.3
16	126	1.70	1.00	1.90	16.2	10.1	118	119	32.9	11.9
17	135	1.70	1.00	1.90	15.6	9.3	119	117	28.4	7.6
18	141	1.80	1.10	1.90	15.9	9.8	99	108	26.4	10.4
19	140	1.60	1.00	1.90	14.1	8.7	110	120	33.8	10.8
20	148	1.80	1.10	2.10	16.2	10.7	117	114	28.9	9.7
21	137	1.60	1.00	1.90	14.3	9.1	129	120	35.5	9.3
22	136	1.70	1.00	2.10	15.6	9.5	135	137	35.1	8.5
23	123	1.50	1.00	1.90	13.9	8.6	115	116	29.0	6.3
24	131	1.70	1.00	1.90	15.6	9.9	135	137	28.8	7.7
25	117	1.60	1.00	1.80	14.6	9.6	108	115	32.7	9.5
26	136	1.60	1.00	2.00	13.7	8.9	111	117	36.2	7.4
27	109	1.90	1.10	2.00	16.7	11.3	132	130	31.9	12.3
28	128	1.70	1.00	1.90	15.7	9.1	123	131	35.3	11.6
29	123	1.70	1.00	2.00	15.3	9.7	118	121	30.7	8.9
30	138	1.80	1.10	2.10	16.8	10.8	125	134	32.2	7.0
31	120	1.80	1.10	2.20	15.8	10.6	138	129	34.0	8.3
32	116	1.60	1.00	1.80	14.5	8.8	116	121	31.9	5.8
33	133	1.50	0.90	1.70	13.3	8.3	106	112	34.9	5.4
34	101	1.60	1.00	1.90	15.1	9.6	121	123	35.8	11.7
35	123	1.80	1.10	2.00	14.4	8.7	134	143	31.4	8.7
36	127	1.60	1.00	1.90	14.1	8.8	117	119	29.0	7.8
37	123	1.70	1.00	2.10	15.2	8.5	123	131	32.0	8.9
38	124	1.60	1.10	1.90	13.6	9.3	120	118	37.7	7.6
39	128	2.00	1.20	2.10	18.0	11.5	126	128	34.2	11.1
40	132	1.80	1.10	2.00	14.5	9.2	132	134	33.9	8.0
41	152	1.80	1.10	2.20	16.5	10.4	118	125	27.0	7.8
42	105	1.70	0.90	2.00	14.9	10.4	131	133	33.9	7.5
43	130	1.50	1.00	1.90	15.4	10.3	109	119	22.0	7.9
44	126	1.60	1.00	1.90	14.2	9.0	106	112	27.3	6.5
45	144	1.60	1.00	2.10	13.4	8.0	138	141	28.9	5.5
46	133	1.70	1.10	2.10	16.2	9.9	131	137	29.5	9.1
47	122	1.70	1.00	2.10	15.8	9.1	130	135	39.2	7.9
48	107	1.60	1.00	1.90	14.4	8.2	118	125	34.7	7.5
49	112	1.60	1.00	2.00	14.2	8.5	136	141	35.4	5.3
50	132	1.60	1.00	2.00	13.0	8.3	128	128	31.6	4.7
51	138	1.60	1.00	1.90	13.7	8.3	131	136	32.6	7.3
52	93	1.50	1.00	1.80	16.8	10.0	127	128	29.4	6.1

Table 2. — Analyses of variance, mean values, variance components and heritability estimates for needle characteristics of black Pine.

Source of variation	D.F.	MEAN SQUARES 1/ Needle length	Needle width	Needle thick.	Sheath thick.	Rows st. dorsal	Rows st. ventral	Stom./ cm dorsal	Stom./ cm ventral	Serra-tions/ cm.	Resin canals
Between clones	51	1716.97**	9.53**	2.88**	11.33**	161.67**	73.93**	7264.34**	2984.89**	998.60**	336.08**
Within clones	364	258.90	0.87	0.35	1.92	12.34	6.19	239.48	254.76	61.23	11.69
Overall mean		12.9	1.68	1.02	1.99	15.0	9.4	120.8	124.6	82.1	8.4
Coef. of var. (C.V%)		12.4	5.51	5.1	6.9	7.4	8.3	4.0	4.0	7.7	12.8
Total genetic var. σ_C^2		182.25	1.080	0.320	1.170	18.670	8.470	878.11	716.140	117.17	405.49
Environmental var. σ_W^2		258.90	0.870	0.350	1.920	12.340	6.190	239.48	254.76	61.23	116.96
Broad sense heritabil. (H_1)		0.41	0.56	0.47	0.38	0.60	0.58	0.78	0.74	0.66	0.77
Broad sense heritabil. on clone mean basis (H_2)		0.85	0.91	0.88	0.83	0.92	0.92	0.97	0.96	0.94	0.97

1/** Statistically significant at the 0.01 probability level

2/ $H_1 = \frac{\sigma_C^2}{\sigma_C^2 + \sigma_W^2}$ $H_2 = \frac{\sigma_C^2}{\sigma_C^2 + \sigma_{W/n}^2}$; n= number of ramets per clone= 8

within-clone variance, which is explained by the differential response of the ramets of the same clone to microenvironmental changes, was in all cases quite small. Lee (1968), studying a four-year-old provenance test of black pine, found that the overall mean for needle length was 83 mm with a maximum mean value of 122 mm (provenance from Spain). Bassiotis (1967) has also reported a mean needle length of 99 mm in data obtained from 23 naturally- grown provenances of black pine in Greece. However, these results are not directly comparable with the longer needles found in the present study because they refer to different materials.

When clones were classified in groups according to their origin it was found that those from the southern part of the range (Parnon and Taygetos mountains) had shorter needles (123 and 125 mm respectively) while those from the northern part (Zarouchla) had a mean length of 137 mm. This is in agreement with the results reported by Bassiotis (1967) and indicates that shorter needles are found in trees from the southern xeric part of Peloponnesos. Wheeler *et. al.* (1976) in a 15-year-old range-wide provenance test of black pine grown in the USA found that eastern seedlots had shorter needles. Wright (1962) did not find differences among 29 provenances in the length of mature needles, although in his provenance test 13 seed lots from Greece had been included.

Differences among clones in needle width and needle thickness were also quite large. The overall means were; 1.7 mm for needle width with a range among the clones from 1.5 to 1.8 mm, and 1.0 mm for needle thickness with a range of 0.9 to 1.1 mm. The needle width (1.7 mm) is larger than the value of 1.31 mm reported by Lee (1968) for 4-year-old provenances from central Greece grown in Michigan, and from the 1.4 mm found by Bassiotis (1967) in the whole natural Greek population of black pine. Clones also differed in sheath thickness of the needles; mean sheath thickness was 2.0 mm with a range of 1.8 to 2.2 mm and a coefficient of variation of 6.9 percent.

Needle Stomata

Number of Rows: It is known that the number of rows of stomata are not continous along the length of the needle. Because partial rows were not counted the number of rows gives only a comparison among rows and cannot be used to evaluate the total number of stomata, along the length of the needle.

The overall mean for the number of rows on the dorsal face was 15.0 with a range among clone means ranking from 11.8 to 18.3 rows *(Table 1)* and a coefficient of variation of 7.4 percent. Lower numbers of rows were found on the ventral face: the overall mean number for this was 9.4 with range among clones from 7.6 to 11.6.

The differences among clones were statistically significant in number of rows of stomata on both sides of the needle *(Table 2)*. A comparison made in the number of rows of stomata between dorsal and ventral faces using a "t" test *(Table 3)* showed that significant differences exist between the two sides, with the dorsal face having more rows. Clone number 6, which has a lower number of rows on the dorsal and ventral faces, had 11.8 rows on the former face (range among ramets 10.4 to 13.4) and 7.6 rows on the latter face (range among ramets 7.2 to 8.2), while clone number 3 which originated from the same area, had 18.3 rows on the dorsal face with a range among ramets 16.8 to 20.6.

The number of rows of stomata found in this study are higher than those reported by other investigators in black pine (Lee 1968; Mergen 1959; Bassiotis 1967).

Number of stomata per cm of Row Length: The variation between clones in the number of stomata per cm of row length were considerable. There were 120.8 stomata per cm of row length on the dorsal face with a range among clones from 99.0 to 138.6 stomata and 124.6 (range among clones 107.7 to 143.0) on the ventral face. The difference between the two means was statistically significant *(Table 3)*. The ventral face has more stomata per cm of row length than the dorsal face, while the latter has more rows of stomata because it has a greater surface.

Geographic variation in the number of stomata per unit of needle length has been reported by Bassiotis (1967) and Mergen (1958) in black pine, Lamontagne (1971) in slash pine and Burley and Burrows (1972) in *Pinus kesiya*.

Table 3. — Comparison of the number of rows of stomata and number of stomata per cm of row length in dorsal (X_1) and ventral (X_2) faces using "t" test.

Characteristic	$\bar{x}_1$	$\bar{x}_2$	$s_{\bar{x}_1}$	$s_{\bar{x}_2}$	$s_{\bar{x}_1 - \bar{x}_2}$	t
Rows of stomata	15	9.4	1.75	1.2	0.1667	33.77**
Number of sto/cm	121	124	9.78	10.49	0.994	3.77**

MERGEN was able to verify hybrids on the basis of stomata analysis and recommended such analyses as valid tests for hybrid studies. BURLEY and BURROWS (1972) found significant differences between provenances of *Pinus kesiya* in the number of rows of stomata and number of stomata per row; they concluded that individual stomata traits permit the best discrimination of provenances on a univariate basis among 12 varieties studied.

In the present study the number of stomata were more abundant in clones of southern origin; clones from the Taygetos and Parnon mountains had mean number of 125.9 and 121.7 stomata per cm of row on the dorsal face respectively, with coresponding values on the ventral face of 130.3 and 125.1. The clones of northern origin (Zarouchla) had 121 stomata per cm on the ventral and 116 on the dorsal face. BASSIOTIS (1967) found similar results and surmised that the higher numbers of stomata in the southern populations is related to climatic adaptation of the trees to xeric conditions. However, the opposite view has also been suggested by THOMES (SQUILLACE, 1966). He considers that low stomatal frequency may be an adaptation to xeric conditions. It seems that these contradictions are rationalised by KOZLOWSKI (1971) who considers that the primary factor in drought tolerance is related to the capacity of the species or genotypes for rapid stomatal closure under developing stress.

Number of Serrations

The differences among the clones in the number of serrations per cm of needle length were significant at the 0.01 probability level. Clone number 47, which had the highest number of serrations, averaged 39 while clone number 43 had only 22. The overall mean of the 52 clones was found to be 32 serrations with a coefficient of variation of 7.7 percent. This mean is a little lower in comparison with the 33 serrations reported by BASSIOTIS (1967) for the whole natural range of black pine in Greece. The lower number found in the present study may be explained by the longer needles in the intensively managed seed orchard; a significant negative correlation was found between total needle length and the number of serrations per cm. FOWLER (1964) has found differences among provenances of red pine in the number of serrations but the differences did not appear to be related to latitude or any other environmental factor of the provenances.

Resin Canals

The variation among clones in the number of resin canals of the needle was very large. Clone number 9 had only 4.0 resin canals with range among ramets of 2.8 to 5.2 and among needles of 2 to 6, while clone number 3 from the same area had 12.7 resin canals with range among ramets from 11.4 to 13.8 and among needles 11—16 *(Table 1)*. The large variation that has been observed among clones makes it possible to distinguish some of them based on this characteristic alone. The overall mean of the 52 clones was 8.4 resin canals.

The differences among the provenances from which the clones originated were statistically significant with a trend of increasing number of resin canals with increasing latitude. Thus the southernmost provenance of Taygetos had 7.0 resin canals while the northern provenance of Feneos had 9.5. More resin canals in northern populations of black pine in Greece have been also reported by BASSIOTIS (1967) while CHIRA (1966) and LAMONTAGNE (1971) found more resin canals in populations originating from areas of higher precipitation. An examination of 77 geographic seed sources of ponderosa pine made by DENEKE and FUNSCH (1972) showed

Table 4. — Matrix of phenotypic correlations among twelve needle characteristics of black pine.

Characteristics	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}
Needle length X_1	0.49	0.49	0.49	0.19	0.18	-0.31	-0.26	-0.30	0.21	-0.04	0.01
Needle width X_2		0.83	0.74	0.65	0.63	0.02	0.05	-0.02	0.57	0.54	0.58
Needle thick. X_3			0.65	0.60	0.47	0.02	0.05	-0.07	0.47	0.50	0.45
Sheath thick. X_4				0.53	0.47	0.07	0.08	-0.09	0.38	0.48	0.46
Rows of stom. in dorsal face X_5					0.76	-0.05	0.01	-0.04	0.58	0.79	0.68
Rows of stom. in ventral face X_6						-0.04	0.01	-0.04	0.49	0.60	0.84
Number of st/cm in dorsal X_7							0.84	0.32	-0.17	0.57	0.41
Number of st/cm in ventral X_8								0.29	-0.14	0.52	0.46
Number of serrat. X_9									0.10	0.13	0.12
Number of resin canals X_{10}										0.37	0.36
$X_5 \cdot X_7$ X_{11}											0.81
$X_6 \cdot X_8$ X_{12}											

that one seed source (near Arnold, Nebraska) had a consistently higher number of resin canals; they suggested that this seed source may be a potential new ecotype. Although the evolutionary significance of the number of resin canals is not well understood some relationship between them and the resistance of genotypes to specific insect attack has been reported (FOWLER and HEIMBURGER, 1958; STROH and GERHOLD, 1965; KRIEBEL, 1972; OVERHULSEN and CARA, 1981).

Although the overall mean of the 52 clones studied was high (8.4 resin canals), ungrafted seedlings of the same age as the grafts had consistently two resin canals. This makes it possible to identify (at least at young ages) a tree in which grafting has failed or to recognize a branch which has developed from the root-stock. This fact is of practical importance in seed orchard management activities where the pruning of the branches of the root-stock or the early replacement of failed grafts is important.

Correlation among Traits

The correlation coefficients among the traits studied are shown in *Table 4*. The most important relationships are briefly discussed below:

Thicker needles have more rows of stomata on both faces (r = 0.60 for the dorsal face and 0.65 for the ventral face). The number of stomata per cm of row length is not related to the thickness or width of the needle. Needle length was found negatively related to the number of rows of stomata on the dorsal and ventral faces as well as to the number of serrations per cm; the r values were −0.31 for the dorsal face; −0.26 for the ventral face; and −0.30 for the number of serrations. These results indicate that the formation of stomata and serrations are genetically predetermined. In favorable microenvironments where needles become longer the distance between stomata (in the row) or between serrations increases and therefore number per cm needle length decreases.

The number of rows of stomata was found to be unrelated to the number of stomata per cm needle length (r = 0.07 for the dorsal face and 0.08 for the ventral face). This does not conform with results reported by BURLEY and BURROWS (1972) in *Pinus kesiya* who found more stomata per row in provenances with a lower number of rows.

The number of resin canals are positively correlated with the needle width and needle thickness, with r values of 0.57 and 0.47 respectively. They are also related to the number of rows of stomata (r = 0.58 for the dorsal face and 0.49 for the ventral face). Thus thicker and wider needles form more rows of stomata and more resin canals.

Abnormalities in Needle Structure

Fascicles with three needles were observed in 10 out of the 52 clones studied. The highest frequency of this abnormality was recorded in clone number 11: half of the ramets of this clone (4 out of 8) had some needles with three fascicles. Nine other clones also had fascicles with three needles in at least one of their ramets; the proportions of three-needle fascicles in the ramets were not determined. Abnormalities in needle structure of black pine have been also reported by other investigators, (READ, 1976; LEE and ANDERSEN, 1968).

The position of resin canals was normal (medial). Deviations of minor importance were observed in clones 4, 33, 38, and 29. In the three first clones only one resin canal of a needle was externally positioned, while in one needle of clone number 29 there were two. It is known that medial and external resin canals are formed in needles of the hybrids *Pinus nigra* × *Pinus sylvestris* (VIDACOVIC, 1958); the latter is a species with external resin canals. However there is no *Pinus sylvestris* grown in the area of Peloponnesos and therefore the possibility of genetic introgression cannot be hypothesized.

Variance Components and Heritability Estimates

The variance among clones (σ^2_c) as well as the variance among the ramets within clones (σ^2_w) have been presented in *Table 2*.

The broad sense heritability values on an individual tree basis were from 0.35 for sheath thickness up to 0.78 for the number of stomata per cm of row on the dorsal face (*Table 2*). Higher values were obtained for the number of stomata per cm. row, number of serrations (0.66) and number of resin canals (0.77) indicating that these characteristics are strongly inherited.

Needle length, needle width, needle thickness and sheath thickness are moderately strongly inherited characteristics with heritability values of 0.41, 0.56, 0.47 and 0.38 respectively. The results in the present study show that the number of stomata per cm of row are more strongly inherited than number of rows of stomata on any of the two faces of the needle. LAMONTAGNE (1971) using the same method of estimation in grafted material of slash pine, also found a lower heritability value for the number of rows of stomata (0.2) and a higher value for the number of stomata per cm of row (0.44).

Vegetative propagation in forestry has limited application especially in coniferous species. *Cryptomeria japonica* is the best known species in which a number of selected clones have been widely planted in Japan (TODA, 1974). Preliminary clonal testing has been also carried out in radiata pine (SHELBOURNE, 1969; SHELBOURNE and THULIN, 1974). Improvement of black pine by cloning at present is rather impractical. However assuming that vegetative propagation techniques can be developed in the future, then capitalizing on the total genetic variance in the improvement of any one characteristic studied can be expected to result in rapid progress.

Conclusions

From the study 5 years after grafting of 10 morphological and anatomical needle characteristics in 52 black pine clones grown in a seed orchard the conclusions are:

1. There are significant differences among clones in all characteristics studied.
2. Number of resin canals was found to be a characteristic with large variation. Mean values among clones ranged from 4 to 12.7 (overall mean 8.4). The differences were so large, that it was possible to distinguish high from low resin canal clones based on this characteristic alone.
3. Resin canals in needles of ungrafted seedlings of the same age as the grafts were constantly 2. This makes it possible to identify a tree in which grafting has failed, or to recognize a branch which has developed from the stock; important information in seed orchard management activities.
4. The number of serrations and the number of stomata per cm of needle length are genetically predetermined characteristics; longer needles have lower numbers of stomata and number of serrations per cm of needle length.
5. Number of stomata per cm of needle length in dorsal and ventral faces, serration number and number of resin canals are strongly inherited characteristics, with broad sense heritability values on an individual tree

basis (under the environmental conditions studied) of 0.78, 0.74, 0.66 and 0.77 respectively.

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Determination of the Origin of an Isolated Group of Trees of Pinus nigra through Enzyme Gene Markers

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Summary

Pinus nigra is a collective species which consists of four subspecies. Previous taxonomic studies on European black pine have used biochemical methods: monoterpene contents and isoenzyme systems. The problem of a differentiation between the subspecies *laricio* and *nigricans* was here examined using isoenzyme gene markers.

In order to characterize the allelic structure on the basis of shikimate dehydrogenase (SKDH), eleven populations of *Pinus nigra* ARN. were studied *(Table 1)*.

Zone starch gel electrophoresis of haploid endosperms of dormant seeds was used. Results were analyzed by genetic distance *(Table 3, 4)*: they show that Corsican provenances of laricio pine clearly differ from the other populations in the high frequency of the B_1 allele *(Table 2)*.

A differentiation was successful within laricio pine, between the Corsican and the Calabrian populations. Therefore, it was possible to analyze an unknown population of *Pinus laricio* (Monti Pisani area) to determine if this tree sample belongs to the Corsica or the Calabria group. Since the results obtained on the genetic structure of the trees from the Monti Pisani area were similar to the allelic frequency in the Corsica populations, it is probable that this group of laricio pines originates from Corsica.

Key words: Pinus nigra, taxonomy, isoenzymes, SKDH.

Zusammenfassung

Pinus nigra ist eine aus 4 Unterarten bestehende Kollektivart. Frühere taxonomische Untersuchungen bei der europäischen Schwarzkiefer bedienten sich biochemischer Methoden: Monoterpengehalte und Isoenzymsysteme. Das Problem einer Differenzierung zwischen den Unterarten *laricio* und *nigricans* wurde hier mit Hilfe von Enzymgenmarkern bearbeitet.

Zur Charakterisierung der allelischen Struktur an den Shikimat-Dehydrogenasen (SKDH) kodierenden Genloci wurden 11 Populationen von *Pinus nigra* ARN. untersucht *(Tab. 1)*.

Die Labormethodik verwendete Stärkegel-Zonenelektrophorese der haploiden Endosperme ruhender Samen. Die Ergebnisse wurden mit Hilfe genetischer Abstandsmaße analysiert *(Tab. 3, 4)*: Sie zeigen, daß die korsischen Herkünfte der Unterart *laricio* von den übrigen Populationen durch die hohe Frequenz des Allels B_1 unterschieden sind *(Tab. 2)*.

Innerhalb der Unterart *laricio* war eine Differenzierung zwischen den korsischen und den kalabrischen Populationen erfolgreich. Daher war es möglich, ein fragliches Vorkommen von *Pinus laricio* in den Pisaner Bergen auf seine Zugehörigkeit zu der korsischen bzw. der kalabrischen Gruppe zu bestimmen. Da die genetischen Merk-