



HAL
open science

Geographic variation in terpene composition of *Pinus nigra* ARN

Sophie Gerber, Philippe Baradat, Anne Marpeau, Michel Arbez

► **To cite this version:**

Sophie Gerber, Philippe Baradat, Anne Marpeau, Michel Arbez. Geographic variation in terpene composition of *Pinus nigra* ARN. *Forest genetics*, 1995, 2 (1), pp.1-10. hal-02708138

HAL Id: hal-02708138

<https://hal.inrae.fr/hal-02708138>

Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

GEOGRAPHIC VARIATION IN TERPENE COMPOSITION OF *PINUS NIGRA* ARN.Sophie Gerber¹⁾, Philippe Baradat²⁾, Anne Marpeau³⁾ & Michel Arbez⁴⁾¹⁾ E. N. S. A. T., 145 avenue de Muret, F-31076 Toulouse Cedex, France²⁾ C. I. R. A. D., 2477 avenue du val de Montferrand, B.P. 5035, F-34032 Montpellier Cedex 01, France³⁾ Laboratoire de physiologie cellulaire végétale, Bordeaux I, Avenue des Facultés, F-33405 Talence, France⁴⁾ I. N. R. A., Station de Recherches Forestières, F-33610 Cestas, France

Received April 21, 1995; accepted June 20, 1995

ABSTRACT

The geographical variation in terpene composition of *Pinus nigra* was studied from 109 samples representing 72 natural and 11 planted populations. Relative amounts of 7 terpenes (camphene, β -pinene, myrcene, Δ^3 -carene + terpinolene, β -phellandrene and caryophyllene) were used for discrimination of populations. Five geographical groups could be distinguished: Spanish, French continental, Corsican, Calabrian and Eastern (comprising Austria, Balkan, Turkey, and Crimea). Evidence from our study of terpenes and from other data on anatomical, morphological and growth characters, and on terpenes, flavonoids and allozyme frequencies, support the use of the classification system of WHEELER *et al.* (1976).

Keywords: *Pinus nigra*, terpenes, taxonomy

INTRODUCTION

European black pine has been considered as a single species only since 1957 when Röhrig recommended the retention of the name "*Pinus nigra*", given by Arnold in 1785. This species covers a discontinuous area of 2,300,000 ha, its range extends up to the northern Mediterranean coast, through 13 countries (Spain, France, Italy, Austria, Yugoslavia, Bulgaria, Albania, Romania, Greece, Turkey, Crimea, and isolated occurrence in Morocco and Algeria). *Pinus nigra* is highly polymorphic. There have been many attempts of classification, and more than a hundred different Latin names have been given to populations of the species, according to WRIGHT and BULL (1962). In 1974, VIDAKOVIĆ considered that no general consensus could be found about the taxonomy of the species. The confusion still exists since various classifications have been used in recent publications such as BONNET-MASIMBERT and BIKAI-BIKAI (1978), NIKOLIĆ and TUCIĆ (1983) and FARJON (1984).

We have investigated the geographical variation of *Pinus nigra* throughout its natural range by analyzing the terpene composition of cortical oleoresin (monoterpenes and sesquiterpenes) by gas-liquid chromatography. The relative amount of some mono- and sesquiterpenes has been found to show simple monogenic inheritance with a pair of alleles ('richness' and 'poorness' alleles) exhibiting variable dominance relationships. This simple genetic control seems to be rather general especially in the genus *Pinus*, e.g. in *Pinus*

pinaster for Δ^3 -carene, limonene, myrcene, longifolene and caryophyllene (BARADAT *et al.* 1972, 1975, MARPEAU *et al.* 1975, MARPEAU-BÉZARD *et al.* 1983), in *Pinus taeda* for limonene, myrcene, β -pinene, and β -phellandrene (SQUILLACE *et al.* 1980), and in *Pinus sylvestris* for Δ^3 -carene, β -pinene, β -phellandrene, limonene and myrcene (YAZDANI *et al.* 1985). Although inheritance of terpenes was not studied in the case of *Pinus nigra*, we assumed that their synthesis was under similar genetic control. As such, they should constitute a reliable criterion for studies on population variation.

MATERIAL AND METHODS

Material

The study concerned 109 origins representing 72 natural and 11 planted populations of *Pinus nigra* (one population can be represented by more than 1 original sample, see table 1). Samples No 73, 74 and 75 were the result of controlled crosses, made with mixed pollen of five trees, of Corsican \times Corsican, Calabrian \times Calabrian and Corsican \times Calabrian trees. Locations of origins are given in Table 1 and Figure 1. Sampling was carried out in origin trials planted in 4 different locations in France: Amance (Meurthe et Moselle), Verdun (Meuse), Cendrieux and Cadouin (Dordogne); trees were between 5 and 13 years old when sampled. This age ensured a good expression of the genotypes for all the terpenes studied (BERNARD-DAGAN *et al.* 1972).

Table 1 Geographical location of the native stands of *Pinus nigra* from which seed was collected for the provenance tests

No.	Abbreviation	Provenance *	Country	Latitude	Longitude
1	TIKJ	Tikjda	Algeria	36°21 N	3°55 E
2	CAZO	Cazorla	Spain	37°50 N	3°00 W
3	RO-MA	Rio Madera (2)	Spain	37°50 N	3°00 W
4	FRES	Fresneda	Spain	39°50 N	2°10 W
5	OLRE	Olmedadel Rey	Spain	39°50 N	2°10 W
6	PRIE	Priego	Spain	40°30 N	2°20 W
7	AINS	Ainsa	Spain	42°30 N	0°12 E
8	SGUL	Saint Guilhem (2)	Corsica	43°41 N	3°35 E
9	GAGN	Gagnères	Corsica	44°07 N	4°17 E
10	TART	Tartagine (5)	Corsica	42°29 N	8°57 E
11	VALD	Valdoniello (4)	Corsica	42°18 N	8°57 E
12	AITO	Aitone	Corsica	42°17 N	8°51 E
13	GHIS	Ghisoni	Corsica	42°05 N	9°12 E
14	PALN	Palneca	Corsica	41°59 N	9°12 E
15	MARM	Marmano	Corsica	42°01 N	8°70 E
16	VEZZ	Vezzani (2)	Corsica	42°09 N	9°15 E
17	SORB	Col de Sorba	Corsica	42°08 N	9°12 E
18	VIZZ	Vizzavona (3)	Corsica	42°13 N	8°67 E
19	ASPR	Aspromonte	Italy	38°05 N	16°00 E
20	CANT	Catanzaro	Italy	38°54 N	16°34 E
21	COZE	Cosenza (2)	Italy	39°15 N	16°17 E
22	TR-CO	Trenta Coste	Italy	39°25 N	16°35 E
23	MA-TA	Macchia del la Tav	Italy	39°25 N	16°35 E
24	GRAN	Grancia (2)	Italy	39°25 N	16°35 E
25	VI-BA	Villeta Barrea (2)	Italy	41°47 N	13°46 E
26	HOHE	Hohewand	Austria	47°49 N	16°00 E
27	DOBL	Doblhoff	Austria	**	**
28	WOLF	Wolfsohl	Austria	47°43 N	16°03 E
29	LEDE	Ledererkogel	Austria	47°16 N	16°53 E
30	MONO	Moldova Noua	Rumania	45°41 N	21°40 E
31	BANA	Banat	Rumania	45°02 N	20°40 E
32	DE-PE	Deliblatski Pešak	Yugoslavia	44°48 N	21°12 E
33	TRBO	Trbovle	Yugoslavia	46°10 N	15°03 E
34	BUGO	Bugojno (2)	Yugoslavia	44°05 N	17°25 E
35	TAZL	Tara Zlatibor	Yugoslavia	43°40 N	19°35 E
36	VGRA	Višegrad (3)	Yugoslavia	43°90 N	19°20 E
37	STUDII	Studenica	Yugoslavia	43°30 N	20°33 E
38	PLEV	Pljevlja	Yugoslavia	43°22 N	19°23 E
39	NIKS	Nikšić	Yugoslavia	42°46 N	18°56 E
40	BRAC	Brač	Yugoslavia	43°20 N	16°40 E
41	GR-AN	GradeskiAndek	Yugoslavia	41°27 N	20°38 E
42	PO-LV	Popova-Livada	Yugoslavia	41°10 N	21°55 E
43	TCHE	Tchernikov Borum	Bulgaria	**	**
44	KUST	Kustendil (3)	Bulgaria	42°16 N	22°40 E
45	RILA	Rila	Bulgaria	42°08 N	23°33 E
46	TZAR	Tzavaritza	Greece	**	**
47	THAS	Thassos (2)	Greece	40°40 N	24°40 E
48	PARA	Paramestion	Greece	40°04 N	24°04 E
49	KOSA	Kosani	Greece	40°18 N	21°47 E
50	GREV	Grevena	Greece	40°05 N	21°25 E
51	METS	Metsovon	Greece	39°46 N	21°11 E
52	MILE	Mile	Greece	39°46 N	21°11 E
53	ALON	Alonia	Greece	**	**
54	BASP	Barkazorema Spilaki	Greece	**	**
55	KA-ZE	Kapidag Zemliker	Turkey	39°35 N	27°00 E

Table 1 (continued)

No.	Abbreviation	Provenance *	Country	Latitude	Longitude
56	KARA	Karabelen	Turkey	40°06 N	29°02 E
57	ALAC	Alaçam (2)	Turkey	39°35 N	28°35 E
58	SIMA	Simav	Turkey	39°00 N	29°00 E
59	MUGL	Mugla	Turkey	37°20 N	28°24 E
60	YILA	Yilanli	Turkey	37°00 N	28°00 E
61	KAYM	Kaymakçı	Turkey	36°52 N	29°22 E
62	EGRD I	Egridir	Turkey	37°40 N	31°10 E
63	AFYO	Afyonkaras	Turkey	38°07 N	30°06 E
64	DEMR	Demirören	Turkey	39°53 N	29°46 E
65	CATA	Catacik	Turkey	39°44 N	31°04 E
66	DEGR	Degirmendere	Turkey	39°57 N	31°05 E
67	KURB	Kurbacik	Turkey	40°30 N	31°42 E
68	GEBE	Gebeler	Turkey	40°30 N	32°40 E
69	KOPR	Köprücek	Turkey	37°00 N	33°00 E
70	CANG	Cangal	Turkey	42°00 N	34°00 E
71	AYANI	Ayancik I	Turkey	41°43 N	34°31 E
72	CRIM	Crimea Yalta (5)	Ukraine	44°40 N	34°20 E
Planted populations					
73	LRCO*LRCO	Les Barres (VALD*VALD)			
74	LRCA*LRCA	Les Barres (COZE*COZE)			
75	LRCO*LRCA	Les Barres (CCOZE*VALD)			
76	LBAR IIG	Les Barres	(France)	47°50 N	2°45 E
77	KOEK II	Koekelare II	Belgium	51°04 N	2°58 E
78	CAUS	Les Causses	France	44°05 N	3°07 E
79	EMBR	Embrunais	France	44°34 N	6°28 E
80	LARA	Laragnais	France	49°28 N	3°84 E
81	MEND	Mende	France	44°30 N	3°30 E
82	VL-HA	Ville Haute	France	43°58 N	6°52 E
83	LBPA	Les Barres	France	47°50 N	2°45 E

* in brackets: number of samples (if more than one)

** unknown location

Constitution of a mean sample

For each origin, at least 30 trees from different plots were sampled. From a top lateral branch of each tree, the shoot of the previous year's whorl was collected. Such shoots are assumed to be in the same physiological state. Weak trees, or trees attacked by insects were not sampled because terpene composition might be locally modified. Every shoot was stripped of a ring of cortical tissue and the 30 samples from one origin were mixed together to form the mean sample (fresh weight between 10 and 15 g). Individual terpene composition were not taken into account since individual terpene percentages are often not normally distributed, causing troubles for the tests realized after ANOVA.

Terpene composition analysis

Tissues were minced and macerated for three hours in 30 to 50 ml of pentane (99%). The liquid phase was then purified on silica gel and analyzed by gas chromatography as described in MARPEAU *et al.* (1989).

Choice of terpenes

The proportions of 8 monoterpenes (α -pinene, camphene, β -pinene, myrcene, Δ -3-carene, limonene, β -phellandrene and terpinolene) and one sesquiterpene (caryophyllene) were plotted on the chromatogram. Peaks were identified by comparison with reference

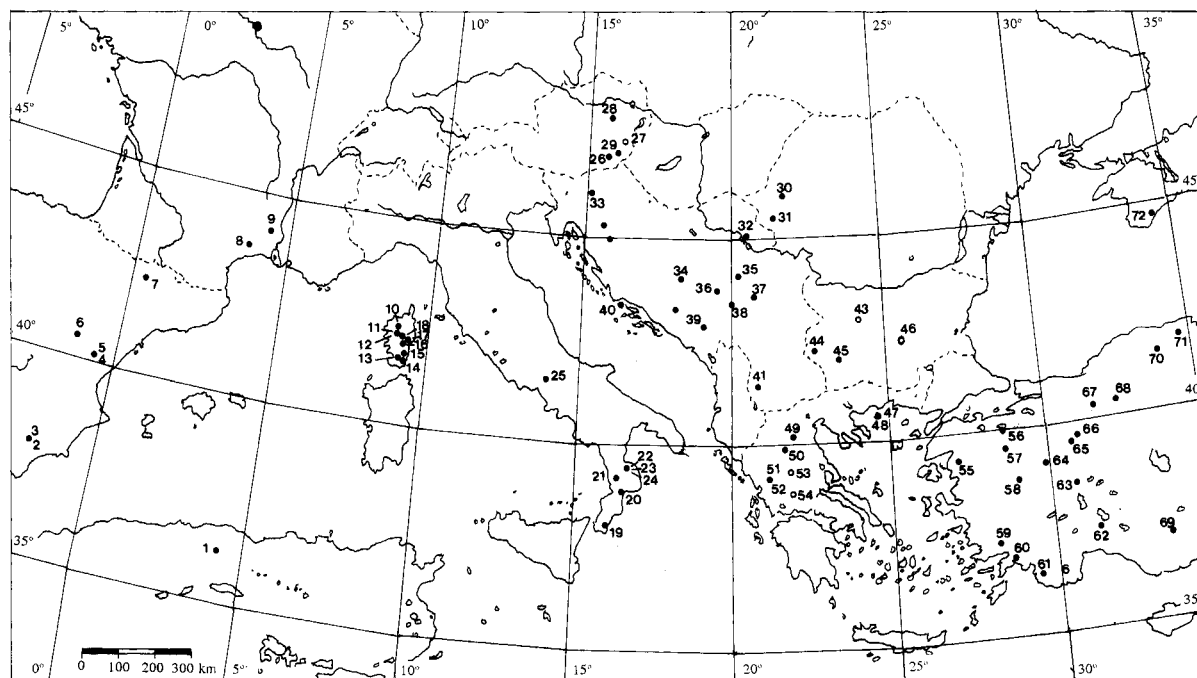


Figure 1 Geographical location of the 72 native stands from which seed was collected for provenance tests

chromatograms run under the same conditions with known terpenes. The relative amount of each monoterpene was expressed as a percentage of the total monoterpenes and the amount of caryophyllene as a percentage of total terpenes. As the proportion of the ninth terpenes entirely determined by the 8 others, we decided to set apart one of the 9 terpenes. α -pinene is present in great quantity (a mean of 67% in the present analysis) in the oleoresin of conifers in general and pines in particular (see, for instance, VON RUDLOFF (1975), MIROV (1961)). Therefore it is the most affected by autocorrelation and its relative amount is mainly the consequence of variation for other terpenes. These correlations between terpenes are partially due to their expression in percentage values. α -pinene has also often been found to exhibit low variation between populations for different species (VON RUDLOFF 1975, YAZDANI *et al.* 1985). For these reasons, the relative amount of α -pinene was not used in this study. The amount of limonene was also not taken into account, since its stable genotypic expression seems to be reached only at the age 8 to 10 in *Pinus piaster* Ait. (BARADAT *et al.* 1972); the situation tends to be the same for *Pinus nigra* (GERBER, unpublished data).

Since Δ -3-carene and terpinolene are under the same genetic control (ZAVARIN 1970, BARADAT & MARPEAU-BÉZARD 1988), the relative amounts of the two terpenes were added to form a single character.

Statistical analysis

Individual percentages were transformed into

$\sqrt{\arcsin}$ (proportion) to normalize distribution of errors and to avoid a relationship between the error variance and the proportion of each terpene in the mean sample.

Each of the 109 mean samples was therefore represented by a set of 6 characters: relative amounts of camphene, β -pinene, myrcene, Δ -3-carene + terpinolene, β -phellandrene and caryophyllene. A principal component analysis was performed, based on the correlation matrix computed with the 98 samples corresponding to the natural origins. The samples from planted populations were not included in the calculation of the axes but were considered as supplementary data.

RESULTS

Natural populations

Table 2 gives the general results of the principal component analysis for the 6 traits studied. The first 3 axes of the analysis, which represented 80 % of the variance, were retained. With the exception of 4 origins¹, the stands cluster into distinct geographical groups. We considered the relative positions of the groups, on

- 1)
- one from Spain (No.3, Rio Madera) and one from Corsica (No.12, Aitone) situated among origins of the eastern part of the natural area,
 - one from Villeta Barrea (No.25, Italy) situated among Corsican populations,
 - one from Turkey (No.58, Simav) rather isolated.

Table 2 General results of the principal component analysis: eigenvalues in % of the total variance and correlation with initial traits for the first three axes

	Axis 1	Axis 2	Axis 3
Eigenvalues (%)	34	29	17
Cumulative eigenvalues (%)	34	63	80
Initial traits	Correlations		
camphene	0.67	-0.16	-0.60
β -pinene	0.25	0.64	0.55
myrcene	-0.87	0.17	-0.17
Δ 3-carene + terpinolene	-0.70	-0.21	-0.30
β -phellandrene	-0.33	-0.72	0.46
caryophyllene	-0.36	0.83	-0.18

Table 3 Mean amounts of terpenes in the groups from the principal components analysis

Group	camphene	β -pinene	myrcene	Δ 3-carene	β -phellandrene	caryophyllene
Major groups						
Spain (7) ¹⁾	1.16	13.02	16.78	2.31	0.89	7.97
Corsica (19)	1.63	5.38	3.98	1.12	7.72	1.87
East (60)	1.74	15.28	2.47	0.40	2.04	4.09
Middle groups						
Calabria (8)	2.04	4.46	1.44	0.30	0.63	1.53
France (3)	1.77	5.47	6.95	0.32	1.50	9.23
Minor groups						
East (38) ²⁾	1.70	10.57	2.37	0.37	1.16	4.36
Turkey (18)	1.63	22.69	2.49	0.49	1.46	4.60
Austria (4)	1.88	12.58	2.55	0.34	3.49	3.31
Algeria (1)	1.73	16.97	5.63	0.35	0.99	5.41

¹⁾ in brackets: number of samples in the group

²⁾ without Turkey and Austria

Figure 2 and 3, combined with the correlations between axis and initial characters (Table 2). The mean amounts for the 6 terpenes calculated for each group are given in Table 3. The main features of the terpene composition discriminating these groups from each other can be derived from these data (table 4).

Three "major" groups are clearly distinct: a Spanish group, a Corsican group and an Eastern group (Austrian, Rumanian, Yugoslavian, Bulgarian, Greek, Turkish and Crimean origins). They are especially differentiated from each other on the first plane (axis 1 and 2). Their main distinctive features are summarized on Table 4.

Two additional "middle" groups can be distinguished, relative to the major groups. A Calabrian group between the Corsican and the Eastern group, in the first plane and a French continental group, comprising only three samples but clearly clustered between Spanish and Eastern groups, though closer to the latter

group. ARBEZ *et al.* (1974), studying 3 mean samples of Calabrian and Corsican origins established the same results for the differences of terpene composition between these two groups. French continental origins are radically different from Spanish origins in their terpene composition on the basis of their values on the first coordinate (see Table 4). These differences for myrcene and camphene were also found by ARBEZ *et al.* (1974) between one French and two Spanish samples. Our single sample from Algeria (No.1), is part of the Eastern group.

"Minor" groups could be detected within the Eastern group. Austrian origins are represented by only four samples but tend to have lower values on the first coordinate than the other members of the Eastern group (i.e. higher β -pinene, Δ 3-carene + terpinolene and lower β -phellandrene, see Table 3), and tend to be closer to Calabrian samples.

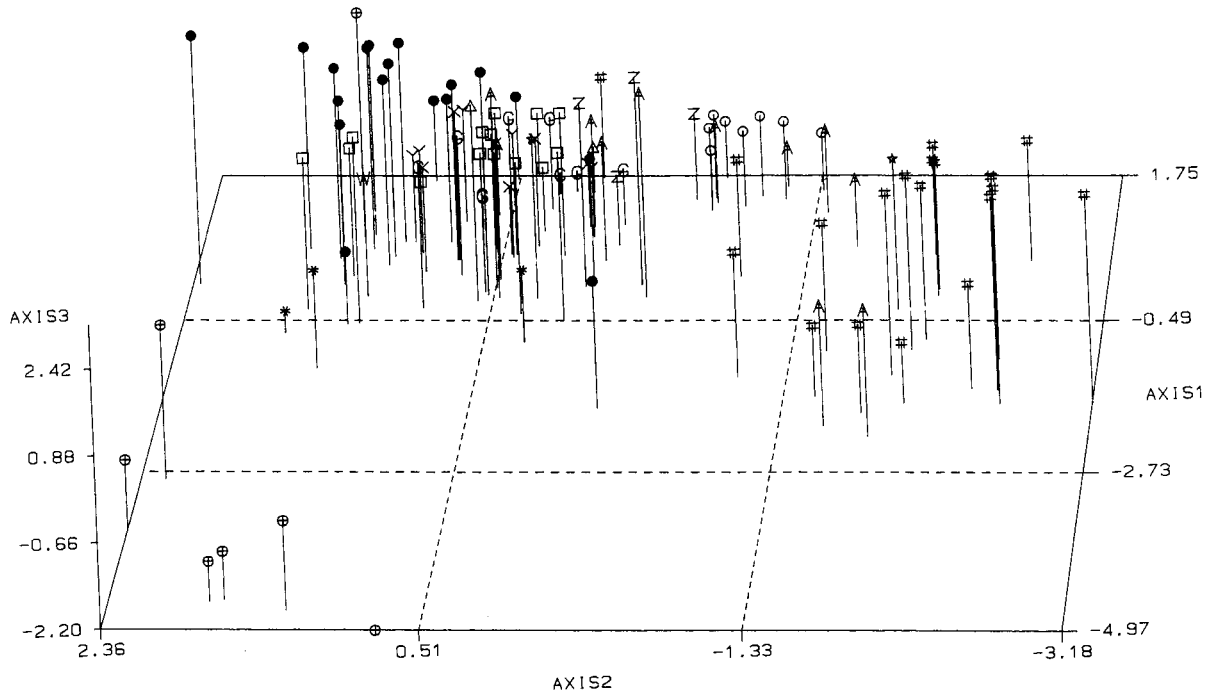


Figure 2 The first three axes of the principal component analysis: samples identified by their origin: WWW – Algeria; ooo – Calabria, *** – France, $\Delta\Delta\Delta$ – Rumania, ZZZ – Austria, ### – Corsica, GGG – Greece, @@@ – Spain, xxx – Bulgaria, YYY – Crimea, ☆☆☆ – Italy, ●●● – Turkey. □□□ – Yugoslavia, AAA – artificial

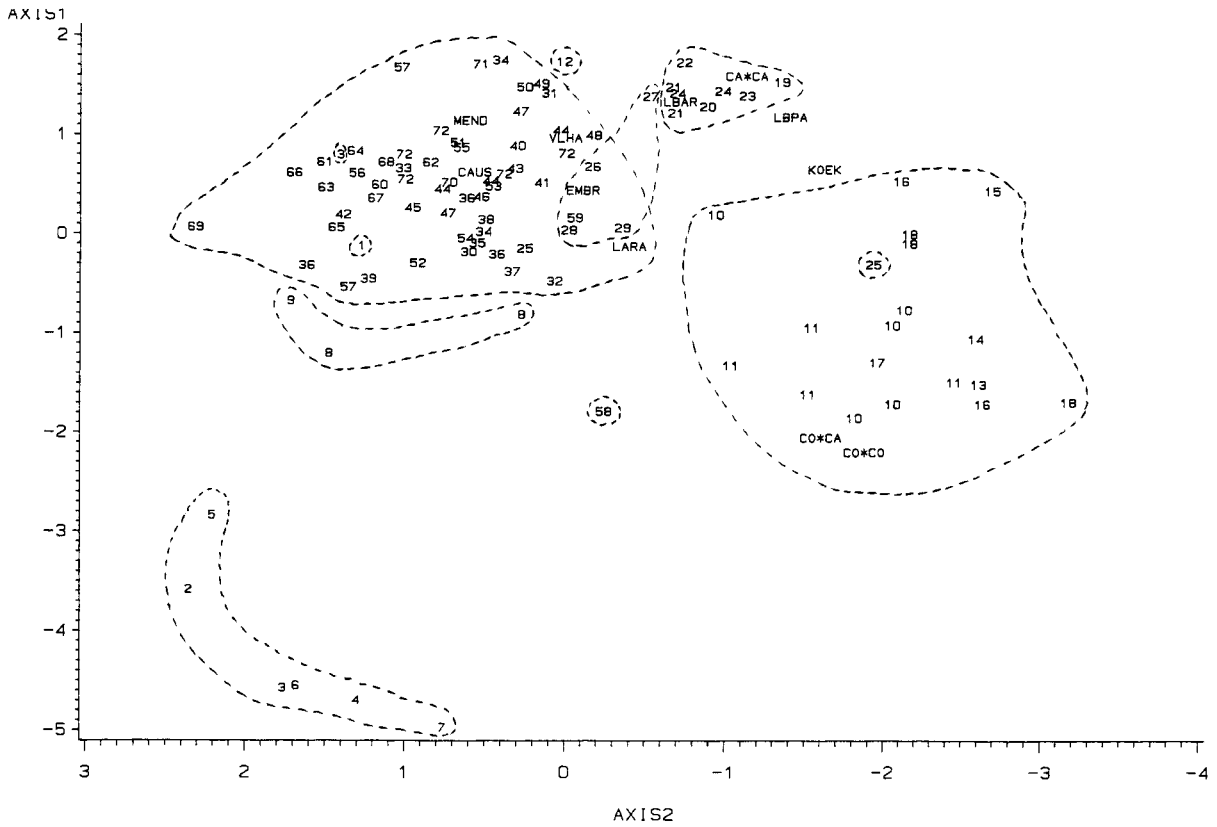


Figure 3 Identification of the samples on the first plane the principal component analysis: 11 samples of the artificial origin by their name, 98 samples of natural origin by their number

Some Turkish samples show a tendency to have higher values on the third axis and thus higher β -pinene than the Yugoslavian samples (clearly seen on table 3). Yugoslavian stands clustered together with Greek, Crimean, Bulgarian and Rumanian origins. A principal component analysis carried out only with these Eastern origins did not reveal any clearer geographical pattern. A slight differentiation can be noticed among these origins, but they are nevertheless close to each other in terpene composition.

Planted populations

Terpene composition of the planted populations is given in table 5. These samples were processed as supplementary data (figures 2 and 3):

- French planted stands (Nos. 78 to 82), known to be of Austrian origin (a variety which was largely used for reforestation in France) are actually part of the Eastern group;
- The progeny of a stand called "les Barres *pallasiana*" (No. 83) originating reputedly from the extreme East of the natural area, is more likely to be of Calabrian or Corsican origin according to its terpene composition, and also from its morphology (ROMAN-AMAT 1984). This result may be due to

the close proximity of Calabrian and Corsican stands in the area from which the progeny comes, which may consequently have been the parents by natural pollination;

- Koeklare (No. 77), a Belgian origin, known to have been introduced during the invasion of the country by Napoleon, but supposed to be intermediate between Calabrian and Corsican pines (NANSON 1972), could indeed be so according to its intermediate position between these two groups;
- The population resulting from a controlled cross of Corsican (No. 73) and Calabrian (No. 74) pines are part of the parental group. The mean hybrid between Corsican and Calabrian pines clusters with the Corsican group.

DISCUSSION

Terpenes are known to be involved in plant-insect relationships (*Pinus nigra*: CHARLES *et al.* 1982) but the possible importance of selection pressure on genes which control their biosynthesis has not been quantified. Their neutrality as markers is thus often disputed. In contrast, allozymes or DNA markers are considered to be neutral markers, providing weak geographical discrimination, compared to terpenes. However, in several

Table 4 Major features of the terpene composition discriminating the geographical groups on the principal component analysis

(a)	Spanish group: (axis 1)	higher myrcene higher $\Delta 3$ -carene + terpinolene lower camphene
(b)	Corsican group (axis 1)	lower caryophyllene higher β -phellandrene lower β -pinene
(c)	Eastern group on axis 1 relative to Spanish group	lower myrcene lower $\Delta 3$ -carene + terpinolene higher camphene
	on axis 2 (relative to Corsican group)	higher caryophyllene lower β -phellandrene higher β -pinene
(d)	Calabrian group (relative to Corsican group)	lower myrcene and $\Delta 3$ -carene + terpinolene higher camphene lower β -phellandrene
(e)	French continental group	lower myrcene and $\Delta 3$ -carene + terpinolene higher camphene

Table 5 Mean amount of terpenes (in %) in samples from planted populations

Population	camphene	β -pinene	myrcene	Δ 3-carene+ terpinolene	β -phellandrene	caryophyllene
EMBR	1.87	11.93	2.09	0.52	2.66	3.80
KOEK	2.24	4.55	5.07	0.40	2.29	0.81
LBAR	2.13	7.12	2.05	0.32	0.93	1.63
VLHA	2.04	12.19	1.97	0.36	1.89	3.50
LBPA	2.21	6.43	2.44	0.40	2.08	1.02
MEND	2.11	13.93	2.53	0.49	0.10	2.93
CO*CO	1.65	3.68	11.93	0.89	10.96	2.71
CA*CA	2.31	3.09	1.47	0.35	0.59	1.47
CO*CA	1.66	3.51	12.33	0.65	9.48	2.95
LARA	1.34	16.09	2.35	0.44	5.20	2.74
CAUS	1.61	15.83	2.29	0.20	1.83	3.96

instances, the patterns of geographical variation displayed by terpenes and isozymes are the same, for example in a study of the native range of Douglas-fir (LI & ADAMS 1988). Using 20 enzyme loci, these authors found a large scale geographical discrimination in agreement with the one found with terpenes with only minor local differences. Terpene information seems therefore to be suitable for taxonomic purposes, comparable with data using other markers with high or complete genetic control.

In classical, descriptive taxonomy developed in 1750 by the Swedish botanist Linnaeus, a plant name should above all permit to identify the plant, to classify it and not to indicate its phylogeny (LAWRENCE 1955, cited in MIROV 1967). A taxonomist is obviously still asked to be able to identify plant material. This is usually possible with morphological characteristics but today, genetic markers can be added to these criteria. Actually, as WALTERS (1963) and CRAWFORD (1983) emphasize, "difference" or "resemblance" are relative terms, established by the context. No taxonomic character is representative of a whole genome so that a variety of approaches can be followed to clarify confused taxonomic situations, such as are found, for instance, in the genus *Pinus* (MIROV 1967) and *Salix* (WALTERS 1963). The ecological and geographical barriers between differentiating populations of *Salix* were destroyed during the glacial period. The present distinctions between taxa of recent origin, which have not diverged significantly, are difficult to define (CRONQUIST 1973). There is consequently no general agreement on the classification of the genus *Salix*. On a species and subspecies level, we are faced with the same difficulties for *Pinus nigra* Arn. We tried to interpret our results for *Pinus nigra*, in the context of those of other authors using different techniques.

The classification proposed in 1958 by FUKAREK for *Pinus nigra* and cited by DEBAZAC (1964), in his handbook on conifers, is generally used. As DEBAZAC (1964) wrote, it is essentially based on geographical distribution, and has the advantage of simplicity:

- subsp. *clusiana* in Northern Africa, Spain and France;
- subsp. *laricio* in Corsica and Calabria;
- subsp. *nigricans* in Austria, central Italy and Balkan;
- subsp. *pallasiana* in Turkey and Crimea.

The distinction between the last two subspecies is not clear. DEBAZAC hesitates to place Greek population in the first or in the second subspecies. ARBEZ and MILLIER (1971) did not find any difference for four needle-characters between the subspecies. Small differences were found for the content of β -pinene by ARBEZ *et al.* (1974), and for the frequency of one allele at the enzyme locus GOT by BONNET-MASIMBERT and BIKAY-BIKAY (1978). Our conclusions tend to discriminate the Turkish populations but cannot provide any clearer distinction between (and within) these subspecies. Moreover, results of earlier papers can be seen as underlying the absence of distinction between origins of this Eastern part of the natural area.

WRIGHT and BULL (1962), from an analysis of seed weight and 12 seedling characteristics, could only differentiate the Corsican populations from a large origin collection. In 1968, LEE measured 3 growth, 19 anatomical and 18 chemical traits in the same collection. He found trends of variation, but he grouped together Yugoslavian, Greek, and Turkish origins, setting apart Crimean and Austrian pines (with a single seedlot for each). WHEELER *et al.* (1976) measured height, mortality and winter injury on the same material, and added the last two origins, constituting one single group. An origin from Cyprus had different characteristics. This result is in agreement with the apparent distinction of a

population of the same origin reported by BONNET-MASIMBERT and BIKAY-BIKAY (1978), based on allozyme frequencies at 4 loci. NIKOLIĆ and TUCIĆ (1983), studying allozyme variation at 4 loci on a large sample from this area, did not agree with the classification of the Eastern part of the natural range proposed by VIDAKOVIĆ in 1974. In fact, the different subspecies supposed to constitute these populations appear to be very close to each other, according to their new genetic distances. This lack of clear discrimination among populations distributed on a wide area may be explained by human intervention, acting over a long period on these forests, a point previously emphasized by DEBAZAC (1971).

CONCLUSION

Calabrian and Corsican pines are clearly differentiated from the other origins and from each other on the basis of growth, anatomical and chemical characters (LEE 1968, WHEELER *et al.* 1976), number of cotyledons (RÖHRIG 1969), needle-characters (ARBEZ & MILLIER 1971), monoterpene composition (ARBEZ *et al.* 1974, FINESCHI & GROSSONI 1981), flavonoid compounds (LAURANSON 1989), and isozymes (BONNET-MASIMBERT & BIKAY-BIKAY 1978, FINESCHI 1983, NIKOLIĆ & TUCIĆ 1983, SCALTSOYIANNES *et al.* 1994). Our results lead to the same conclusions.

French and Spanish origins, included in the 1958 classification of FUKAREK into one single group are also clearly discriminated by different studies. LEE (1968), and subsequently WHEELER *et al.* (1976), clustered them in separate groups from their phenotypical features. Spanish populations appear to contain the most vigorous trees, on a 9 year old origin trial in New Zealand (WILCOX & MILLER 1974). From the discriminant analysis of BONNET-MASIMBERT and BIKAY-BIKAY (1978), one can see a clustering of Spanish origins clearly distinct from the french ones.

The relationships of Northern African origins within the species have not been ascertained. The information available is not consistent, since a Moroccan population appeared to be distinct from other populations (NIKOLIĆ & TUCIĆ 1983) whereas our Algerian sample can be included among Eastern origins. This populations supposed to be native but we could wonder whether it has been introduced during colonial times.

On the principal component analysis, the clustering of the planted populations included in our study with controls of natural origin is an illustration of the use of terpene composition to verify or specify the probable geographical origin of unknown stands. This technique is used in routine varietal identification of *Pinus pinaster* (BARADAT & MARPEAU-BÉZARD 1988).

In their conclusion, WHEELER *et al.* (1976), attempted to clarify the taxonomy of *Pinus nigra*, combining their results with those of RÖHRIG (1966) and WILCOX & MILLER (1974). We are inclined to join these authors in their classification proposal. Except for the Eastern part of the natural range, which does not correspond to a precise region, the classification suggested is close to an earlier idea of Röhrig who proposed, in 1957, to identify the different populations of *Pinus nigra* by their origin ("origin of" Calabria, Corsica,...). The classification is as follows:

Pinus nigra Arn.

- var. *pyreneica* (La Peyrouse) Godron for Spanish populations,
- var. *salzmanii* (Dunal) Asch. and Graebner for French trees,
- var. *poiretiana* (Ant.) Schneider for Corsican populations,
- var. *calabrica* (Loud.) Schneider for Calabrian populations
- var. *nigra* (Hoess) Asch. and Graebner for Central Italy, Austria, Yugoslavia, Albania, Greece, Romania, Bulgaria, Crimea, and Turkey.

Additional studies from all Eastern areas with genetic markers would lead to a better understanding of the geographical variation of this last and largest variety.

This study should help to define international policies for the preservation of genetic diversity for this species.

REFERENCES

- ARBEZ, M., BERNARD-DAGAN, C. & FILLON, C., 1974: Variabilité intraspécifique des monoterpènes de *Pinus nigra* Arn. Bilan des premiers résultats. *Ann. Sci. Forest.* **31**(1):57-70.
- ARBEZ, M & MILLIER, C. 1971: Contribution à l'étude de la variabilité géographique de *Pinus nigra* Arn. *Ann. Sci. Forest.* **28**(1):23-49.
- BARADAT, PH., BERNARD-DAGAN, C., FILLON, C., MARPEAU, A. & PAULY, G., 1972: Les terpènes du Pin maritime: aspects biologiques et génétiques. II. Hérité de la teneur en monoterpènes. *Ann. Sci. For.* **29**(3):307-334.
- BARADAT PH., BERNARD-DAGAN, C. & PAULY, G., 1975: Les terpènes du Pin maritime: aspects biologiques et génétiques. III. Hérité de la teneur en myrcène. *Ann. Sci. For.* **31**(1):29-54.
- BARADAT, PH. & MARPEAU-BÉZARD, A., 1988: Le Pin maritime - *Pinus pinaster* Ait. Biologie et génétique des terpènes pour la connaissance et l'amélioration de l'espèce. Thèse d'Etat, Université de Bordeaux I, 443 pp.
- BERNARD-DAGAN, C., FILLON, C., PAULY, G., BARADAT, PH. & ILLY, G., 1971: Les terpènes du Pin maritime: aspects biologiques et génétiques. I. Variabilité de la composition terpénique dans un individu, entre individus et entre provenances. *Ann. Sci. For.* **28**(3):223-258.

- BONNET-MASIMBERT, M. & BIKAY-BIKAY, V., 1978: Variabilité intraspécifique des isozymes de la glutamate-oxaloacétate transaminase chez *Pinus nigra* Arnold. Intérêt pour la taxonomie des sous-espèces. *Silvae Genetica* **27**(2):71-78.
- CHARLES, P.J., DELPLANQUE, A., MARPEAU, A., BERNARD-DAGAN, C. & ARBEZ, M., 1982: Susceptibility of European black pine (*Pinus nigra*) to the European pine shoot moth (*Rhyacionia buoliana*): variations of susceptibility at the provenance and individual level of the pine and effect of terpene composition. In: Resistance to Diseases and Pests in Forest Trees. Proceedings of the Workshop 1980, PUDOC Wageningen: 206-212.
- CRAWFORD, D.J., 1983: Phylogenetic and systematic inferences from electrophoretic studies. In: Isozymes in Plant Genetics and Breeding, Part A. (ed. S.D. Tanksley & T.J. Orton). pp. 257-287. Elsevier Science Publishers B.V., Amsterdam.
- CRONQUIST, A., 1973: Chemical plant taxonomy: a generalist's view of a promising speciality. In: Chemistry in Botanical Classification. (ed. G. Bendz & J. Santesson) Nobel Symposia - Medicine and Natural Science. pp. 29-39. Nobel Foundation Stockholm, Academic Press, New York and London.
- DEBAZAC, E.F., 1964: Manuel des conifères. pp. 93-97. Ecole Nationale des Eaux et Forêts, Nancy, 136 pp.
- DEBAZAC, E.F., 1971: Contribution à la connaissance de la répartition et de l'écologie de *Pinus nigra* Arn. dans le Sud de l'Europe. *Ann. Sci. For.* **28**(2):91-139.
- FARJON, A., 1984: Pines, Drawings and Descriptions of the Genus *Pinus*. E.J. Brill / DR.W. Backhuys Leiden, 220 pp.
- FINESCHI, S., 1983: Variabilità intraspecifica in *Pinus nigra* Arn. Risultati di analisi su alcuni sistemi isoenzimatici. *Italia forestale e montana* **38**(4):200-213.
- FINESCHI, S. & GROSSONI, P., 1981: Contenuto in monoterpeni di oleoresine xilematiche in provenienze diverse di pino laricio. *Italia forestale e montana* **36**(5):232-239.
- LAURANSON, J., 1989: Exploration de la diversité biochimique chez les conifères: contribution à l'étude de l'hybridation de *Pinus uncinata* × *Pinus sylvestris* et à la connaissance du complexe spécifique *Pinus nigra*. Thèse Univ. Lyon I, 245 pp.
- LEE, C.H., 1968: Geographic variation in European black pine. *Silvae Genetica* **17**:165-172.
- LIP, & ADAMS, W.T., 1988: Range-wide patterns of allozyme variation in Douglas-fir (*Pseudotsuga menziesii*). *Can. J. For. Res.* **19**:149-161.
- MARPEAU, A., BARADAT, PH. & BERNARD-DAGAN, C., 1975: Les terpènes du Pin maritime: aspects biologiques et génétiques. IV. Hérité de la teneur en deux sesquiterpènes: le longifolène et le caryophyllène. *Ann. Sci. For.* **32**(4):185-203.
- MARPEAU, A., WALTER, J., LAUNAY, J., CHARON, J., BARADAT, PH. & GLEIZES, M., 1989: Effects of wounds on the terpene content of twigs of Maritime pine (*Pinus piaster* Ait.) II. Changes in the volatile terpene hydrocarbon composition. *Trees* **4**:220-226.
- MARPEAU-BEZARD, A., BARADAT, PH. & BERNARD-DAGAN, C., 1983: Les terpènes du Pin maritime: aspects biologiques et génétiques. V. Hérité de la teneur en limonène. *Ann. Sci. For.* **40**(2):197-216.
- MIROV, N.T., 1961: Composition of Gum Turpentine of Pines. Pacific Southwest Forest Range Experiment Station, U.S. Department of Agriculture, Forest Service. Technical Bulletin No. 1239.
- MIROV, N.T., 1967: The Genus *Pinus*. The Ronald Press Company, New York, 602 pp.
- NANSON, A., 1972: Peuplements, arbres "plus" et vergers à graines de *Pinus nigra* cv Koeklare. Station de recherche des eaux et forêts, Groenendaal-Hoeilaart, Belgique. Travaux - Série E, No. 5.
- NIKOLIĆ, D. & TUCIĆ, N., 1983: Isoenzyme variation within and among populations of European black pine (*Pinus nigra* Arnold). *Silvae Genetica* **32**(3-4):80-89.
- RÖHRIG, E., 1957: Über die Schwarzkiefer (*Pinus nigra* Arn.) und ihre Formen. I. Teil: Die natürlichen Standorte. *Silvae Genetica* **6**(1-2):39-53.
- RÖHRIG, E., 1966: Die Schwarzkiefer (*Pinus nigra* Arn.) und ihre Formen. II. Erste Ergebnisse von Provenienzversuchen. *Silvae Genetica* **15**(1):21-26.
- RÖHRIG, E., 1969: Die Schwarzkiefer (*Pinus nigra* Arn.) und ihre Formen. III. Untersuchungen an Sämlingen verschiedener Herkünfte. *Silvae Genetica* **18**(3):92-94.
- ROMAN-AMAT, B., 1984: Contribution à l'exploration et à la valorisation de la variabilité intraspécifique et individuelle du pin laricio de Corse, *Pinus nigra* Arn. ssp. *laricio* var. *corsicana* Loud. Thèse de Docteur Ingénieur. Université de Paris Sud. Centre d'Orsay No. 679, 135 pp.
- SCALTSOYIANNES, A., ROHR, R., PANETSOS K.P. & TSAKTIRA M., 1994: Allozyme frequency distributions in five European populations of black pine (*Pinus nigra* Arnold). *Silvae Genetica* **43**(1):20-30.
- SQUILLACE, A.E., WELLS, O.O. & ROCKWOOD, O., 1980: Inheritance of monoterpene composition in cortical oleoresin of loblolly pine. *Silvae Genetica* **29**(3-4):141-152.
- VIDAKOVIĆ, M., 1974: Genetics of European black pine (*Pinus nigra* Arn.). *Annales Forestales (Zagreb)* **6**(3):57-86.
- VON RUDLOFF, E., 1975: Volatile leaf oil analysis in chemosystematic studies of North American conifers. *Biochemical Systematic and Ecology* **2**:131-167.
- WALTERS, S.M., 1963: Methods of classical plant taxonomy. In: Chemical Plant Taxonomy. (ed. T. Swain). pp. 1-15. Academic Press - London and New York.
- WHEELER, N.C., KRIEBEL, C.H., LEE, C.H., READ, R.A. & WRIGHT, J.W., 1976: 15-year performance of European black pine in provenance tests in North Central United States. *Silvae Genetica* **25**(1):1-6.
- WILCOX, M.D. & MILLER, J.T., 1974: *Pinus nigra* provenance variation and selection in New Zealand. New Zealand Forest Service, Forest Research Institute, Genetics and Tree Improvement Report No. 68.
- WRIGHT, J.W. & BULL, W.I., 1962: Geographic variation in European black pine - two year results. *Forest Science* **8**(1):32-42.
- YAZDANI, R., NILSSON, J.E. & ERICSSON, T., 1985: Geographical variation in the relative proportion of monoterpenes in cortical oleoresin of *Pinus sylvestris* in Sweden. *Silvae Genetica* **34**(6):201-208.
- ZAVARIN, E., 1970: Qualitative and quantitative co-occurrence of terpenoids as a tool for elucidation of their biosynthesis. *Phytochemistry* **9**:1049-1063.