Foliar and Cortex Oleoresin Variability of Silver Fir (*Abies alba* Mill.) in Albania[§]

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Terpene composition of needle and cortical oleoresin from lateral shoots were analyzed by GC/MS for four Silver fir (*Abies alba* Mill.) populations scattered in natural species range in Albania. More than sixty compounds were detected in the needle oleoresin, which was characterized by a high content of α-pinene, camphene, β-pinene, limonene and bornyl acetate. Three monoterpenes, α-pinene, β-pinene and limonene, and two sesquiterpenes, β-caryophyllene and germacrene D, comprised the majority of cortical oleoresin. The terpene composition differences among the populations that led to the recognition of two chemotypes. The needle oleoresin from the provinces of Puka, Bulqiza and Llogara were characterised by high amounts of β-pinene, camphene and α-pinene and low amounts of limonene, while that from Drenova had high amounts of β-pinene and limonene. A similar pattern was found in the cortical oleoresin with the exception of camphene that was a minor contributor. Geographical and seasonal variation between the populations was, also, investigated. Multivariate analysis of both needle and cortical oleoresin separated Drenova (southeastern population) from the other sites. When both major monoterpenes and sesquiterpenes were considered four chemical profiles could be attributed. Based on their chemical profiles, the populations can be divided into two groups:

- Populations with high content of β-pinene and α-pinene but a low content of limonene (Puka, Bulqiza and Llogara), typical of most of *A. alba* populations in all its distribution range.
- Population with a high content of limonene and a moderate content of β-pinene and α-pinene (Drenova).

Introduction

The genus *Abies*, which is regarded to be complex in comparison with other genera of the family *Pinaceae*, was established by Miller in 1754 [type species: European Silver fir (*Abies alba*)]. There are 39 species, 23 varieties and 8 hybrids of the genus *Abies* (Liu, 1971). All these taxa are native to cool temperate and boreal regions of the Northern Hemisphere and, like other genera of the *Pinaceae*, are widely distributed across both Eastern and Western parts of the world.

Silver fir, an ecologically valuable and indigenous tree species in many European mountain forests, is currently one of the most important conifers in Albania, occupying an area of about 16060 ha or 9.3% of conifer forests. Natural occurring fir forest in Albania, comprise pure fir forest and mixed forest with other conifers or broadleaf. Silver Fir populations of the southeastern area of distribution are very variable species and regularly had a higher variation than other populations (Wolf, 1990; Wolf, 1994).

Extensive studies have been conducted on the morphological, anatomical characters and iso-

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zymes of fir species in an attempt to examine the genetic structure of fir populations in Albania and the kind of existing variation (Mitrushi, 1955; Mitrushi, 1966; Habili, 1985; Dano, 1998; Misiri 1999).

Recently, several classes of chemical compounds have increasingly been used at all levels of the taxonomic hierarchy, the exact level depending upon the particular compounds employed. Terpenoids, in general, are most useful at the lower level (specific and infraspecific) (Stuessy, 1990). Conifer oleoresin composition affords a readily available source of genotypic information, which is much less susceptible to environmental variation. It represents a useful device in chemotaxonomy of conifers and in characterizing trees of different seed or even clonal origin within a single species (Kossuth *et al.* 1988; Lockhart, 1990; Lang, 1994; Gallis and Panetsos, 1997; Canard *et al.* 1997; Gallis *et al*, 1998). Needle oleoresin, on the other hand, which is a primary resin, synthesized in primary tissues, represents another resin system and constitutes a very powerful tool for the study of variation among populations and chemotaxonomic differences between species from the same family or genus (Fady *et al.*, 1992). The value of the resin from different tissues as taxonomic tools lies in the fact that many of them are under the strong genetic control of relatively few number of genes and they are not greatly influenced by environmental factors (Hanover, 1990).

Terpenes have been used to solve problems related to population and ecological genetics of fir trees as well as markers for genetic research into inheritance (Mitsopoulos and Panetsos, 1987; Wolf, 1992; Fady *et al.*, 1992; Lang 1994). Monoterpenes have also been used as a tool in the studies of general adaptation and growth of certain genotypes of fir (Gaudlitz *et al.,* 1988; Wolf, 1992). The monoterpene composition of the volatiles from the fir needle and cortical oleoresin show a high degree of variation from tree to tree (Paule *et al.*, 1988; Wolf, 1992). To our knowledge, however, no studies have been conducted on terpene variation of Silver fir grown in Albania. The aim of the present study was to determine qualitative and quantitative differences in the needle and cortical oleoresin of the naturally grown Silver fir located in different geographical regions in Albania during two seasons. Chemotaxonomic investigation on this species was also attempted in order to interpret the origin of variation.

Experimental

Sampling

Collection Sites

Plant material from natural distributions of silver fir forest was sampled at four locations: Lumebardhe (Puka district), Liqeni i Zi (Bulqiza district), Drenova – Bozdovec (Korca district) and Llogara (Vlora district). These areas cover a representative sample of the natural population of this species in Albania (Table I).

Trees, 15–30 years of age, at least 50 m apart, in good health and without visible symptoms of disease or insect infestation, were selected for this study. Samples were taken considering only last year branches starting from the lower longitudes and progressing to the higher ones.

The first sampling was performed during the dormancy period, while the second sampling was conducted when the trees were in full vegetation. Winter sampling took place between 15th Novem-

Table I. Geographical and topographic characteristics of collection sites.

	Geographic Position		Topographic Characteristics		
Location*	Longitude	Latitude	Altitudinal range (m)	Orientation of compartment**	Aspect of Slope (°)
Puka	42° 07′	20° 10′	800–850	SW	23
BULQIZA	41° 27′	20° 18′	1540–1600	W	20–25
LLOGARA	40° 12′	19° 35′	980–1050	W	25
DRENOVA	40° 35′	20° 48′	900–1000	SW	21–27

* Plant material from natural distributions of silver fir forest was sampled at four locations: Lumebardhe (Puka district), Liqeni i Zi (Bulqiza district), Drenova – Bozdovec (Korca district) and Llogara (Vlora district).
** W west, SW south-west.

ber and 5th December 1998. Summer sampling started on 24th May 1999 and was completed on 8th June. Twenty trees per area were sampled and each of them was labelled with numbers that were used for the second collection. The samples (branches one or two years old) were kept in dry ice during transportation and then in a deep freezer (–20° C).

Laboratory procedures

Cortical oleoresin sample collection

Cortical rather than xylem oleoresin was sampled as many previous investigations showed that the former contained more major constituents and was less affected by the location in the tree. After the branch was removed from the freezer, drops of oleoresin were collected within 2–3 minutes from cortical tissue by excising branch buds at about 10 mm or less from the tips. Exuded oleoresin was placed in screw-cap vials, diluted with 0.25 ml cyclohexane and stored in a refrigerator (4° C) until analysed.

Extraction of needle oleoresin via hydrodistillation

The needles were manually separated from the branches and 50 grams of fresh needles were distilled in 65 ml of water. A steam distillation method with a Clevenger apparatus was used for distillation of the needles after removal of woody parts. The distillation process lasted three hours. The needle oleoresin collected in cyclohexane (Holubova *et al.*, 2000) was dried over Na_2SO_4. Subsequently, the samples were stored in a refrigerator (4 °C) until analysed.

Quantitative and qualitative analyses

Quantitative oleoresin analysis was carried out on a Hewlett Packard Gas Chromatoghraph HP 5890 series II equipped with a flame ionization detector (FID). The GC was equipped with a polar fused silica capillary column HP-FFAP (30 m × 0.25 mm i.d., 0.33 μm film thickness). Helium was used as the carrier gas (flow rate = 1.7 ml/min at 60° C). Initial column temperature was 60° C for 3 min, then increased at a rate of 3° C/min up to 240° C, and finally held at 240° C for 20 min. Injector and detector temperature were 230° C and 260° C, respectively. 0.5 μl of each sample were injected using an HP 7673 auto-sampler (split ratio 1/20).

The qualitative analysis was performed on a HP5890 GC coupled with a VG TRIO 2000 Mass Spectrometer. Chromatographic conditions were similar to those described above. Mass spectra recorded at 70 eV. Identification of the chemical constituents was based on comparison of the retention times (RT) values and mass spectra with those obtained from authentic standards and/or the NIST/NBS, Wiley 275 library spectra and Adams's Book (1995).

Statistical procedures

The Statistical Package for Social Sciences (SPSS) for Windows version 8.0 software (SPSS Inc. Chicago, 1997) was used for the statistical analysis.

The following differential statistical methods were employed:

- One way analysis of variance (one way ANOVA) was used to test the hypothesis of equality of all population means, while multivariate analysis of variance (MANOVA) was used to test differences among the groups
- Hierarchical cluster analysis with Ward methods and Squared Euclidean distance was used to classify groups of objects judged to be similar according to a distance or similarity measure.
- Canonical variate analysis by Wilks-Lambda method was used to analyse the correlation between two groups of variables (attributes) in the same set of objects simultaneously.

Results and Discussion

Needle oleoresin

Hydrodistillation of the needles of *A. alba* resulted to a pleasant smelling, colorless to pale yellow oil. The geographical variation in the needle oleoresin yield for the investigated populations, is presented in Table II. The yields reported in the present study are within the range previously reported for the fir species (Guenther, 1952; Habili 1985; Glowacki, 1994). The different yield obtained from trees of different geographical sites can be explained by the influence of ecological factors (Fady *et al.*, 1992; Simic *et al.*, 1996). In the

Table II. Variation in essential oil yield from different provenances.

Provenance*	Winter Collection[#]		Summer Collection[$]	
	Range (ml/g)	Mean (%) (ml/g fresh tissue)	Range (ml/g)	Mean (%) (ml/g fresh tissue)
Bulqiza	0.50–0.88	0.656	0.13–0.40	0.280
Drenova	0.40–0.78	0.566	0.17–0.40	0.278
Llogara	0.36–0.58	0.484	0.17–0.33	0.207
Puka	0.42–0.76	0.560	0.17–0.40	0.260

*: LSD test detected that significant variations exist among the provenances under investigation.

[#]: One way analysis of variance (Anova) showed that the yield of essential oil of the Bulqiza provenance differed significantly from that of the Llogara and the Drenova provenances, but no significant differences existed among Puka and other provenances.

[$]: The yields of essential oil from the summer collection showed that only Bulqiza and Llogara differed significantly from each other.

present study, the variation may be attributed to abrupt changes in climatic conditions prevailing in the mainland of Albania. The decrease in needle resin yield from autumn to spring is also supported by other previous investigations (Guenther, 1952; Schonwitz *et al.*, 1990).

More than sixty compounds were detected and identified in the needle oleoresins of *A. alba*, representing approximately 92–96% of the oleoresin (Table III).

The two major classes, mono- and sesquiterpenoids, were equally represented by 34 and 36 compounds, respectively, accounting together for more than 75% of the total needle oleoresin, as is depicted in Table III. Most of the previous studies dealt only with monoterpenes (Wolf, 1990; Wolf, 1992; Paule *et al.*, 1988). Sesquiterpenes in the needle oleoresin of *A. alba* have only been studied by Isidorov *et al.* (1996) and Roussis *et al.* (2000). Within the class of monoterpenoids, 13 monoterpene hydrocarbons, 9 monoterpenes alcohol and 12 other oxygenated monoterpenes (including ketones, esters, ethers and miscellaneous compounds) were detected. The second class, sesquiterpenoids, was represented by 21 sesquiterpene hydrocarbons and 15 oxygenated sesquiterpenes. No qualitative variation was recorded between populations. Generally, only a few compounds were present in all individuals and these major contributors are the compounds present in the highest proportions.

Monoterpenes are abundant in all needle oleoresins. Positive correlation is found to exist between the relative amounts of α- and β-pinene and β-pinene and limonene and this correlation is significant at alpha 0.01%. The correlation of the relative amounts of α-pinene and limonene is negative but not significant. β-Pinene was the major constituent in needle oleoresin in both seasons and all populations. A decrease in the amount of this compound was observed from winter to summer. In general, the content of monoterpenes decreases from winter to summer while the concentration of sesquiterpenes increases. The α-pinene content among the investigated populations in both seasons was low. It seems that, from the major compounds, limonene is the one that can distinguish the populations. The Tukey HSD test showed that individuals of the Drenova population, which yielded the highest amount of this compound, differed significantly from all the populations in the winter collection, and significantly from Puka and Bulqiza but not significantly from Llogara in the the summer collection.

Oxygenated monoterpenoids, although represented by many compounds (21), they account for only 10.3% to 22% of the total percentage. Among them, borneol and α-terpineol and bornyl acetate were the only ones present in all populations and individuals analyzed. Bornyl acetate, which was found in high quantities in the winter samples and shows significant variation between the northern populations (Bulqiza and Puka) and southern populations (Drenova and Llogara), does not show any significant variation among the populations in the summer collection. The mean concentration of this compound greatly decreased in the summer collection. There are only a few

Table III. Percentage contribution of the identified metabolites in the needle oleoresin from *A. alba*.

Compound*	Kovats indices	Bulqiza Winter	Bulqiza Summer	Drenova Winter	Drenova Summer	Llogara Winter	Llogara Summer	Puka Winter	Puka Summer
Santene	888	3.2 (1.33)^	1.0 (0.07)	2.1 (0.13)	0.3 (0.06)	2.9 (0.45)	0.8 (0.09)	2.6 (0.45)	0.9 (0.1)
Tricyclene	926	1.8 (0.24)	0.7 (0.23)	1.3 (0.53)	0.3 (0.08)	1.7 (0.25)	0.7 (0.1)	1.6 (0.64)	0.7 (0.09)
α-Pinene	939	9.5 (0.45)	15.2 (2.61)	10.1 (2.1)	8.7 (1.15)	11.7 (0.85)	8.4 (0.9)	10.9 (0.95)	9.9 (1.14)
Camphene	953	17.2 (1.65)	5.4 (0.72)	10.8 (1.43)	3.0 (0.75)	16.0 (1.37)	4.8 (0.12)	15.5 (1.14)	5.2 (0.94)
β-Pinene	980	24.7 (1.74)	16.4 (2.32)	31.6 (2.72)	14.5 (1.67)	30.7 (1.17)	18.4 (1.4)	29.5 (1.64)	16.0 (1.45)
Myrcene	991	1.0 (0.06)	0.6 (0.09)	1.2 (0.07)	0.6 (0.09)	1.0 (0.67)	0.8 (0.1)	1.1 (0.42)	0.7 (0.04)
α-Phellandrene	1005	0.2 (1.11)	0.2 (0.14)	0.2 (0.72)	0.1 (0.31)	0.2 (0.34)	0.2 (0.08)	0.1 (0.06)	0.2 (0.11)
Limonene	1031	11.4 (2.73)	6.5 (0.73)	17.5 (2.11)	10.2 (1.91)	11.5 (0.09)	9.8 (0.5)	9.6 (1.12)	6.9 (0.74)
Z-β-Ocimene	1040	0.2 (0.14)	tr#	tr	tr	0.1 (0.06)	tr	0.2 (0.06)	tr
α-Terpinolene	1088	0.8 (0.06)	0.8 (0.06)	0.6 (0.07)	1.0 (0.5)	0.8 (0.24)	1.7 (0.25)	0.8 (0.12)	1.6 (0.46)
Monoterpene hydrocarbons		**70.3**	**47.9**	**77.0**	**40.3**	**77.2**	**46.1**	**72.2**	**42.7**
Linalool	1098	0.2 (0.11)	tr	0.1 (0.33)	tr	0.2 (0.18)	tr	0.92 (0.11)	0.2 (0.11)
endo-Fenchol	1112	tr	0.2 (0.12)	tr	0.2 (0.13)	tr	0.4	tr	0.4 (0.16)
α-Campholenal	1125	0.1 (0.24)	0.5 (0.09)	tr	0.3 (0.23)	tr	0.5	tr	0.5 (0.13)
trans-Pinocarveol	1139	tr	1.0 (0.23)	tr	0.6 (0.11)	tr	0.4	tr	0.9 (0.11)
Camphor	1144	0.1 (0.23)	0.2 (0.16)	tr	0.1 (0.18)	tr	0.1 (0.13)	tr	0.2 (0.18)
Camphene hydrate	1148	0.2 (0.11)	0.2 (0.19)	tr	0.2 (0.10)	0.2 (0.16)	0.2 (0.17)	0.16 (0.13)	0.4 (0.38)
Borneol	1165	1.0 (0.09)	5.5 (0.45)	0.4 (0.68)	3.4 (0.25)	0.6 (0.13)	2.3 (0.35)	1.7 (0.19)	5.4 (0.62)
cis-Pinocamphone	1173	0.1 (0.25)	0.2 (0.13)	0.1 (0.45)	0.1 (0.08)	tr	0.1 (0.43)	tr	0.3 (0.18)
Terpin–4-ol	1177	tr	tr	tr	tr	tr	tr	0.1 (0.56)	0.4 (0.14)
α-Terpineol	1189	1.6 (0.45)	3.5 (0.46)	1.4 (0.45)	4.0 (0.43)	1.21	5.8 (0.63)	2.0 (0.41)	6.5 (0.78)
Linalool acetate	1257	0.2 (0.08)	tr	0.1 (0.12)	tr	0.11	tr	1.0 (0.19)	tr
Bornyl acetate	1285	17.6 (1.42)	1.8 (0.11)	7.1 (0.73)	1.6 (0.16)	5.68	2.1 (0.42)	13.0 (1.13)	2.4 (0.18)
α-Terpinyl acetate	1350	0.6 (0.08)	0.2 (0.16)	0.4 (0.46)	0.2 (0.13)	0.35	0.2 (0.13)	1.4 (0.13)	0.4 (0.18)
Oxygenated monoterpenes		**22.05**	**14.4**	**10.5**	**11.3**	**10.1**	**12.7**	**20.9**	**21.4**
Neryl acetate	1365	tr	0.3 (0.11)	–	0.6 (0.14)	tr	0.2 (0.18)	tr	0.3 (0.24)
E-Caryophyllene	1418	0.7 (0.11)	10.8 (1.18)	0.6 (0.12)	12.8 (1.65)	1.2 (0.14)	7.2 (0.65)	0.8 (0.21)	9.4 (1.12)
p-Menth–1-en–9-ol-acetate	1420	tr	0.2 (0.18)	tr	0.3 (0.13)	0.2 (0.16)	0.3 (0.13)	0.2 (0.16)	0.7 (0.11)
α-Himachalene	1447	tr	0.1 (0.33)	tr	tr	0.1 (0.28)	0.1 (0.28)	0.2 (0.18)	0.2 (0.14)
α-Humulene	1454	0.4 (0.18)	5.7 (0.46)	0.3 (0.18)	7.2 (0.74)	0.6 (0.12)	4.7 (0.44)	0.3 (0.13)	5.2 (0.44)
9-epi-*E*-Caryophyllene	1467	tr	tr	tr	0.4 (0.10)	tr	0.3 (0.14)	tr	0.2 (0.18)
γ-Gurjunene	1473	tr	tr	tr	0.4 (0.11)	tr	0.3 (0.13)	tr	0.4 (0.14)
γ-Muurolene	1476	tr	0.5 (0.11)	tr	0.8 (0.09)	tr	0.9 (0.11)	tr	0.7 (0.16)
Germacrene D	1480	0.1 (0.38)	0.2 (0.11)	0.2 (0.11)	0.1 (0.24)	0.3 (0.13)	0.3 (0.12)	0.1 (0.28)	0.2 (0.09)
β-Selinene	1485	0.1 (0.26)	0.6 (0.13)	tr	1.0 (0.19)	0.2 (0.18)	0.7 (0.10)	tr	0.7 (0.13)
cis-β-Guaiene	1490	tr	0.6 (0.12)	0.2 (0.16)	0.3 (0.14)	0.3 (0.11)	1.2 (0.10)	0.4 (0.10)	0.4 (0.10)
α-Selinene	1494	0.2 (0.14)	0.6 (0.12)	0.1 (0.66)	1.0 (0.18)	0.2 (0.20)	0.7 (0.09)	–	0.5 (0.20)
β-Himachalene	1499	0.2 (0.11)	0.7 (0.10)	0.2 (0.18)	1.2 (0.14)	0.4 (0.21)	1.2 (0.13)	0.2 (0.16)	1.0 (0.19)
Germacrene A	1503	tr	0.2 (0.18)	0.1 (0.48)	0.4 (0.08)	0.1 (0.28)	tr	tr	0.3 (0.13)
γ-Cadinene	1513	tr	0.8 (0.10)	tr	1.2 (0.18)	0.1 (0.26)	0.1 (0.26)	tr	0.7 (0.09)
δ-Cadinene	1524	0.3 (0.12)	2.2 (0.21)	0.5 (0.16)	3.5 (0.31)	0.5 (0.11)	2.8 (0.22)	0.3 (0.13)	2.0 (0.18)
Cadina–1,4 -diene	1532	0.1 (0.28)	0.2 (0.46)	0.1 (0.15)	0.3 (0.11)	0.1 (0.27)	0.2 (0.14)	0.1 (0.26)	0.2 (0.16)
α-Cadinene	1538	tr	0.2 (0.55)	tr	0.3 (0.12)	tr	0.3 (0.11)	tr	0.2 (0.18)
α-Calacorene	1542	tr	0.2 (0.18)	0.1 (0.33)	0.3 (0.11)	tr	0.1 (0.28)	0.3 (0.11)	0.1 (0.24)
Sesquiterpene hydrocarbons		**2.8**	**25.6**	**3.1**	**32.6**	**5.1**	**23.0**	**3.3**	**25.2**
E-Nerolidol	1564	tr	0.1 (0.28)	tr	0.2 (0.18)	0.4 (0.08)	0.4 (0.15)	tr	0.2 (0.17)
10-epi-γ-Eudesmol	1619	2.3 (0.31)	1.8 (0.19)	5.4 (0.55)	0.7 (0.08)	4.8 (0.41)	3.9 (0.34)	1.4 (0.14)	1.1 (0.14)
Epi-α-Cadinol	1640	0.1 (0.25)	0.2 (0.16)	tr	0.3 (0.12)	0.6 (0.10)	0.2 (0.18)	tr	0.2 (0.18)
Cubenol	1642	0.3 (0.14)	tr	0.3 (0.11)	0.1 (0.25)	0.2 (0.19)	tr	0.5 (0.09)	tr
Himachalol	1647	0.4 (0.22)	tr	0.8 (0.12)	tr	0.6 (0.11)	0.2 (0.19)	0.3 (0.12)	tr
α-Cadinol	1653	0.3 (0.10)	2.8 (0.24)	0.3 (0.14)	4.2 (0.52)	0.3 (0.14)	4.2 (0.44)	0.42 (0.07)	2.7 (0.21)
E,E-Farnesol	1722	tr	0.7 (0.09)	0.3 (0.13)	1.0 (0.17)	tr	0.3 (0.11)	tr	0.8 (0.09)
Oxygenated sesquiterpenes		**4.8**	**12.1**	**9.4**	**15.7**	**7.7**	**18.2**	**3.5**	**10.7**
Total (%)		**100**	**100**	**100**	**99.9**	**100**	**100**	**99.9**	**100**

* : Every compound was present at least in one individual tree in amounts ≥0.1%.

^ : Numbers in parenthesis represent standard deviation.

: Compound present in less than 0.1%.

$: The LSD (least significant difference test) showed that significant differences existed among the provenances.

reports on the presence of bornyl acetate in genus *Abies* (Guenther, 1952; Baĝci *et al.*, 1999; Ross *et al.*, 1996). Strong positive correlation existed between camphene and bornyl acetate, which was significant at 0.1 level. On the other hand, a decrease in the mean concentrations of camphene and bornyl acetate was associated with an increase in the content of borneol and α-terpineol and a negative correlation existed between these compounds.

The most important sesquiterpene hydrocarbons are, namely, *E*-caryophyllene, α-humulene, α- and γ-cadinene and β-himachalene. Drenova differed significantly from Llogara in both seasons when the content of *E*-caryophyllene was considered. ANOVA revealed that there was no significant variation among the investigated populations for the remaining sesquiterpenes. All populations had a low content of *E*-caryophyllene and α-humulene with a strong positive correlation, but their mean concentrations increased during the summer. *E*-Caryophyllene had been reported only by Roussis *et al.* (2000) for *A. abies* individuals. To our knowledge there has been no other report on its presence in European Silver Fir.

From oxygenated sesquiterpenes, 10-epi-γ-eudesmol was characterized by significant variation among populations, as shown in Tukey HDS multiple's range and its tendency to increase from north to the south could be observed.

The investigated populations in Albania produced higher amount of β-pinene than those from Southeastern Europe and Calabria (Wolf and Bungart, 1992), whereas the production of α-pinene and camphene was lower compared to that of Eastern and South-Eastern Europe (Wolf, 1990; Wolf, 1994; Wolf and Bungart, 1992). It is interesting to note the total absence of β-phellandrene. This compound was found to be a major one in the study reported by Wolf (1994) with a mean concentration of 5.4% but with very high amplitude (0% to 54% of total needle oleoresin content).

As it was generally observed, very high variation existed within each population both in summer and winter collection. Nevertheless, based on the major contributors, a chemical profile could be deduced for each of the populations. While considering the chemical profiles in this investigation, trace components were largely ignored as they could be probably the result of incomplete blocking of recessive alleles, low level activities of non-specific enzymes or chemical ärtefacts" caused by chemical transformations during extraction and isolation (Franz, 1993). These chemical profiles, particularly those of the winter collection, appeared qualitatively the same for the populations of Puka, Bulqiza and Llogara, and agreed with results previously reported (Roussis *et al.*, 2000):

β-*pinene* > *camphene* > α-*pinene* > *limonene*

On the contrary, the chemical profile of Drenova population was different, but in accordance with a chemical profile for the Southeastern European populations reported by Wolf (1990):

β-*pinene* > α-*pinene* > *limonene* > *camphene*

Multivariate analysis was carried out using Canonical Discriminant Analysis and Cluster Analysis for the major compounds (> 1%) and for two classes of terpenes both for winter and summer collection. The results for the winter collection showed significant differences up to the third function. The total variance proportion for the first two functions was 88%, with 65.9% for the first (canonical correlation 0.889) and 22% for the second (canonical correlation 0.889). Appropriate plotting indicated several groupings of the populations. The distribution centers of Llogara and Drenova appeared very close, whereas Puka and Bulqiza were very well separated both from each other and from Drenova and Llogara. On the other hand, the results from the summer collection didn't produce a good separation for the investigated populations, as several cases were misclassified.

Cortical oleoresin

Cortical oleoresin composition was different from the needle oleoresin profile. This study confirmed the existence of two resins that have different terpene proportions, which has been noted previously for other conifer species (Sjőrdin *et al.*, 1996; Dormont *et al.*, 1998; Kleinhentz *et al.*, 1999; Wainhouse *et al.*, 2000). The results of the analyses revealed the presence of thirty-seven compounds, including mono-and sesquiterpenoids. Thirty-three of these compounds were identified, representing approximately 95–97% of the cortex oleoresin.

Generally, the number of compounds in the summer collection samples was smaller than the number of compounds found in the winter collection. This decrease in the number of compounds was higher in Puka and smaller in Drenova. The highest variation in terms of the number of compounds was observed for the sesquiterpenes, while oxygenated sesquiterpenes exhibited the highest seasonal variation.

The cortical oleoresin of silver fir in both seasons was found to contain mainly monoterpene hydrocarbons with a mean yield of 62–82%. The compositions of the studied populations of silver fir were similar to those reported by Lang (1994) and Vendramin *et al.* (1997), all stressing the importance of α-pinene, β-pinene and limonene. Oxygenated monoterpenes had a mean concentration smaller than 2%. As shown in Table IV, Llogara had the highest mean concentration of monoterpenes and regardless the change in the mean concentration of single compounds, there were no significant differences between the seasons. Drenova shows the highest variation in monoterpene content as the mean increased from 64.23% in the winter collection to 85.83% in the summer collection.

α-Pinene is one of the major compounds found in all populations. A general increase in the content of this compound from winter to summer could be observed. Drenova was the lowest pro-

Table IV. Percentage contribution of the identified metabolites in the cortical oleoresin from *A. alba*.

Compound*	Kovats	Bulqiza		Drenova		Llogara		Puka	
	indices	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
α-Pinene	939	26.3 (1.45)^	42.9 (4.11)	12.2 (1.42)	23.1 (2.15)	36.0 (3.11)	38.6 (3.65)	37.3 (3.65)	47.1 (4.23)
Camphene	953	0.7 (0.14)	0.9 (0.10)	0.3 (0.15)	0.4 (0.11)	0.6 (0.09)	0.4 (0.08)	0.9 (0.10)	0.7 (0.11)
β-Pinene	980	35.7 (2.11)	26.6 (3.10)	14.9 (1.22)	18.3 (1.15)	32.6 (3.24)	29.9 (2.33)	36.6 (3.06)	30.0 (3.21)
Myrcene	991	1.4 (0.18)	1.7 (0.18)	1.6 (0.22)	2.6 (0.22)	1.5 (0.18)	1.6 (0.17)	1.4 (0.14)	1.4 (0.13)
Limonene	1031	6.9 (0.64)	10.2 (1.43)	35.3 (3.14)	41.4 (4.44)	10.2 (1.04)	10.3 (1.18)	6.7 (0.63)	5.5 (0.53)
α-Terpinolene	1088	tr[#]	tr	tr	tr	0.1 (0.28)	tr	0.1 (0.26)	tr
Monoterpene hydrocarbons		**71.0**	**82.5**	**64.2**	**85.8**	**81.1**	**80.9**	**83.1**	**84.7**
Bornyl acetate	1285	0.2 (0.16)	tr	1.1 (0.24)	tr	tr	–	0.2 (0.18)	tr
Oxygenated monoterpenes		**0.2**	**tr**	**1.1**	**tr**	**tr**	**–**	**0.2**	**tr**
α-Longipinene	1351	0.3 (0.12)	0.1 (0.26)	0.3 (0.13)	tr	tr	tr	0.3 (0.11)	tr
Neryl acetate	1365	0.2 (0.31)	0.1 (0.28)	tr	0.1 (0.27)	tr	tr	0.1 (0.26)	tr
E-Caryophyllene	1418	5.0 (0.51)	3.6 (0.33)	8.9 (0.86)	3.9 (0.33)	3.9 (0.31)	3.2 (0.38)	3.4 (0.33)	3.6 (0.33)
α-Himachalene	1447	0.3 (0.12)	tr	tr	tr	tr	tr	0.3 (0.14)	tr
α-Humulene	1454	2.4 (0.21)	1.8 (0.19)	3.9 (0.35)	1.9 (0.11)	1.8 (0.18)	1.6 (0.18)	1.6 (0.18)	1.8 (0.11)
γ-Muurolene	1477	0.6 (0.09)	0.1 (0.27)	0.3 (0.11)	tr	0.4 (0.10)	tr	0.3 (0.13)	tr
Germacrene D	1480	12.5 (1.23)	9.2 (0.95)	15.2 (1.54)	6.9 (0.66)	6.6 (0.61)	12.1 (1.26)	5.9 (0.56)	8.4 (0.81)
β-*cis*-Guaijene	1490	0.6 (0.09)	tr	0.2 (0.16)	tr	tr	tr	0.7 (0.11)	0.1 (0.23)
α-Selinene	1494	0.3 (0.13)	0.2 (0.19)	tr	tr	0.1 (0.22)	tr	tr	tr
β-Himachalene	1499	0.2 (0.19)	0.1 (0.21)	tr	tr	0.1 (0.23)	tr	0.2 (0.16)	tr
γ-Cadinene	1513	0.6 (0.08)	0.3 (0.10)	0.2 (0.17)	0.1 (0.29)	0.3 (0.16)	0.1 (0.26)	0.2 (0.17)	0.2 (0.18)
δ-Cadinene	1524	1.1 (0.16)	0.3 (0.11)	0.9 (0.15)	0.1 (0.22)	0.6 (0.09)	tr	0.6 (0.09)	0.1 (0.25)
α-Cadinene	1538	0.6 (0.07)	0.2 (0.16)	0.6 (0.09)	0.3 (0.13)	0.5 (0.10)	0.4 (0.13)	0.3 (0.14)	0.2 (0.16)
Sesquiterpene hydrocarbons		**25.7**	**16.0**	**30.8**	**13.5**	**15.2**	**18.0**	**14.8**	**14.8**
E-Nerolidol	1564	0.2 (0.16)	tr	0.1 (0.26)	tr	0.2 (0.16)	tr	tr	tr
10-epi-γ-Eudesmol	1619	1.4 (0.16)	1.1 (0.14)	2.0 (0.24)	0.2 (0.18)	1.4 (0.32)	0.4 (0.24)	0.8 (0.16)	tr
epi-α-Cadinol	1640	0.2 (0.15)	tr	tr	tr	0.4 (0.16)	tr	0.2 (0.12)	tr
E,E-Farnesol	1722	tr	0.1 (0.22)	0.1 (0.25)	tr	0.2 (0.24)	0.3 (0.15)	0.2 (0.18)	0.2 (0.11)
Oxygenated sesquiterpenes		**2.11**	**1.2**	**3.4**	**0.3**	**3.3**	**1.0**	**1.4**	**0.2**
Total (%)		**99.0**	**99.7**	**99.5**	**99.6**	**99.6**	**99.9**	**99.5**	**99.7**

* : Every compound was present at least in one individual tree in amounts $\geq$0.1%.
^ : Numbers in parenthesis represent standard deviation
[#] : Compound present in less than 0.1%.

ducer of β-pinene and the highest of limonene. Llogara showed the highest within-population variation for α-pinene and β-pinene for both the winter and summer collection. A tendency for an increase in limonene content from north to south was observed whereas a north to south decreasing trend could be observed for camphene. A strong correlation existed between the relative amounts of α-pinene, β-pinene and limonene. This correlation was positive and significant at alpha = 0.05% for α-pinene and β-pinene. Negative correlation existed between the content of β-pinene and limonene and between α-pinene and limonene. This correlation was significant at the 0.01 level. Strong positive correlation between monoterpene proportions may indicate common biosynthesis (Shaw *et al.*, 1982). In grand fir (*Abies grandis* Mill) the common biosynthetic route was found to be catalyzed by a single enzyme and led to the formation of the major monoterpenes (Bohlmann *et al.*, 1997).

A large number of sesquiterpenes were present in low quantities in the cortical oleoresins. High variation existed in the quantities of these compounds in all populations and between seasons. *E*-Caryophyllene was among the most important sesquiterpenes. Significant variation existed among populations. Individuals from Drenova contained the highest amounts of *E*-caryophyllene in both the summer and winter collection. Other important sesquiterpenes detected were germacrene D and the three isomers of cadinene: α-cadinene, γ-cadinene and δ-cadinene. It is interesting to note the strong positive correlation found between *E*-caryophyllene and α-humulene. These compounds come from the same biosynthetic route and have similar structures (Croteau, 1986). A strong positive correlation is also found between these two compounds in the needles of *Pinus caribaea* (Barnola *et al.*, 1997), the needles of *Cedrus libani* (A.) Rich. and *Cedrus atlantica* Manet (Canard *et al.*, 1997).

Among the group of oxygenated sesquiterpenes, only 10-epi-γ-eudesmol was found in amounts higher than 1% of the total content.

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