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ARTICLE *in* CHEMISTRY & BIODIVERSITY · JUNE 2007

Impact Factor: 1.52 · DOI: 10.1002/cbdv.200790079 · Source: PubMed

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Variability of the Needle Essential Oils of *Pinus heldreichii* from Different Populations in Montenegro and Serbia

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The essential-oil compositions of *Pinus heldreichii* CHRIST. from Montenegro and Serbia are reported at the population level. Whitebark pine is a sub-endemic high-mountain Balkan pine relict of an anthropogenically reduced area, with large morphological diversity and insufficiently clear taxonomic position. In the pine-needle terpene profile from three populations from Montenegro, and one from Serbia, 101 compounds were detected, 72 of which could be identified (*Table 3*). The dominant constituents are limonene (26.3%), α-pinene (17.5%), germacrene D (13.5%), and β-caryophyllene (10.4%), comprising *ca.* 67.7% of the essential oil. Medium-to-high contents (0.5–10%) of the following 16 additional components were found: β-pinene, β-myrcene, α-humulene, δ-cadinene, α-muurolene, (*E*)-hex-2-enal, β-gurjunene, γ-muurolene, isopimarol, camphene, γ-cadinene, aromadendrene, β-bisabolene, *trans*-β-farnesene, α-cadinene, and (*Z*)-hex-3-en-1-ol. The similarity of the populations and the within-population variability was visualized by principle-component analysis (PCA) of eleven selected terpenes in 97 tree samples. Cluster and genetic analyses suggest closest connection between the two spatially most-distant populations I (Montenegro) and IV (Serbia). Based on the profile of the main sesquiterpene components, the studied populations from Montenegro and Serbia are more similar to the populations from Greece and the Central Balkan peninsula (Bosnia and Serbia–Kosovo) than to those on the furthest eastern margin of their natural range (Bulgaria).

Introduction. – *Pinus heldreichii* CHRIST. (whitebark pine or Bosnian pine), a Tertiary relict, is a Balkan sub-endemite of the high mountains of southern Italy, Bosnia–Herzegovina, Serbia, Montenegro, Albania, Macedonia, Bulgaria, and Greece. It mostly forms pure stands, although it can also appear in mixed ones, especially with *Pinus peuce*. The taxonomic position of *P. heldreichii* is still undefined [1–3]. Based on morphological characteristics, several varieties and horticultural forms have been differentiated [4][5]. Discoveries of transitional forms between the varieties *typica* and *leucodermis* in Macedonia [6], and spontaneous whitebark-pine hybrids with *P. mugo* and *P. nigra* in Bosnia–Herzegovina [7], have undoubtedly contributed to past uncertainties. Whitebark pine was for a long time considered a variety of black pine, an assumption that was refuted by terpene analysis [8].

Many articles have been published concerning the essential-oil composition in different *Pinus* species, with a focus on geographic variability and taxonomic

significance [9–16]. Recent analyses claim that whitebark pine holds a 'divider' position between the Mediterranean and the rest of the pines of *Sylvestres* subsection [17], or even that it is closely related to Mediterranean taxa [18–20]. *Otto* and *Wilde* [21] have pointed out the importance of sesqui-, di-, and triterpenoids in chemotaxonomy and phylogeny of conifers, as well as their applicability as genetic markers at various taxonomic levels.

The oleoresin composition and essential oils of the twigs or needles of whitebark pine were intensively studied [22–32]. However, all of these studies dealt with a small number of samples per population (less than ten trees). The variability and divergence within a significant population were only studied in Bulgarian populations [33–35].

In this work, we present a detailed study of the compositions of terpenes and their derivatives in the needles of four natural whitebark-pine populations from the central part of the Balkan, the ultimate goal being to extend our knowledge of the diversity and taxonomy of this species. A map of the study area in Montenegro and Serbia is shown in *Fig. 1*, and the corresponding geographic and geologic data are collected in *Table 1*.

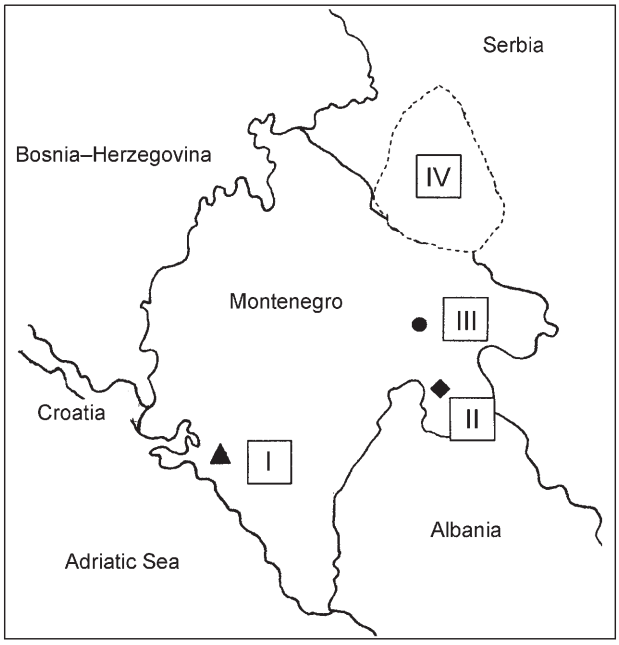

Fig. 1. *Location of analyzed populations of* P. heldreichii *in Montenegro and Serbia*. Population I: Mt. Lovćen; II: Mt. Zeletin; III: Mt. Bjelasica; IV: Mt. Zlatibor to Mt. Pešter.

Results and Discussion. – A summary of the terpene distribution of compounds in three whitebark-pine populations from Montenegro (I–III) and in one population from Serbia (IV) is given in *Table 2*. A total of 72 individual components out of 101 compounds could be identified, as listed in *Table 3*.

Table 1. *Geographic and Geologic Characteristics of the Study Area* (see also *Fig. 1*)

Feature	Montenegro			Serbia
	Population I (Mt. Lovcen)	Population II (Mt. Zeletin)	Population III (Mt. Bjelasica)	Population IV (Mt. Zlatibor to Mt. Pester)
Latitude (N)	42° 25′	42° 37′	42° 53′	43° 15′–43° 30′
Longitude (E)	18° 50′	19° 50′	19° 45′	19° 30′–19° 55′
Altitude (m)	1700–1800	1700–1900	1700	1100–1430
Exposure	S, SE	E, NE	S, SE	S, SW, NW
Terrain inclination	<30°	<30°	<35°	<30°
Geologic substratum	limestone	limestone serpentinite schist	limestone dolomite	limestone dolomite hornestone tuff neogene sediments

In the overall terpene profile, mono- and sesquiterpenes were found to dominate, comprising 92.9% of the essential oil. Two monoterpenes, limonene (*16*)[1]) and α-pinene (*6*), as well as two sesquiterpenes, germacrene D (*53*) and β-caryophyllene (*44*) were the dominant constituents, together comprising 67.7% of the essential-oil mass. The following 16 additional components were found to be present in medium-to-high amounts (0.5–10%) [10]: β-pinene (*9*), β-myrcene (*10*), α-humulene (*48*), δ-cadinene (*58*), α-muurolene (*54*), (*E*)-hex-2-enal (*1*), β-gurjunene (*45*), γ-muurolene (*52*), isopimarol (*94*), camphene (*7*), γ-cadinene (*57*), aromadendrene (*46*), β-bisabolene

Table 2. *Compositions* (in %) *of Different Classes of Terpenes in the Needles of* P. heldreichii. For individual compounds, see *Table 3*.

Class	Population[a])				Average
	I	II	III	IV	I–IV
Monoterpene hydrocarbons	56.08	48.11	57.60	50.34	53.70
Oxygenated monoterpenes	0.90	2.26	0.62	0.74	1.21
Total monoterpenes	56.98	50.37	58.22	51.08	54.91
Sesquiterpene hydrocarbons	37.29	37.82	34.36	43.28	36.96
Oxygenated sesquiterpenes	1.15	1.09	0.93	0.83	1.06
Total sesquiterpenes	38.44	38.91	35.29	44.11	38.02
Diterpene hydrocarbons	0.21	0.50	0.29	0.20	0.32
Oxygenated diterpenes	1.28	1.97	1.45	1.40	1.55
Total diterpenes	1.49	2.47	1.74	1.60	1.87
Others[b])	2.02	5.83	3.72	1.80	3.73
Unknown	1.07	2.42	1.03	1.41	1.47
Total [%]	100.00	100.00	100.00	100.00	100.00

[a]) See *Table 1*. [b]) Mainly aliphatic or aromatic alcohols, aldehydes, and acids and their esters.

[1]) Numerals in italics refer to the entries in *Table 3*.

Table 3. *Terpene Compositions* (in %) *in the Needles of Four Populations of* P. heldreichii. For geographic and analytic details, see *Table 1* and the *Exper. Part*. Standard deviations are given in parentheses.

Entry	Compound[a])	Population				Average
		I	II	III	IV	
1	(*E*)-Hex-2-enal	0.61 (0.35)	2.60 (2.26)	2.02 (1.54)	0.79 (0.46)	1.68 (1.74)
2	(*Z*)-Hex-3-en-1-ol	0.09 (0.10)	1.14 (1.25)	0.33 (0.26)	0.00 (0.00)	0.48 (0.84)
3	Hexan-1-ol	0.00 (0.00)	0.57 (1.27)	0.00 (0.00)	0.00 (0.00)	0.18 (0.75)
4	Tricyclene	0.00 (0.00)	0.04 (0.15)	0.00 (0.00)	0.00 (0.00)	0.01 (0.09)
5	α-Thujene	0.23 (0.21)	0.23 (0.10)	0.23 (0.09)	0.10 (0.08)	0.22 (0.14)
6	α-Pinene	20.25 (10.13)	16.05 (4.12)	17.30 (6.41)	12.85 (5.49)	17.51 (7.42)
7	Camphene	0.94 (0.40)	1.09 (0.47)	1.05 (0.29)	0.63 (0.27)	1.00 (0.40)
8	Sabinene	0.09 (0.09)	0.01 (0.03)	0.07 (0.07)	0.00 (0.00)	0.05 (0.07)
9	β-Pinene	6.46 (2.11)	4.84 (1.44)	5.96 (2.70)	4.52 (2.50)	5.66 (2.26)
10	β-Myrcene	2.18 (0.57)	1.80 (0.41)	2.50 (0.50)	2.17 (0.27)	2.16 (0.55)
11	Unknown 1	0.00 (0.00)	0.03 (0.09)	0.00 (0.02)	0.00 (0.00)	0.01 (0.05)
12	α-Phellandrene	0.02 (0.05)	0.09 (0.09)	0.03 (3.00)	0.00 (0.00)	0.04 (0.07)
13	Δ^3-Carene	0.00 (0.01)	0.00 (0.00)	0.21 (1.15)	0.00 (0.00)	0.06 (0.60)
14	α-Terpinene	0.03 (0.10)	0.08 (0.10)	0.02 (0.07)	0.00 (0.00)	0.04 (0.10)
15	*p*-Cymene	0.00 (0.03)	0.02 (0.09)	0.00 (0.00)	0.00 (0.00)	0.01 (0.05)
16	Limonene	25.12 (9.81)	23.23 (4.71)	29.70 (6.57)	29.54 (5.72)	26.30 (7.67)
17	*trans*-β-Ocimene	0.33 (0.41)	0.11 (0.15)	0.14 (0.16)	0.36 (0.36)	0.21 (0.29)
18	γ-Terpinene	0.03 (0.08)	0.05 (0.07)	0.02 (0.05)	0.00 (0.00)	0.03 (0.07)
19	α-Terpinolene	0.40 (0.40)	0.47 (0.34)	0.37 (0.14)	0.17 (0.26)	0.40 (0.32)
20	Phenethyl alcohol	0.00 (0.00)	0.06 (0.12)	0.00 (0.00)	0.00 (0.00)	0.02 (0.07)
21	Myrcenol	0.00 (0.02)	0.30 (0.29)	0.00 (0.00)	0.00 (0.00)	0.09 (0.21)
22	Unknown 2	0.00 (0.01)	0.06 (0.13)	0.00 (0.00)	0.00 (0.00)	0.02 (0.07)
23	Unknown 3	0.00 (0.00)	0.25 (0.39)	0.00 (0.00)	0.00 (0.00)	0.07 (0.20)
24	Unknown 4	0.00 (0.00)	0.07 (0.13)	0.00 (0.00)	0.00 (0.00)	0.02 (0.08)
25	*endo*-Borneol	0.00 (0.00)	0.10 (0.23)	0.00 (0.00)	0.00 (0.00)	0.03 (0.14)
26	Terpinen-4-ol	0.00 (0.02)	0.04 (0.07)	0.00 (0.00)	0.00 (0.00)	0.01 (0.04)
27	α-Terpineol	0.02 (0.05)	1.35 (1.70)	0.02 (0.08)	0.00 (0.00)	0.43 (1.13)
28	Unknown 5	0.00 (0.00)	0.26 (0.45)	0.00 (0.00)	0.00 (0.00)	0.08 (0.27)
29	Bornyl acetate	0.13 (0.34)	0.02 (0.05)	0.02 (0.08)	0.05 (0.12)	0.06 (0.20)
30	Vinylguaiacol	0.02 (0.03)	0.01 (0.03)	0.01 (0.03)	0.00 (0.00)	0.01 (0.03)
31	Terpinen-4-ol-acetate	0.06 (0.09)	0.06 (0.08)	0.04 (0.06)	0.23 (0.16)	0.06 (0.09)
32	α-Terpinyl acetate	0.55 (0.28)	0.34 (0.22)	0.49 (0.18)	0.19 (0.20)	0.44 (0.25)
33	Citronellyl acetate	0.14 (0.23)	0.05 (0.06)	0.05 (0.06)	0.27 (0.29)	0.09 (0.17)
34	α-Ylangene	0.01 (0.06)	0.13 (0.17)	0.03 (0.07)	0.00 (0.00)	0.05 (0.12)
35	α-Copaene	0.24 (0.11)	0.29 (0.15)	0.23 (0.16)	0.36 (0.11)	0.26 (0.14)
36	β-Bourbonene	0.32 (0.09)	0.35 (0.16)	0.31 (0.17)	0.48 (0.13)	0.33 (0.15)
37	Unknown 6	0.01 (0.06)	0.00 (0.02)	0.00 (0.01)	0.00 (0.00)	0.01 (0.04)
38	β-Cubebene	0.05 (0.07)	0.06 (0.08)	0.06 (0.08)	0.08 (0.10)	0.05 (0.07)
39	β-Elemene	0.33 (0.14)	0.28 (0.13)	0.28 (0.13)	0.34 (0.07)	0.27 (0.14)
40	Unknown 7	0.03 (0.05)	0.00 (0.00)	0.00 (0.03)	0.04 (0.07)	0.02 (0.04)
41	Unknown 8	0.03 (0.06)	0.06 (0.09)	0.04 (0.08)	0.02 (0.05)	0.04 (0.07)
42	α-Gurjunene	0.01 (0.03)	0.20 (0.23)	0.14 (0.27)	0.02 (0.05)	0.11 (0.22)
43	Unknown 9	0.00 (0.00)	0.03 (0.08)	0.00 (0.00)	0.00 (0.00)	0.01 (0.05)
44	β-Caryophyllene	8.92 (2.06)	11.01 (2.84)	11.36 (2.37)	10.07 (2.89)	10.41 (2.65)
45	β-Gurjunene	1.57 (0.44)	1.45 (0.51)	1.22 (0.63)	1.79 (0.31)	1.44 (0.54)
46	Aromadendrene	0.91 (0.24)	0.86 (0.27)	0.73 (0.36)	1.13 (0.22)	0.85 (0.31)

Table 3 (cont.)

Entry	Compound[a])	Population				Average
		I	II	III	IV	
47	Unknown 10	0.13 (0.06)	0.15 (0.14)	0.17 (0.08)	0.18 (0.10)	0.15 (0.10)
48	α-Humulene	1.91 (0.35)	2.22 (0.51)	2.15 (0.48)	2.18 (0.51)	2.10 (0.47)
49	Unknown 11	0.00 (0.00)	0.03 (0.08)	0.00 (0.02)	0.04 (0.10)	0.01 (0.05)
50	*trans*-β-Farnesene	0.60 (0.17)	0.50 (0.11)	0.48 (0.30)	0.77 (0.14)	0.55 (0.23)
51	γ-Gurjunene	0.00 (0.00)	0.25 (0.52)	0.03 (0.17)	0.00 (0.00)	0.09 (0.32)
52	γ-Muurolene	1.21 (0.30)	1.12 (0.47)	1.01 (0.55)	1.74 (0.39)	1.16 (0.48)
53	Germacrene D	15.41 (4.90)	13.13 (6.53)	11.37 (6.15)	16.33 (2.68)	13.53 (5.93)
54	α-Muurolene	1.61 (0.53)	1.83 (0.87)	1.50 (0.92)	2.33 (0.64)	1.69 (0.80)
55	β-Bisabolene	0.68 (0.28)	0.53 (0.39)	0.49 (0.33)	0.81 (0.14)	0.59 (0.34)
56	δ-Amorphene	0.12 (0.05)	0.10 (0.16)	0.09 (0.09)	0.13 (0.09)	0.11 (0.11)
57	γ-Cadinene	1.03 (0.28)	0.93 (0.36)	0.87 (0.46)	1.32 (0.27)	0.98 (0.38)
58	δ-Cadinene	1.69 (0.58)	1.91 (0.76)	1.45 (0.88)	2.51 (0.73)	1.74 (0.79)
59	*trans*-Cadina-1(2),4-diene	0.19 (0.07)	0.16 (0.13)	0.14 (0.12)	0.27 (0.12)	0.17 (0.11)
60	α-Cadinene	0.48 (0.15)	0.51 (0.21)	0.42 (0.28)	0.62 (0.15)	0.48 (0.21)
61	Lauric acid	0.11 (0.12)	0.24 (0.18)	0.12 (0.15)	0.20 (0.11)	0.16 (0.16)
62	Unknown 12	0.03 (0.04)	0.03 (0.07)	0.04 (0.06)	0.03 (0.09)	0.03 (0.06)
63	Germacrene D-4-ol	0.32 (0.24)	0.29 (0.16)	0.21 (0.15)	0.29 (0.10)	0.28 (0.19)
64	Ethyl laurate	0.07 (0.07)	0.03 (0.06)	0.08 (0.07)	0.02 (0.04)	0.06 (0.07)
65	τ-Muurolol	0.16 (0.11)	0.13 (0.15)	0.05 (0.08)	0.02 (0.06)	0.11 (0.13)
66	α-Cadinol	0.13 (0.08)	0.07 (0.10)	0.03 (0.06)	0.02 (0.06)	0.08 (0.09)
67	Unknown 13	0.02 (0.06)	0.10 (0.16)	0.02 (0.08)	0.12 (0.13)	0.05 (0.12)
68	Eudesma-4(15),7-diene-1-β-ol	0.18 (0.10)	0.10 (0.15)	0.11 (0.11)	0.00 (0.00)	0.12 (0.13)
69	*trans,trans*-Farnesol	0.21 (0.37)	0.24 (0.20)	0.24 (0.42)	0.33 (0.70)	0.24 (0.37)
70	Benzyl benzoate / miristic acid	0.01 (0.04)	0.02 (0.06)	0.01 (0.03)	0.04 (0.08)	0.02 (0.05)
71	*cis*-α-Copaene-8-ol	0.00 (0.00)	0.04 (0.12)	0.07 (0.15)	0.00 (0.00)	0.03 (0.11)
72	Farnesyl acetate (isomer)	0.06 (0.06)	0.18 (0.14)	0.13 (0.20)	0.15 (0.10)	0.13 (0.14)
73	2-*trans*,6-*trans*-Farnesyl acetate	0.05 (0.09)	0.01 (0.03)	0.07 (0.13)	0.00 (0.00)	0.04 (0.10)
74	Unknown 14	0.00 (0.00)	0.05 (0.10)	0.00 (0.01)	0.08 (0.11)	0.02 (0.07)
75	2-Methylbenzyl benzoate	0.05 (0.06)	0.16 (0.15)	0.09 (0.13)	0.00 (0.00)	0.09 (0.12)
76	Unknown 15	0.00 (0.00)	0.06 (0.12)	0.00 (0.00)	0.00 (0.00)	0.02 (0.07)
77	Unknown 16	0.19 (0.08)	0.09 (0.09)	0.09 (0.09)	0.16 (0.12)	0.12 (0.10)
78	Octadeca-9,12,15-trienal	0.02 (0.08)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.01 (0.04)
79	Unknown 17	0.13 (0.09)	0.23 (0.20)	0.21 (0.26)	0.22 (0.14)	0.18 (0.19)
80	Unknown 18	0.11 (0.07)	0.25 (0.14)	0.20 (0.16)	0.14 (0.11)	0.18 (0.14)
81	Unknown 19	0.00 (0.00)	0.14 (0.14)	0.06 (0.11)	0.02 (0.06)	0.06 (0.11)
82	Unknown 20	0.02 (0.04)	0.03 (0.11)	0.03 (0.07)	0.00 (0.00)	0.03 (0.08)
83	Unknown 21	0.01 (0.04)	0.03 (0.11)	0.01 (0.03)	0.00 (0.00)	0.02 (0.07)
84	Unknown 22	0.05 (0.11)	0.00 (0.00)	0.00 (0.01)	0.04 (0.10)	0.01 (0.07)
85	Palmitic acid	0.28 (0.12)	0.22 (0.19)	0.29 (0.17)	0.29 (0.15)	0.27 (0.16)
86	Fusicoca-2,5-diene (M_r 272)	0.21 (0.12)	0.50 (0.33)	0.29 (0.33)	0.20 (0.22)	0.32 (0.30)
87	Unknown 23	0.03 (0.06)	0.00 (0.00)	0.01 (0.03)	0.00 (0.00)	0.01 (0.04)
88	Manool	0.21 (0.08)	0.64 (0.37)	0.34 (0.23)	0.35 (0.16)	0.39 (0.30)
89	Phytol	0.03 (0.05)	0.04 (0.12)	0.00 (0.02)	0.00 (0.00)	0.02 (0.08)
90	Linoleic acid	0.11 (0.07)	0.18 (0.11)	0.27 (0.26)	0.04 (0.12)	0.18 (0.18)
91	Geranylgeraniol	0.04 (0.07)	0.03 (0.09)	0.02 (0.05)	0.02 (0.06)	0.03 (0.07)

Table 3 (cont.)

Entry	Compound[a])	Population				Average
		I	II	III	IV	
92	Methyl arachidonate	0.62 (0.76)	0.40 (0.25)	0.49 (0.25)	0.42 (0.17)	0.02 (0.07)
93	Unknown 24	0.03 (0.08)	0.03 (0.09)	0.00 (0.00)	0.00 (0.00)	0.50 (0.07)
94	Isopimarol	1.07 (0.48)	1.00 (0.60)	1.11 (0.66)	1.05 (0.45)	1.06 (0.57)
95	Unknown 25	0.16 (0.15)	0.15 (0.28)	0.14 (0.18)	0.32 (0.44)	0.16 (0.23)
96	Unknown 26	0.04 (0.08)	0.00 (0.00)	0.01 (0.04)	0.00 (0.00)	0.01 (0.05)
97	Unknown 27	0.02 (0.07)	0.05 (0.21)	0.00 (0.02)	0.00 (0.00)	0.02 (0.12)
98	Unknown 28	0.03 (0.07)	0.01 (0.06)	0.00 (0.00)	0.00 (0.00)	0.01 (0.05)
99	Unknown 29	0.00 (0.02)	0.23 (1.13)	0.00 (0.00)	0.00 (0.00)	0.07 (0.63)
100	Methyl (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-4,7,10,13,16,19-hexaenoate	0.00 (0.00)	0.16 (0.24)	0.01 (0.05)	0.00 (0.00)	0.05 (0.15)
101	Pimaric acid	0.00 (0.02)	0.33 (0.37)	0.00 (0.00)	0.00 (0.00)	0.10 (0.25)
	Total [%]	100	100	100	100	100

[a]) Literature names rather than fully systematic names are given.

(*55*), *trans*-β-farnesene (*50*), α-cadinene (*60*), and (*Z*)-hex-3-en-1-ol (*3*). Trace components were found to comprise only 8.6% of the total essential oil.

Population I (Mt. Lovcen, Montenegro) was found to have more α-pinene (*6*) and β-pinene (*9*), but less β-caryophyllene (*44*) and α-humulene (*48*), compared to the other populations. Population II (Mt. Zeletin, Montenegro) was shown to have the lowest content of limonene (*16*) and β-myrcene (*10*). Population III (Mt. Bjelasica, Montenegro) exhibited the highest content of β-myrcene (*10*), and the least of germacrene D (*53*) and β-gurjunene (*45*). Population IV (Mt. Zlatibor to Mt. Pester, Serbia) was found to be distinguished from the Montenegro populations I–III by higher contents of germacrene D (*53*), α-muurolene (*54*), and δ-cadinene (*58*), and by lower contents of α-pinene (*6*) and camphene (*7*).

According to *Simić et al.* [30], *P. heldreichii* is one of only few conifer species with high limonene (*16*) content, such as *P. sabiniana* (19%) and *P. resinosa* (29.1%). This general observation was also confirmed in this study. Notably, the limonene amounts in the Montenegro populations I and II were found to be similar to those from populations investigated at Mt. Šara in Kosovo [30]. Further, the limonene amounts in the populations III and IV from Montenegro and Serbia, respectively, were found to be similar to those from adult whitebark pines of Mt. Rujišta in Bosnia [29]. The limonene content in our populations is lower compared to those in whitebark pines from Mt. Prokletije (Kosovo) [31], adult whitebark pines from Mt. Trebević (Bosnia) [29], and whitebark pines from both Greece [32] and Bulgaria [35]. Based on the chemical profile of five main terpenes in the needles (limonene $\gg$ α-pinene > germacrene D > β-caryophyllene > β-pinene)[2]) and an abundant myrcene content, whitebark-pine

[2]) The following symbols are used to denote differences in content: 0.1–1.0% (=); 1.1–5.0% (>); 5.1–15.0% ($\gg$); more than 15.1% ($\ggg$) [32].

populations from both Montenegro and Serbia (this work) are most similar to Greek whitebark pines. Whitebark pines from Serbia (population IV), besides a high percentage of germacrene D (*53*), are also distinguished by the concentrations of α-muurolene (*54*) and δ-cadinene (*58*), and, along with our population from Montenegro, by the quantity of β-gurjunene (*45*). The four most-abundant terpene components (limonene, α-pinene, germacrene D, and β-caryophyllene) are also present in most of the other geographically close populations from Bosnia and Kosovo [29–31], but in different relative orders. Our populations are the least similar to whitebark pines from Bulgaria, which represent the eastern areal border, in the sesquiterpene part of the profile (limonene $\ggg$ γ-muurolene $>$ α-pinene $\gg$ *trans*-β-farnesene $=$ β-pinene). It was already shown for the Bulgarian populations of *P. heldreichii*, *P. sylvestris*, and *P. nigra* [35–37] that sesquiterpenes reflect inter-population diversity better than monoterpenes.

Out of 101 terpene components from Montenegro and Serbia, those eleven compounds were selected for multi-variation principle-component analysis (PCA) and for cluster analysis, whose distribution was normal (χ^2 test; $P \geq 0.05$) and which did not display statistically important differences between the standard deviations of the populations (*Levene*'s test; $P \geq 0.05$). PCA was performed on a correlation matrix, computed with all 97 tree samples, with individual compounds expressed as percentage of the total terpene fraction (*Fig. 2*). This analysis revealed that the first two principal axes represent 67% of the total information. PCA visualizes variability within the populations and their overlapping, *i.e.*, similarity. Cluster analysis (*Fig. 3*) showed the closest connection between populations I and IV, population III being the most distant.

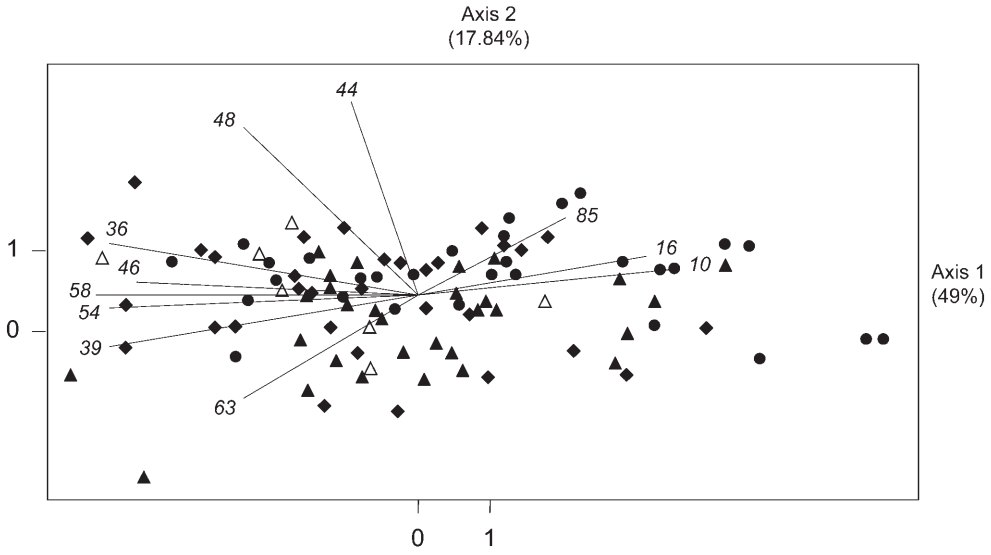

Fig. 2. *Principle-component analysis of eleven selected terpenes isolated from 97 pine-tree samples from four populations.* Terpenes[1]): β-myrcene (*10*), limonene (*16*), palmitic acid (*85*), β-caryophyllene (*44*), α-humulene (*48*), β-bourbonene (*36*), aromadendrene (*46*), δ-cadinene (*58*), α-muurolene (*54*), β-elemene (*39*), and germacrene D-4-ol (*63*). Population I: ▲ (Mt. Lovćen); population II: ◆ (Mt. Zeletin); population III: ● (Mt. Bjelasica); population IV: △ (Mt. Zlatibor to Mt. Pešter).

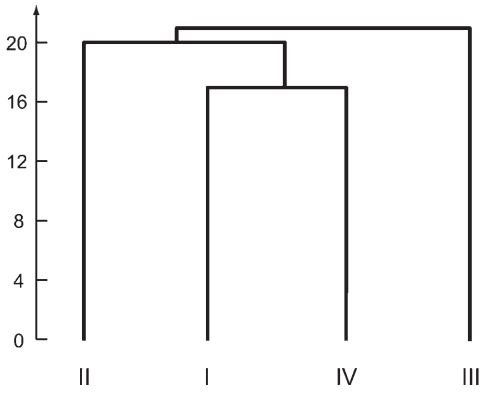

Fig. 3. *Dendrogram based on a 'nearest-neighbor method'* (square Euclidean distance) *of the studied populations I–IV* (mean values) *of* P. heldreichii. The numbers on the vertical axis refer to distance level, calculated on the basis of differences between population contents of selected components.

When testing the normality of the distribution of each of the 101 components, we observed that the α-pinene (*6*) content deviates most from normal distribution (97 values ranging from 3.36–54.92%; $\chi^2 = 13.1$, d.f. = 5, $P = 0.02$). The frequency distribution of α-pinene suggests monogenic type of heredity. In other words, there is a single locus with two alleles (indicated by 'p' and 'P' in *Fig. 4*), leading to three genotypes: homozygous pp and PP, respectively, producing low and high amounts of α-pinene, and heterozygous Pp, producing medium amounts of this terpene. Based on this genetic model, the trimodal α-pinene frequency distribution was accounted for by assigning distribution intervals of 0–20, 20–40, and 40–100% to the pp, Pp, and PP genotypes, respectively (*Fig. 4*). The dendrogram shown in *Fig. 5* is based on genetic distances from estimated allele frequencies of α-pinene. It confirms the similarity of populations I and IV, and of populations II and III.

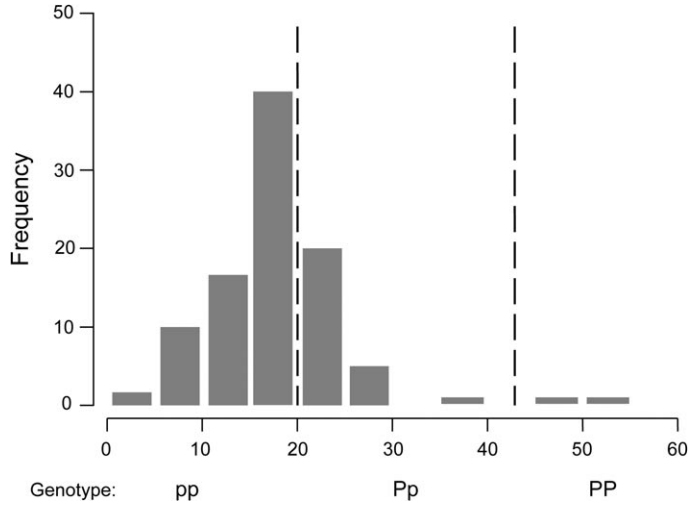

Fig. 4. *Frequency distribution of α-pinene content in the four populations* (97 trees) *suggesting trimodality.* For details, see text.

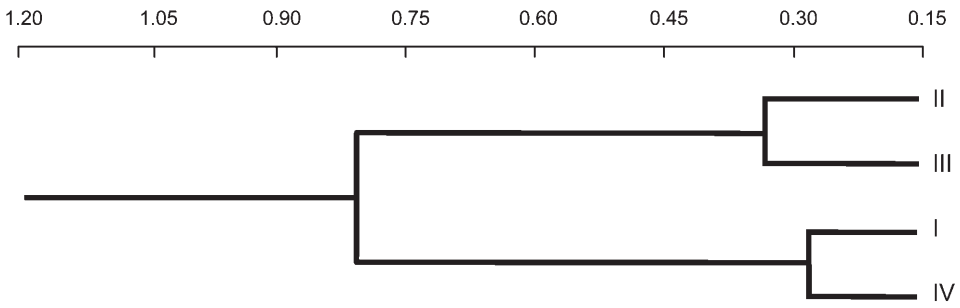

Fig. 5. *Dendrogram based on genetic distances from estimated allele frequencies of α-pinene in the populations I–IV.* Based on UPGMA clustering of *Rogers* genetic distances, as modified by *Wright* [52].

The genetic difference between the whitebark-pine populations, regarding α-pinene content, is approximately the same as that based on the β-pinene content in black pine [38]. This confirms that species of narrower distribution area are not necessarily species of lesser genetic variability. Research on geographically close, endemic species of *Picea omorika*, whose distribution area is even narrower than that of whitebark pine, revealed a high level of variation, based on analysis of isozymes [39][40]. Some genetic analyses of whitebark-pine populations from Italy [41][42] and Bulgaria [35] further confirm this claim. The pronounced divergence of whitebark-pine populations from Italy [43] can be rationalized by the fact that populations located on the margin of a species' natural range are expected to diverge by a larger extent, based on the distance from the main gene pool (situated in Greece). The Quaternary climatic history may also significantly affect diversity within populations: endemic eastern Mediterranean conifers sheltered from harsh glacial conditions are significantly more genetically diverse than their western congeners [44].

Conclusions. – A direct ancestor of whitebark pine is the long-extinct *Pinus thomasiana* var. *tomskiana* [45]. Fossile remains of *P. thomasiana* found in Germany originate from early Oligocene [46] and late Miocene [47], and those found in Poland from middle and late Miocene [48]. To the best of our knowledge, this study is the first to report the essential-oil composition of *Pinus heldreichii* Christ. from Montenegro and Serbia at the population level. All whitebark-pine populations we investigated are found on the spurs of the Dinaric mountain range, with mostly Tertiary limestone. Both cluster analyses (*Figs. 3* and *4*) show the greatest similarity between the geographically, geologically, edaphically, and climatically most-distant populations I and IV (150-km air distance). The two other geographically close populations, II and III, belong to the Prokletije massif. The direction of these, also Dinaric, mountains is specific compared with other parts of the Dinaric range, because it was changed by a tectonic clash with the Scardo-Pindic mountains in Pliocene. Tectonic changes caused the complexity of geomorphologic and ecologic conditions on Prokletije and the creation of ecologic niches during glaciation in Pleistocene [49]. During this period, many plant species were created, survived, evolved and/or diverged, and remained until today, but their survival was constantly endangered by human influences and by climate changes (increasing average temperature), which rationalizes the areal disjunction today [50].

The divergence of whitebark-pine populations from Montenegro and Serbia, as well as those throughout the Balkans and the Apennine peninsula, is most probably the consequence of the influence of tectonic and climatic changes in Tertiary and Quaternary. These assumptions should be confirmed by further genetic analyses.

We thank Dr. *Ljubinko Rakonjac* (Institute of Forestry, Belgrade) and Dr. *Mihailo Tošić* for their help in our investigations. This work is part of a research project (Code 143049) sponsored by the Serbian *Ministry for Science and Environmental Protection*.

Experimental Part

Plant Material. Twigs with needles from the lowest third of the tree crown were collected in late summer to early fall 2003 from 30 randomly selected trees in each of the three populations from Montenegro (Mt. Lovćen (I), Mt. Zeletin (II), Mt. Bjelasica (III)), and from seven selected trees in Serbia (Mt. Zlatibor to Mt. Pešter (IV). The collected twigs were stored at $-20°$. Voucher specimens were deposited at the Institute of Forestry, Belgrade, Serbia.

Isolation of Essential Oils. Two-year-old needles, stored until extraction in a freezer at $-20°$, were cut into pieces of 2–3 mm, and extracted with pentane (1 g of needles per milliliter of solvent). The extracts were kept at 4–6° for 24 h, then filtered, and stored in chromatography vials with solid caps in a refrigerator until analyzed chromatographically.

Compound Identification. The chemical analysis of the pine-needle extracts was performed by GC/FID and GC/MS. The oil components were identified by comparison of their mass spectra to those reported previously [51], or by searching the *Wiley-275* and the *NIST/NBS* libraries. The obtained results were correlated with retention indices.

GC/FID Analysis. A *Hewlett Packard 5890-II* gas chromatograph was used, equipped with a split–splitless injector, an automatic liquid sampler (ALS), an *HP-5* fused-silica capillary column (25 m × 0.32 mm; 0.52 μm film thickness), and an FID. The injector was heated at 250°, the detector at 280°, and the column temp. was linearly raised from 40 to 280° at 4°/min, H_2 (1 ml/min) being used as carrier gas. Samples (1 μl) were injected in splitless mode using ALS.

GC/MS Analysis. GC/MS Analyses were carried out on a *Hewlett Packard G1800C-GCD* apparatus, equipped with split–splitless and ALS, *HP-5MS* fused-silica capillary column (30 m × 0.25 mm; film thickness 0.25 μm), and mass-selective detector. The chromatographic conditions were the same as those described above for GC/FID, except for the carrier gas, which was He. Electron-impact mass spectra (EI-MS; 70 eV) were acquired in the *m/z* range 40–450. For quantification purposes, area-percent values were determined by GC/FID.

Statistical Treatment. The calculation of arithmetic means and standard deviations (SD) of the populations, frequencies, histograms, test for normality (χ^2 test), one-way analyses of variance (ANOVA), *Levene*'s test, principal-component analyses (PCA) as a descriptive multivariate method capable of suggesting the structure and tendency of the data set, and cluster analysis were all carried out with the software Statgraphics Plus (version 5.0; *Statistical Graphics Corporation*, USA). Genetic distances were computed by means of NTSYS [52] using estimated allele frequencies from frequency histograms.

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Received December 22, 2006