The composition of the leaf essential oils of *J. sabina* var. *balkanensis*: chemotypes high in transsabinyl acetate and methyl eugenol discovered in three natural populations

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#### Abstract

The composition of the leaf oil of *J. sabina* var. *balkanensis* is reported. *J. sabina* var. *balkanensis* oils contained chemotypes in all three populations examined. The Greece population contained chemotypes in the concentration of methyl eugenol (13.2%, trace) and elemicin (6.0%, trace). Both chemotypes were low in trans-sabinyl acetate. The eastern Rhodopes and Rila Mtns. had chemotypes for high and low trans-sabinyl acetate (39.9, 0.4%, and 39.8, 0.4%, respectively). Associated with these chemotypes were: low-high  $\alpha$ -pinene (7.5, 41.2%, and 3.7, 41.3%); and high-low trans-thujone (12.7, 0.2% and 6.7, 0.1%), respectively. A comparison is presented of the compositions of the leaf essential oils of *J. sabina* var. *sabina*, from Pyrenees, Switzerland and Kazakhstan. The composition of the volatile leaf oils of *J. s.* var. *balkanensis* do not appear to be different in any major components from that of *J. s.* var. *sabina*, Switzerland. Due to the chemotypes, it is not possible to discern any geographic differentiation among the var. *balkanensis* populations sampled. Published on-line www.phytologia.org *Phytologia 100(1): 45-50 (Mar 16, 2018)*. ISSN 030319430. **KEY WORDS**: *Juniperus sabina* var. *balkanensis*, volatile leaf oils, terpenes, composition. *J. sabina*.

Recently, Adams, Schwarzbach and Tashev (2016) discovered a new variety of *Juniperus sabina* L., *J. s.* var. *balkanensis* R. P. Adams & A. N. Tashev from three locations in Bulgaria and one population in northern Greece. This new variety appears to morphologically nearly identical to *J. s.* var. *sabina.* var. *balkanensis* appears to have the nrDNA of *J. sabina*, but has the chloroplast of *J. thurifera.* It appears var. *balkanensis* is of ancient hybrid origin (Adams, Schwarzbach and Tashev, 2016). Additional sampling revealed var. *balkanensis* also occurs in far western Turkey (Adams et al. 2017) and in the Croatia-Macedonia region (Adams et al. 2018, in press).

Previously, Adams, Nguyen and Liu (2006) reported on the composition of the leaf oils from seven populations of *J. sabina* and one population of *Juniperus sabina* var. *arenaria* (E. H. Wilson) Farjon (now *J. davurica* var. *arenaria* (E. H. Wilson) R. P. Adams) as well as the oils of *J. chinensis* L. and *J. davurica* Pall. They found considerable differentiation in populations of *J. sabina* from the Iberian peninsula and far eastern populations (Kazakhstan, China). The amounts of cedrol, citronellol, safrole, trans-sabinyl acetate, terpinen-4-ol and trans-thujone were found to be polymorphic in several populations.

The leaf oils of the aforementioned species, except var. *balkanensis*, have been reported, and reviewed: *J. chinensis* (Adams, Chu and Zhong, 1994); *J. davurica* (Adams, Shatar and Dembitsky, 1994), *J. sabina* (Adams, Dembitsky and Shatar, 1998, Adams, 2014).

The purpose of this paper is to make the initial report on the composition of the volatile leaf oil(s) of *J. sabina* var. *balkanensis* and compare it with that of *J. s.* var. *sabina*.

### **MATERIALS AND METHODS**

Specimens used in this study (species, popn. id., location, collection numbers):

# J. sabina var. balkanensis

# **Bulgaria and Greece**

- BeR: Eastern Rhodopes. In protected site "Gumurdjinsky Snejnik", locality "Madzharsky Kidik". On limestone rocks above the upper border of a forest of *Fagus sylvatica* ssp. *moesiaca* with *Juniperus communis*. 41° 14' 44.7" N; 25° 15' 31.9" E. elev. 1270 m, 13 Aug. 2012, Adams 13725-13729 (A. Tashev 2012-1-5);
- BSk: Central Stara Planina (the Balkan). National Park "Central Balkan". Reserve "Sokolna". On a steep, rocky limestone slope, with Sorbus aucuparia, S. aria, S. borbasii, Amelanchier ovalis, Carpinus orientalis, Sesleria latifolia, Pastinaca hirsute, Cephalanthera rubra, Laserpitium siler, Hieracium alpicola etc. near a forest of Fagus sylvatica. 42°42'13.3" N,25°08'10.4" E, 1501 m, 22.08.2015. Bulgaria, Adams 14721 (A. Tashev 2015 Balkan 1;
- BkR: Rila Mountain, National Park "Rila". On the eco-path, Beli Iskar", near river Beli Iskar, in a forest with Pinus sylvestris, P. peuce, Picea abies, Abies alba, Juniperus communis, J. sibirica, Vaccinium myrtillus, Rosa canina, Sorbus aucuparia, Acer hyrcanum, Chamaespartium sagittale, Hypericum perforatum, Thymus sp. etc. 42°14'26.5" N, 23°32'33.8" E, 1242 m, 24.06.2015. Bulgaria, Adams 14722-14726 (A. Tashev 2015 Rila 1.1-1.3, 2.1-2.2);

BkG: Mt. Tsena, Greece, Adams 14727-14731 (A. Tashev 2015 So. 1-5 Tsena);

## Turkey

Bk/Turk: Spil Daği Milli Parki (National Park), Turkey, Manisa, N38°, 57', E 27° 41', 1024 m. Adams 14934, (Tuğrul Mataraci 2016-1)

## Other plants referenced in this paper:

*J. chinensis*, CH, Lanzhou, Gansu, China, Adams 6765-67; *J. davurica*, DV, 15 km se Ulan Bator, Mongolia, Adams 7252, 7253, 7601; *J. sabina*, SN, Sierra Nevada, Spain, Adams 7197, 7199, 7200; PY, Pyrenees Mtns., Spain/ France border, Adams 7573-77; SW, Switzerland, Adams 7611, 7612, 7614, 7615; KZ, 30 km n. of Jarkent, Kazakhstan, Adams 7811-13; AM, Altair Mtns., Mongolia, Adams 7585-88; TS, Tian Shan Mtns., Xinjiang, China, Adams 7836-38; MS, sand dunes, 80 km sw Ulan Bator, Mongolia, Adams 7254-56; AR, sand dunes, Lake Qinghai, Qinghai, China, Adams 10347-52. Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU).

Fresh, air dried leaves (50-100 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at 20 °C until analyzed. The extracted leaves were oven dried (100 °C, 48 h) for determination of oil yields.

Oils from 4-5 trees of each taxon were analyzed and average values reported. The oils were analyzed on a HP 5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

### RESULTS

The compositions of the leaf oils are given in Table 1. The leaf oils in all *J. sabina* var. *balkanensis* populations are dominated by sabinene (34.8 - 59.7%) except in the low trans-sabinyl acetate chemotypes (BeR/hiTS, 7.5%) and BkR/hiTS, 3.7%). Other moderately high components are:  $\alpha$ -pinene (1.3 - 3.7%), myrcene (1.6 - 3.9%),  $\gamma$ -terpinene (0.8 - 3.2%), trans-sabinene hydrate (trace -3.1%), terpinen-4-ol (2.1 - 5.9%) and methyl citronellate (0.6 - 3.9%).

The most obvious characteristic of the *J. sabina* var. *balkanensis* oils is the presence of chemotypes in three populations. The Greece population contained a polymorphism for methyl eugenol and elemicin (both phenolics not terpenes). Two plants were hiME and 3 plants were loME. Comparing BkG/hiME vs. BkG/loME: methyl eugenol (13.2%, trace), elemicin (6.0%, trace). Interestingly, this chemotype seems restricted to these two compounds as BkG/hiME and BkG/loME oils are very similar in their other components composition (Table 1). Both chemotypes are low in trans-sabinyl acetate (Table 1).

The second chemotype is high trans-sabinyl acetate (hiTS) and low trans-sabinyl acetate (loTS) polymorphism. The eastern Rhodopes samples contained 1 hiTS and 4 loTS oils, and the Rila Mtns. samples contained 2 hiTS and 3 loTS oils. The single sample from the Sokolna Reserve, Bulgaria and the sample from Spil Dagi, western Turkey were both loTC, loME chemotype oils. Of course, with such limited sampling, one can not know the frequency of chemotypes in any of the populations sampled in this study.

The hiTS chemotype is characterized by higher concentrations of trans-sabinyl acetate (39.6, 39.8%), cis-thujone (1.7, 12.4%), trans-thujone (12.7, 6.7%), trans-sabinol (5.0, 4.3%) with lower concentrations of sabinene (7.5, 3.7%),  $\gamma$ -terpinene (0.8, 0.6) and terpinen-4-ol (2.1, 1.6%) (Table 1). In contrast, the loTS chemotype has lower concentrations of trans-sabinyl acetate (0.3-1.7%), cis-thujone (0-trace%), trans-thujone (trace-0.4%), trans-sabinol (0-0.5%) with higher concentrations of sabinene (41.2-59.7%),  $\gamma$ -terpinene (2.2-3.2%) and terpinen-4-ol (3.9-5.9%) (Table 1).

Adams et al. (2006) reported that a Sierra Nevada, Spain population was the most polymorphic examined, with variations of: trans-thujone (0.5 to 8.7%), terpinen-4-ol (3.6-14.4%), trans-sabinyl acetate (6.4 - 41.3%), and methyl eugenol (0.01 - 12.1%). In the Pyrenees population (PYR, Table I), citronellol was largest with 4.1%. Safrole (1.8%) was only found in PYR. Several compounds, normally associated with cedarwood oil (cedrol,  $\alpha$ -cedrene,  $\gamma$ -amorphene, allo-cedrol, epi-cedrol,  $\alpha$ -acorenol), were reported only in the Kazakhstan population (KAZ, Table I). However, Adams et al. (2006) found that cedrol (13.3%) and related compounds were also present in Tian Shan, China, *J. sabina* population. Cedrol concentrations were very variable in the *J. chinensis* samples (cedrol, 1.0 - 16.0%, Adams et al. 2006).

It is noteworthy that coahuilensol, methyl ether-coahuilensol, and pinchotene acetate (all containing the 2-ethenyl-3-methyl phenol moiety) were present only in the Bulgaria, eastern Rhodopes, loTS chemotype and *J. s.* var. *sabina*, Switzerland (Table 1). These unusual compounds were discovered (Adams et al. 2007) in *Juniperus pinchotii* and *J. arizonica*, and have been found in several taxa around the world.

The composition of the volatile leaf oils of J. s. var. *balkanensis* do not appear to be different in any major components from that of J. *sabina*, Switzerland. Due to the chemotypes, it is not possible to discern any geographic differentiation among the var. *balkanensis* populations sampled.

### ACKNOWLEDGEMENTS

This research was supported by funds from Baylor University.

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Table 1. Comparisons of the per cent total oil for leaf essential oils for *J. sabina* var. *balkanensis* and *J. sabina* populations. *J. s.* var. *balkanensis* population codes: Bk/Turk, Turkey; BkR/IoTS, Rila Mtn, Bulgaria, low trans-sabinyl acetate; BkR/hiTS, Rila Mtn, Bulgaria, high trans-sabinyl acetate; BeR/loTS, eastern Rhodopes, Bulgaria, low trans-sabinyl acetate; BeR/hiTS, eastern Rhodopes, Bulgaria, high trans-sabinyl acetate; BkG/loME, Greece, low methyl eugenol; BkG/hiME, Greece, high methyl eugenol; *J. sabina* population codes: SN, Sierra Nevada, Spain; PYR Pyrenees Mtns.; SWZ, Switzerland; KAZ, Kazakhstan; Components that tend to separate the species are highlighted in boldface.

RI	Compound	Bk Turk	BkG hiME	BkG IoME	BeR hiTS	BeR loTS	BkR hiTS	BkR loTS	SWZ	PYR	KAZ
931	α -thujene	1.2	1.2	1.7	0.5	1.3	0.3	1.0	0.9	1.0	0.6
939	α-pinene	2.4	3.7	2.6	1.3	3.0	2.2	1.3	2.0	1.8	15.8
953	camphene	t	-	t	t	t	t	t	t	t	0.3
976	sabinene	56.1	42.5	59.7	7.5	41.2	3.7	41.3	34.8	54.9	42.6
980	β-pinene	t	0.3	0.2	0.3	0.4	0.6	t	t	t	0.7
991	myrcene	3.4	2.8	3.9	2.0	2.9	1.6	2.7	4.2	3.1	3.8
1005	α-phellandrene	t	t	t	t	0.1	t	0.1	t	0.1	t
1011	δ-3-carene	0.1	1.5	t	1.0	0.1	0.1	0.1	-	0.1	0.2
1018	α-terpinene	1.2	1.4	1.6	0.4	1.6	0.3	1.6	1.0	1.5	0.7
1026	p-cymene	0.3	0.4	0.5	0.4	0.6	0.6	0.5	0.2	0.4	0.1
1031	limonene	1.5	0.8	1.3	0.6	0.6	0.8	1.0	3.0	2.4	2.1
1031	β-phellandrene	1.6	0.8	1.3	0.9	0.9	0.8	1.0	t	t	1.4
1032	1,8-cineole	t	0.1	0.1	0.2	0.4	t	t	-	t	t
1050	(E)-β-ocimene	0.9	0.3	0.2	0.1	0.5	0.1	t	1.1	0.7	0.1
1062	γ-terpinene	2.2	2.3	2.7	0.8	2.9	0.6	3.2	1.1	2.5	0.1
1068	cis-sabinene hydrate	2.3	1.6	2.1	1.1	3.2	0.5	2.3	0.7	1.4	0.5
1067	cis-linalool oxide(fura)	0.1	t	t	t	0.3	t	t	t	t	-
1088	terpinolene	1.1	1.2	1.3	0.6	1.2	0.3	1.2	0.8	1.0	0.7
1097	trans-sabinene hydrate	0.9	2.1	1.9	1.0	3.1	t	1.0	0.3	1.1	0.4
1098	linalool	1.0	t	t	1.0	3.1	0.4	1.0	1.5	0.3	0.2
1102	nonanal	-	-	t	-	-	-	-	t	-	t
1102	cis-thujone(α-thujone)	t	t	-	1.7	t	12.4	t	0.1	-	1.0
1114	trans-thujone(β- thujone)	0.4	t	t	12.7	0.2	6.7	0.1	0.7	0.1	-
1121	cis-p-menth-2-en-1-ol	0.5	0.4	0.4	0.2	0.5	0.2	0.5	0.2	0.6	0.2
1134	iso-3-thujanol	0.2	-	-	0.6	-	0.2	t	-	-	-
1140	trans-sabinol	-	-	-	5.0	0.5	4.3	0.3	0.7	0.3	-
1140	trans-p-menth-2-en- 1-ol	-	0.2	0.4	-	-	-	-		-	0.2
1153	citronellal	0.1	t	t	-	-	-	-	0.2	0.4	
1156	sabina ketone	0.1	t	t	-	0.2	-	0.1		-	-
1166	coahuilensol	0.3	-	t	-	1.1	-	-	0.4	-	-
1177	terpinen-4-ol	5.0	3.9	4.4	2.1	5.9	1.6	4.8	1.4	7.2	2.9
1183	p-cymen-8-ol	t	-	t	t	0.1	t	t	-	-	-
1189	$\alpha$ -terpineol	0.2	0.2	0.2	0.1	0.5	t	0.2	0.1	0.3	0.2
1193	cis-piperitol	0.1	t	0.2	0.1	0.4	0.2	0.3	t	0.2	0.1
1205	trans-piperitol	0.2	0.2	0.2	0.2	0.3	t	0.3	-	0.2	0.1
1219	methyl coahuilensol	0.2	-	t	-	0.6	-	-	0.4	-	-
1228	citronellol	0.8	0.3	0.4	0.3	2.9	t	0.2	0.6	4.1	0.4
1257	linalyl acetate	t	0.1	0.1	0.6	0.8	t	0.8	0.2	-	0.3
1261	methyl citronellate	0.3	1.0	1.6	0.6	3.3	1.2	3.9	0.7	0.8	0.1
1285	bornyl acetate	t	t	t	-	t	t	t	t	-	0.4
1285	safrole	-	-	-	-	-	-	-	-	1.8	-
1287	trans-linalyl acetate	0.2	0.6	t	0.1	0.2	t	0.1		-	-

RI	Compound	Bk Turk	BkG hiME	BkG IoME	BeR hiTS	BeR loTS	BkR hiTS	BkR loTS	SWZ	PYR	KAZ
1290	trans-sabinyl acetate	1.7	0.5	0.3	39.6	0.4	39.8	0.4	35.0	t	-
1319	(E,E)-2,4-decadienal	t	t	t	t	0.3	0.2	t	-	-	-
1323	methyl geranate	0.4	t	0.3	t	0.4	0.9	0.4	0.3	0.1	0.1
1350	α-terpinyl acetate	0.1	t	t	0.1	0.2	t	0.2	0.1	-	0.2
1376	α-copaene	t	t	t	0.1	0.1	t	t	-	-	-
1389	2E,4E-me-decanoate	-	0.4	0.5	-	-	0.3	0.8	-	-	-
1401	methyl eugenol	-	13.2	t	0.4	0.3	4.1	5.8	-	1.1	-
1409	α-cedrene	-	-	-	-	-	-	-		-	0.2
1418	(E)-caryophyllene(β- caryophyllene)	t	t	0.1	0.1	t	t	0.2	-	-	-
1468	pinchotene acetate	t	-	-		0.5	-	-	0.1	-	t
1477	γ-muurolene	0.1	t	t	0.3	0.2	t	0.1	0.1	t	0.1
1480	germacrene D	0.1	t	t	0.3	t	t	t	-	0.1	-
1491	trans-murrola-4(14),5- diene	-	-	-	-	-	-	-	-	0.6	t
1493	epi-cubebol	0.1	t	t	0.3	0.2	t	0.2	0.1	0.3	-
1495	γ-amorphene	-	-	-	-	-	-	-	-	-	0.1
1499	α-muurolene	0.2	0.1	0.1	0.5	0.3	0.2	0.3	0.1	0.2	0.2
1513	γ-cadinene	0.4	0.5	0.4	1.7	0.8	0.4	0.6	0.3	0.8	0.3
1524	δ-cadinene	0.8	0.8	0.6	1.9	1.1	0.5	0.9	0.5	1.0	0.8
1538	α-cadinene	0.2		t	0.2	0.1	t	0.3	0.1	0.1	0.1
1549	elemol	0.6	t	t	0.1	t	0.5	1.9	0.8	2.1	0.1
1554	elemicin	0.1	6.0	t	0.2	t	6.4	2.3	-	0.4	-
1574	germacrene D-4-ol	2.6	1.4	1.8	2.5	2.2	0.5	2.4	1.4	0.7	1.1
1587	allo-cedrol	-	- 1	-	-	-	-	-	-	-	1.1
1596	cedrol	-	-	-	-	-	-	-	-		15.9
1606	β-oplopenone	0.7	0.7	0.3	0.4	0.6	0.5	1.1	0.1	0.3	-
1611	epi-cedrol	-	-	-	-	-	-	-	-	-	0.1
1627	1-epi-cubenol	0.1	t	t	0.2	t	0.1	0.2	t	0.4	-
1632	α-acorenol	-	-	-	-	-	-	-	-	-	0.2
1640	epi-α-cadinol	0.4	0.3	0.3	0.8	0.5	0.3	0.5	0.2	0.3	0.3
1640	epi-α-muurolol	0.4	0.4	0.3	0.8	0.5	0.3	0.6	0.2	0.2	0.3
1645	α-muurolol	0.1	0.1	t	0.3	0.2	0.1	0.2	0.1	0.1	0.1
1649	β-eudesmol	t	-	-	-	-	0.2	0.6	-	0.2	-
1652	α-eudesmol	t	-	-	-	-	t	0.6	-	0.3	-
1653	α-cadinol	1.0	0.8	0.7	1.8	1.3	0.9	1.2	0.6	0.5	0.9
1689	shyobunol	0.4	t	0.3	0.2	t	0.1	0.5	0.1	t	0.1
2054	abietatriene	t	t	t	t	t	t	t	t	t	t
2080	abietadiene	0.2	0.2	0.4	0.2	0.3	t	t	t	0.1	t
2288	4-epi-abietal	0.4	0.3	0.5	0.5	0.7	0.3	0.7	t	0.1	t
2302	abieta-7,13-dien-3- one	1.0	0.7	1.3	0.7	1.9	0.5	2.0	0.1	0.1	0.2
2325	trans-ferruginol	t	t	t	t	t	t	0.2	t	t	t
2343	4-epi-abietol	t	t	t	t	0.3	t	0.1	t	t	t

RI = Kovat's Retention Index on DB-5(=SE54) column using alkanes. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.



Adams, Robert P., Mataraci, T, and Martínez, Luis A. 2018. "The composition of the leaf essential oils of J. sabina var. balkanensis: chemotypes high in trans-sabinyl acetate and methyl eugenol discovered in three natural populations." *Phytologia* 100(1), 45–50.

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