

**GEOGRAPHIC VARIATION IN LEAF OILS OF  
*JUNIPERUS DELTOIDES* FROM BULGARIA, GREECE,  
ITALY AND TURKEY**

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**ABSTRACT**

The volatile leaf oil of *J. deltoides*, Bulgaria, was compared to *J. deltoides* oils from Greece, Italy and Turkey as well as the oil of *J. oxycedrus*, France. Leaf terpenoids showed a similar pattern somewhat intermediate between Greece and Turkey *J. deltoides*. The oil was unusual in having less  $\alpha$ -pinene (12.6%) and more manoyl oxide (16%) than in any other *J. deltoides* sources examined. The plants had protuberances on the seed cones and leaves with deltate bases, typical of *J. deltoides*. *Phytologia* 94(3): 310 - 318 (December 1, 2012).

**KEY WORDS:** *Juniperus deltoides*, Bulgaria, Greece, Italy, Turkey, *J. oxycedrus*, terpenes, taxonomy.

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Recent studies (Adams, 2004; Adams, et al., 2005) utilizing nrDNA sequencing, RAPDs, leaf terpenoids and morphology, clearly demonstrate that *J. oxycedrus* (*sensu stricto*) is restricted to the western Mediterranean; whereas, another, morphologically similar species, *J. deltoides* R. P. Adams occupies the eastern Mediterranean region. Adams (2011) recognized both *J. deltoides* and *J. oxycedrus* in his monograph of *Juniperus*. However, examination of junipers on the margins of the range of *J. deltoides* on the boundary between the taxa is useful to understand the distribution (Fig. 1).

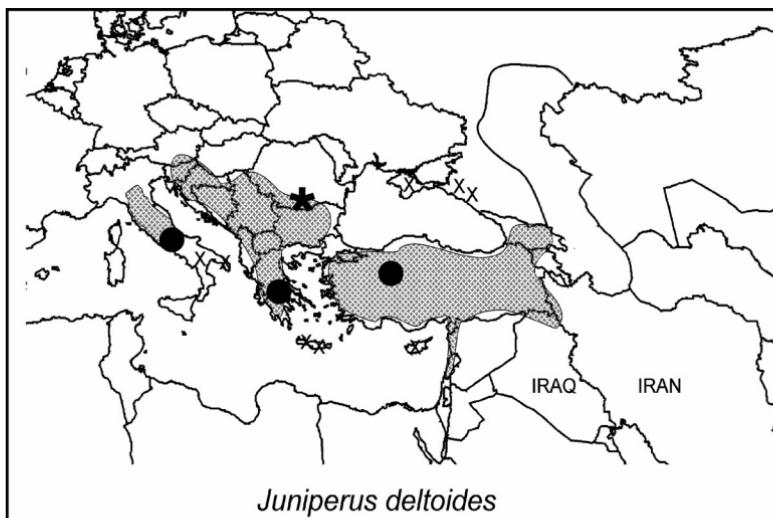


Figure 1. Distribution of *J. deltoides* with the Bulgaria population indicated by a star; Italy, Greece and Turkey populations are indicated by closed circles.

Recent collections of *J. deltoides* from Bulgaria (star, Fig. 1) afforded the opportunity to compare the leaf essential oils with other populations previously sampled (Adams et al. 2005; Adams, R. P. and T. Matraci, 2011; Adams, R. P., S. Terzioğlu and T. Mataraci, 2010; closed circles, Fig. 1). The purposes of this paper are to report on the leaf oil of *J. deltoides* from Bulgaria and compare it with other populations of *J. deltoides*.

## MATERIALS AND METHODS

Plant material: *J. deltoides*: Adams 9445-47, 2 km w Raiano, 42° 05.768' N, 13° 47.757' E, 525 m, Italy, Adams 9436-38, 14 km e Arachova, 38° 26.720' N, 22° 41.678' E, 420 m, Greece, Adams 9430-9432, 30 km n Eskisehir, 39° 57.300' N, 30° 41.027' E, 1064 m, Turkey, Adams and Tashev 13126-13130, Devin region, 47° 44' 54" N, 24° 23' 02" E, 857 m, Bulgaria; *J. oxycedrus*, Adams 9039-9041, 4 km e

Forcalquier, 44° 04.06' N, 5° 59.19' E, 490 m, France. Voucher specimens are deposited at Baylor University (BAYLU).

*Isolation of Oils* - Air dried leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus with an ether trap (Adams, 1991). The oil samples were concentrated (ether reduced to 90% conc.) 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.

*Chemical Analyses* - Oils from each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 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 AND DISCUSSION

Specimens of *J. deltoides* from Bulgaria had the typical protuberances on the seed cones (Fig. 2, left) compared to smooth seed cones of *J. oxycedrus* (Fig. 3). In addition, their leaves were scarcely tapered to deltate at the point of attachment (Fig. 2, right) compared to the leaves of *J. oxycedrus*, that has leaves noticeably constricted (tapered) at the point of attachment (Fig. 3).

The leaf oil from Bulgaria contained all the characteristic components of *J. deltoides* (cis-p-mentha-2,8-dien-1-ol, carvone, (2E)-decenal,  $\alpha$ -copaene,  $\alpha$ -muurolene,  $\alpha$ -copaen-11-ol,  $\alpha$ - and  $\beta$ -calacorene, cis-calamenen-10-ol and cadalene, Table 1) and lacked components typical of *J. oxycedrus* (1-octen-3-ol,  $\alpha$ -terpinyl acetate, 2-tridecanone, germacrene B, salvial-4(14)-en-1-one, hexadecane, sesquiterpene

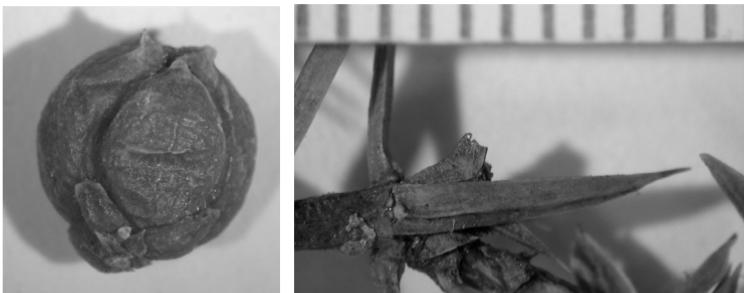


Figure 2. *J. deltoides*, Bulgaria. Left: seed cone showing protuberances. Right: leaf base showing the delta shape of the leaf at the point of attachment.

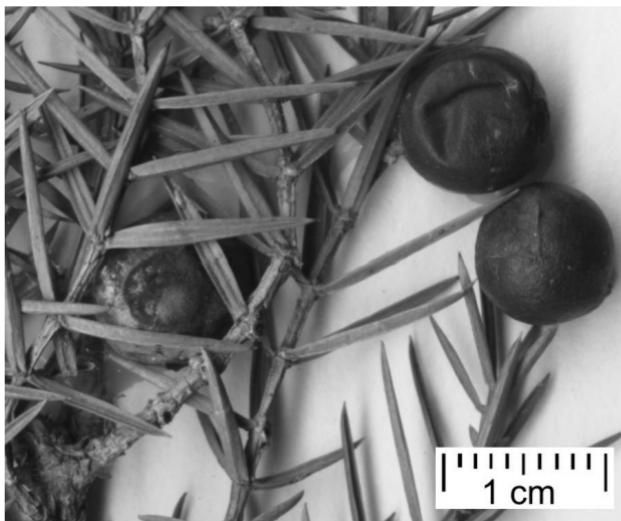


Figure 3. *J. oxycedrus*. Note the lack of protuberances on the seed cones and the leaves that are constricted (tapered) at the point of attachment (from Adams 2011).

alcohol 1619, C15-dienol acetate 1674, nootkatone, nonadecane, epi-13-manoxy oxide, sandaracopimarinal, 1-docosene, docosane, phytol acetate and tricosane, Table 1). However, the Bulgarian oil was unusual in having less  $\alpha$ -pinene (12.6%) and more manoyl oxide (16%) than found in the other populations of *J. deltoides* examined (Table 1).

### ACKNOWLEDGEMENTS

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Table 1. Comparisons of the per cent total oil for leaf oils components of *J. deltoides*, Bulgaria, Turkey, Greece, and Italy compared to *J. oxycedrus*, France. Components that tend to separate the taxa and populations are highlighted in boldface. KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

KI	Compound	delt Turk	delt Bulg	delt Grec	delt Italy	oxy Fra
927	tricyclene	0.1	0.1	t	t	0.1
930	$\alpha$ -thujene	t	-	t	t	t
<b>939</b>	<b><math>\alpha</math>-pinene</b>	<b>32.7</b>	<b>12.6</b>	<b>22.7</b>	<b>15.3</b>	<b>53.2</b>
953	$\alpha$ -fenchene	0.3	-	0.1	0.5	0.1
954	camphene	0.6	0.3	0.2	0.2	0.6
960	thuja-2,4(10)-diene	0.4	-	-	-	t
975	sabinene	0.2	0.1	0.1	0.2	0.5
<b>979</b>	<b>1-octen-3-ol</b>	-	-	-	-	<b>0.1</b>
979	$\beta$ -pinene	3.0	0.3	2.4	1.8	2.1
991	myrcene	3.8	0.3	2.3	1.7	2.8
1002	$\delta$ -2-carene	0.9	-	t	-	t
1003	$\alpha$ -phellandrene	1.8	0.2	0.3	0.5	t
<b>1011</b>	<b><math>\delta</math>-3-carene</b>	<b>3.7</b>	<b>t</b>	<b>3.3</b>	<b>10.0</b>	<b>5.1</b>
1017	$\alpha$ -terpinene	0.1	-	-	-	t
1025	p-cymene	2.3	0.5	0.3	0.6	0.3
<b>1029</b>	<b>limonene</b>	<b>6.0</b>	<b>6.0</b>	<b>23.7</b>	<b>22.8</b>	<b>3.5</b>
1030	$\beta$ -phellandrene	11.5	5.5	4.8	2.5	0.8
1050	(E)- $\beta$ -ocimene	-	-	0.2	0.2	t
1060	$\gamma$ -terpinene	0.2	-	0.1	0.1	0.1
1089	terpinolene	2.0	0.5	1.1	1.6	0.7
1099	linalool	0.7	-	0.3	t	t
1101	n-nonanal	0.5	0.2	0.2	0.2	t
1122	cis-p-menth-2-en-1-ol	0.3	-	-	t	-
1123	trans-p-mentha-2,8-dien-1-ol	t	0.3	0.2	-	-
1126	$\alpha$ -campholenal	1.3	1.3	0.3	0.2	0.8
1137	trans-pinocarveol	1.3	1.3	0.3	-	0.4
<b>1138</b>	<b>cis-p-mentha-2,8-dien-1-ol</b>	<b>0.1</b>	<b>0.1</b>	<b>0.3</b>	<b>0.2</b>	-
1141	cis-verbenol	0.4	0.4	0.1	-	t
1145	trans-verbenol	1.8	3.2	0.5	0.2	0.6
1163	trans-pinocamphone	0.1	-	-	0.2	-
1165	pinocarvone	0.6	0.5	-	-	t

KI	Compound	delt Turk	delt Bulg	delt Grec	delt Italy	oxy Fra
1170	p-mentha-1,5-dien-8-ol	1.1	0.4	0.2	0.3	0.5
1175	cis-pinocamphone	0.1	0.1	-	-	-
1177	terpinen-4-ol	0.6	0.1	0.4	0.3	0.3
1181	naphthalene	0.3	-	0.2	-	0.1
1183	p-cymen-8-ol	1.0	0.3	0.2	0.2	t
1187	trans-p-mentha-1(7),8-dien-2-ol	-	-	0.2	-	-
1189	$\alpha$ -terpineol	1.2	0.4	0.3	0.1	0.6
<b>1196</b>	<b>myrtenal</b>	<b>0.6</b>	<b>0.4</b>	<b>0.3</b>	-	<b>t</b>
1205	verbenone	0.7	0.7	0.3	0.2	0.3
1217	trans-carveol	0.5	1.1	1.1	0.7	0.1
1229	cis-carveol	t	0.5	0.4	0.3	-
1242	cumin aldehyde	0.1	-	-	-	-
<b>1243</b>	<b>carvone</b>	<b>0.3</b>	<b>0.8</b>	<b>0.8</b>	<b>0.7</b>	-
1253	piperitone	t	-	-	-	-
1257	linalyl acetate	t	-	-	-	0.3
<b>1264</b>	<b>(2E)-decenal</b>	<b>0.2</b>	<b>0.1</b>	<b>0.2</b>	<b>0.2</b>	-
1289	bornyl acetate	0.9	0.6	0.4	0.3	0.7
1298	trans-pinocarvyl acetate	0.1	0.1	-	-	-
1298	carvacrol	t	0.1	-	-	-
1299	(2E,4Z)-decadienal	0.4	-	t	t	-
1317	(2E,4E)-decadienal	0.8	-	t	t	0.1
1342	trans-carvyl acetate	t	t	-	t	-
<b>1349</b>	<b><math>\alpha</math>-terpinyl acetate</b>	-	-	-	-	<b>0.2</b>
<b>1377</b>	<b><math>\alpha</math>-copaene</b>	<b>0.2</b>	<b>0.3</b>	<b>0.3</b>	<b>0.3</b>	-
1381	geranyl acetate	t	-	t	-	-
1388	$\beta$ -bourbenene	0.2	0.1	0.2	0.1	0.3
1408	longifolene	0.6	-	-	-	-
1419	(E)-caryophyllene	1.2	0.8	0.2	1.1	0.4
1431	cis-thujopsene	0.1	-	t	-	-
1455	$\alpha$ -humulene	0.8	0.5	0.2	0.8	0.3
1480	$\gamma$ -muurolene	t	-	-	t	0.1
1485	germacrene D	0.5	0.2	0.7	1.4	2.3
1486	ar-curcumene	-	1.0	0.7	0.3	-
<b>1496</b>	<b>2-tridecanone</b>	-	-	-	-	<b>0.3</b>
<b>1500</b>	<b><math>\alpha</math>-muurolene</b>	<b>0.4</b>	<b>1.0</b>	<b>02</b>	t	-
1514	$\gamma$ -cadinene	0.4	1.2	0.9	0.8	0.7
1523	$\delta$ -cadinene	0.4	0.7	1.1	1.1	0.4
<b>1541</b>	<b><math>\alpha</math>-copaen-11-ol</b>	<b>0.1</b>	<b>0.5</b>	<b>1.1</b>	<b>0.6</b>	-

KI	Compound	delt Turk	delt Bulg	delt Grec	delt Italy	oxy Fra
<b>1546</b>	$\alpha$ -calacorene	<b>0.5</b>	<b>0.6</b>	<b>0.7</b>	<b>0.6</b>	-
<b>1561</b>	germacrene B	-	-	-	-	<b>0.1</b>
<b>1566</b>	$\beta$ -calacorene	<b>0.3</b>	<b>0.5</b>	t	<b>0.4</b>	-
1563	(E)-nerolidol	-	-	1.5	-	-
1567	dodecanoic acid	-	0.8	1.4	-	0.4
1583	caryophyllene oxide	3.2	6.0	0.6	5.9	0.4
<b>1595</b>	salvial-4(14)-en-1-one	-	-	-	-	<b>0.4</b>
<b>1600</b>	hexadecane	-	-	-	-	<b>0.3</b>
1601	cedrol	0.1	t	0.3	t	t
1608	humulene epoxide II	1.1	2.8	0.3	2.8	0.3
<b>1619</b>	sesquiterpene alcohol, M226	-	-	-	-	<b>0.3</b>
1627	1-epi-cubenol	0.1	-	-	-	-
1630	muurola-4,10(14)-dien-1- $\beta$ -ol	-	-	0.3	-	-
1640	epi- $\alpha$ -cadinol	t	0.2	0.3	0.3	0.3
1641	epi- $\alpha$ -muurolol	t	0.2	0.3	0.3	0.3
1651	$\beta$ -eudesmol	-	-	-	-	t
1654	$\alpha$ -cadinol	-	-	0.3	0.4	1.6
<b>1661</b>	cis-calamenen-10-ol	t	<b>0.4</b>	<b>0.2</b>	<b>0.5</b>	-
1670	caryophyllene<14-OH-(Z)->	-	0.6	-	-	-
<b>1674</b>	C15-dienol acetate, M+224	-	-	-	-	<b>1.6</b>
<b>1677</b>	cadalene	<b>0.1</b>	<b>0.5</b>	<b>0.5</b>	<b>0.4</b>	-
1686	germacra-4(15),5,10(14)-trien-1-al	-	-	0.3	-	1.6
1700	heptadecane	-	-	t	-	0.3
1702	10-nor-calamenen-10-ene	-	0.1	0.1	t	-
1717	(2E, 6E)-farnesol	-	1.0	t	-	0.3
1722	(2Z, 6E)-farnesol	-	-	1.0	-	0.4
1740	(2E,6Z)-farnesal	-	-	0.3	-	-
1758	tetradecanoic acid	-	-	0.5	0.3	-
1800	octadecane	-	t	0.1	-	t
<b>1807</b>	nootkatone	-	-	-	-	<b>0.1</b>
<b>1900</b>	nonadecane	-	-	-	-	<b>0.1</b>
1959	hexadecanoic acid	-	1.1	-	-	-
1966	sandaracopimara-8(14),15-diene	-	-	0.7	-	0.1
<b>1998</b>	manoyl oxide	<b>1.3</b>	<b>16.0</b>	<b>6.8</b>	<b>5.8</b>	<b>6.2</b>
2014	palustradiene (=abieta-8,13-diene)	-	1.1	-	-	-
<b>2017</b>	epi-13-manoyl oxide	-	-	-	-	<b>0.1</b>

KI	Compound	delt Turk	delt Bulg	delt Grec	delt Italy	oxy Fra
2022	cis-abiet-8,12-diene	-	0.3	-	-	0.1
2026	geranyl linalool	-	-	-	-	-
2057	abietatriene	0.1	5.5	2.0	2.3	1.2
2088	abietadiene	-	7.6	1.4	1.9	1.3
2154	abiet-8(14),13(15)-diene	-	-	0.7	-	0.2
<b>2185</b>	<b>sandaracopimarinal</b>	-	-	-	-	<b>0.2</b>
<b>2190</b>	<b>1-docosene</b>	-	-	-	-	<b>0.1</b>
<b>2200</b>	<b>docosane</b>	-	-	-	-	<b>0.1</b>
<b>2218</b>	<b>phytol acetate</b>	-	-	-	-	<b>0.1</b>
2220	dehydro-totarol*	-	2.3	-	-	-
<b>2300</b>	<b>tricosane</b>	-	-	-	-	<b>0.2</b>
2313	trans-totarol	-	0.6	-	-	-
2313	abietal	-	0.5	0.5	0.4	-
2331	trans-ferruginol	-	0.4	-	-	-