

**GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS
OF *JUNIPERUS OSTEOSPERMA* (CUPRESSACEAE) II.****Robert P. Adams**Biology Department, Baylor University, Box 97388, Waco, TX
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ABSTRACT

The volatile leaf oils of *J. osteosperma* were analyzed from its western range. Four major geographical groups were found: Nevada, San Bernardino Mtns.- Mountain Pass, CA, Thistle, UT and Oak Creek Canyon, AZ. The AZ population is likely a Pleistocene relict that may account for its unusual oil. The terpene data did not indicate hybridization of *J. osteosperma* with *J. grandis* or *J. californica* in the San Bernardino Mtns. Populations from NW Nevada, reported to hybridize with *J. grandis* and *J. occidentalis*, were not included in the study but will be analyzed in a future report. *Phytologia* 94(1): 118-132 (April 2, 2012).

KEY WORDS: *J. osteosperma*, *J. grandis*, *J. occidentalis*, *J. californica*, Cupressaceae, terpenes, geographic variation.

Previously, Adams (1994) analyzed geographic variation in the leaf essential oils of *J. osteosperma* (Torr.) Little and reported differences among the five populations analyzed. More recently, Adams and Kauffmann (2010) analyzed 9 Nevada and California populations of *J. osteosperma* as part of a study on *J. grandis*. They reported some variation in the leaf oils of *J. osteosperma*, but did not delve deeply into geographic variation, as their focus was on *J. grandis* oils.

Terry et al. (2000) found cpDNA (trnL-trnF, trnS-trnG) haplotypes of *J. occidentalis* in Nevada populations of *J. osteosperma*, with lower frequencies occurring in Utah, Colorado, and Wyoming. Subsequently, Terry (2010) analyzed trnL-trnF and trnS-trnG (cpDNA) haplotypes and reported similar results (Fig. 1). Notice, all 15 trees of

J. occidentalis in Oregon have the same haplotype and that this haplotype is also present in northwest Nevada. Hybridization in this area was first reported by Vasek (1966) and confirmed by Terry et al. (2000) and Terry (2010). Subsequently, Terry (2010) also concluded that there was introgression from *J. occidentalis* into *J. osteosperma*.

The present study examines geographic variation in the leaf volatile oil components of *J. osteosperma*. Because terpenes are products of gene expression and interact directly with herbivores, insects and diseases in the environment, they have proved useful in the study of evolution. Our present understanding of nucleotide substitutions and indels in introns and inter-genic regions makes it difficult to discern their actual role, if any, in speciation. The area of

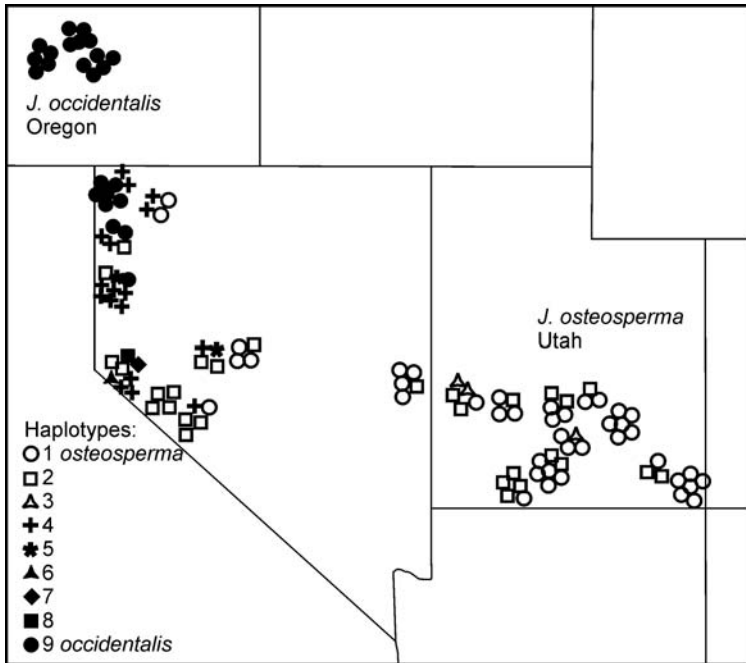


Figure 1. Distribution of haplotypes (trnL-trnF and trnS-trnG) in *J. occidentalis* and *J. osteosperma* (information from Terry, 2010).

putative hybridization in northwest Nevada is excluded from the present study and will be published in subsequent papers.

MATERIALS AND METHODS

Plant material (Fig 2): *J. osteosperma*, Adams 1689-1699, 1701-1705, on US 6, Thistle, 40° 00' 6.9" N, 111° 29' 4.6" W, 1650 m, Utah Co., UT, Adams 12067-12071, 4 km n of Sedona, AZ, at Grasshopper Point,

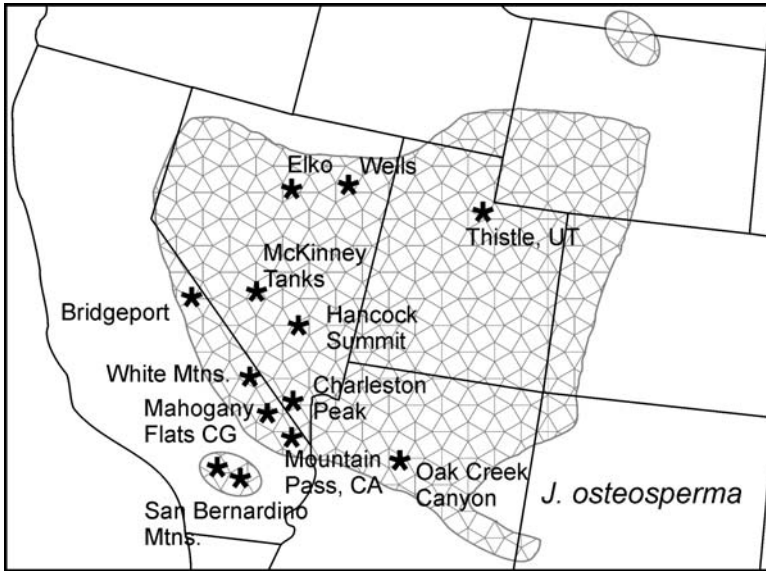


Figure 2. Distribution of *J. osteosperma* with populations sampled in this study.

on Alt US 89, 34.888° N, 111.733° W, 1380m, Coconino Co., AZ, Adams 10272-10276, on NV157, Charleston Mtns., 36° 16.246' N, 115° 32.604' W, 1795 m, Clark Co., NV; Adams 11122-11124, Hancock Summit, mile 38 on US 375, 37° 26.404' N, 115° 22.703' W, 1675 m, Lincoln Co. NV; Adams 11125-11127, McKinney Tanks Summit on US 6, 38° 07.005' N, 116° 54.103' W, 1933 m, Nye Co., NV; Adams 11134-36, 8 km s of Bridgeport, on US395, 38° 12.639' N, 119° 13.846' W, 2004 m, Mono Co., CA; Adams 11141-11143, 13 km w of Elko, on

I 80, 40° 45.598' N, 115° 55.942' W, 1535 m, Elko Co., NV; *Adams 11144-11146*, 8 km e of Wells, on I 80, 41° 06.533' N, 114° 51.441' W, 1876 m, Elko Co., NV; *Adams 11960-11962*, 56 km n of Reno, NV; on US 395, 39° 54.458' N, 120° 00.322' W, 1383 m, Lassen Co., CA; *Adams 11973-11977*, 10 km n of CA 168 on White Mtn. Rd., 37° 20.143' N, 118° 11.346' W, 2607 m, Inyo Co., CA; *Adams 11978-11982*, Mahogany Flats Campground, Panamint Mtns., 36° 13.783' N, 117° 04.102' W, 2477 m, Inyo Co., CA, *Adams 12323-12327*, Basin, San Bernardino Mtns., 34° 16.910' N, 116° 45.306' W, 1820 m, San Bernardino Co., CA, *Adams 12210-12214*, ca. 1 km e of CA 18, ca. 16 km s of jct CA 18 & CA 247, n slope San Bernardino Mtns., 34° 21.213' N, 116° 50.607' W, 1393 m, San Bernardino Co., CA, *Adams 12215-12219*, on I15, at Bailey Rd., 35° 27.938' N, 115° 31.709' W, 1431 m, San Bernardino Co., CA.

J. grandis, *Adams 11963-11967*, Jct. US 50 & CA 89, 38° 51.086' N, 120° 01.244' W, 1937 m, Meyers, El Dorado Co.; CA; *Adams 11968-11972*, 16 km w of Sonora Jct., on CA. 108, 38° 18.289' N, 111° 35.598' W, 2585 m, Tuolumne Co.; CA, *Adams 11984-11988*, Nine Mile Canyon Rd., 20 km w of Jct. with US 395, 35° 54.003' N, 118° 02.078' W, 2059 m, Tulare Co., CA; *Adams 11989-11993*, 5km n Big Bear City on CA 18, 34° 17.533' N, 116° 49.153' W, 2053 m, San Bernardino Co., CA; *Adams 11963-11967*, Jct. US 50 & CA 89, 38° 51.086' N, 120° 01.244' W, 1937 m, Meyers, El Dorado Co.; CA; *Adams 11968-11972*, 16 km w of Sonora Jct., on CA Hwy. 108, 38° 18.289' N, 111° 35.598' W, 2585 m, Tuolumne Co.; CA, *Adams 11984-11988*, Nine Mile Canyon Rd., 20 km w of Jct. with US 395, 35° 54.003' N, 118° 02.078' W, 2059 m, Tulare Co., CA; *Adams 11989-11993*, 5km n Big Bear City on CA 18, 34° 17.533' N, 116° 49.153' W, 2053 m, San Bernardino Co., CA; *Adams 12319-12322*, Onyx Summit on CA 38, 34° 11.524' N; 116° 43.227' W. 2600 m, San Bernardino Co., CA; *Adams 12328-12331*, 12367, Donner Pass Summit on old US50, 39° 18.999' N; 120° 19.581' W. 2180 m, Placer Co., CA; *Adams 12332-12336*, on Stampede Meadows Rd. (Co. rd 894A alt), 5 mi. n of I80. 39° 24.966' N, 120° 05.249' W, 1660 m, Nevada Co., CA; *Adams 12337-12341*, 4.7 mi. n of Beckwourth on Beckwourth-Genesee Rd., 39° 52.433' N, 120° 24.345' W, 1770 m, Plumas Co., CA.

J. occidentalis, *Adams 11940-11942*, 12 km e of Jct. WA 14 & US 97 on WA 14, 45° 44.392' N, 120° 41.207' W, 170 m, Klickitat Co.; WA, *Adams 11943-11945*, 2 km s of jct. US 97 & US 197 on US 97, 38 km

ne of Madras, OR; 44° 53.676' N, 120° 56.131' W, 951 m, Wasco Co., OR; *Adams 11946-11948*, 3 km sw of Bend, OR; on OR 372, 44° 02.390' N, 121° 20.054' W, 1132 m, Deschutes Co., OR; *Adams 11949-11951*, 32 km e of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43° 53.922' N, 120° 59.187' W, 1274 m, Deschutes Co., OR; *Adams 11952-11954*, 14 km e of Jct. OR66 & I 5, on OR66, 42° 08.044' N, 122° 34.130' W, 701 m, Jackson Co., OR; *Adams 11957-11959*, on CA 299, 10 km e of McArthur, CA, 41° 05.313' N, 121° 18.921' W, 1091 m, Lassen Co., CA; *Adams 11995-11998* (*Kauffmann A1-A3, B1*), Yolla Bolly-Middle Eel Wilderness, 40° 06' 34" N, 122° 57' 59" W, 1815-2000 m, Trinity Co., CA, *Adams 12342-12346*, 19 km WSE of Susanville, CA, on CA 36, 40° 22.178' N, 120° 50.211' W, 1570 m, Lassen Co., CA, *Adams 12347-12351*, on US 395, 5 km n of Madeline, 41° 05.867' N, 120° 28.456' W, 1695 m, Lassen Co., CA. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Isolation of Oils - Fresh leaves (200 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.

Chemical Analyses - Oils from 10-15 trees of 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. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The oils of *J. osteosperma* are dominated by camphor (19.7 - 60.2%) and bornyl acetate (4.4 - 19.7%, Table 1), with moderate amounts of sabinene, α -pinene, borneol and terpinen-4-ol. For comparison, typical oils of *J. grandis* and *J. occidentalis* (Table 1) have little camphor (0, 2.5%) or borneol (0, 2.2%). The oil of *J. occidentalis* has large amounts of sabinene, p-cymene, citronellol and bornyl acetate (Table 1), whereas *J. grandis* oil is dominated by δ -3-carene, α -pinene and β -phellandrene (Table 1).

To examine geographic trends in the leaf essential oils, contours of the cluster levels were plotted (Figure 3). The overall trend is that *J. osteosperma* oils in the central portion of Nevada are very uniform (notice contour similarity levels of 0.84 - 88, Fig. 3). The major divergences are the Thistle, Utah population, the San Bernardino

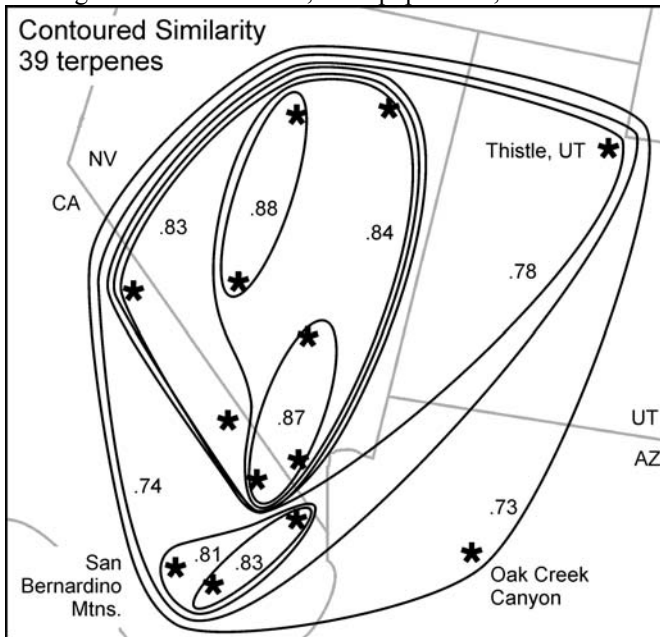


Figure 3. Contoured similarities of populations (see Fig. 2) of *J. osteosperma* based on 41 terpenes.

Mtns. - Mountain Pass, CA populations with the Oak Creek Canyon, AZ population being the most differentiated (Fig. 3). Comparison of the McKinney Tanks, Utah, San Bernardino Mtns., and Oak Creek Canyon AZ oils (Table 1) shows differences in sabinene, myrcene, camphor, terpinen-4-ol and bornyl acetate, but overall, these oils are very similar.

Principal Coordinate analysis of the terpene similarities matrix resulted in eigenroots that accounted for 24, 17, 10 and 9% of the variation among populations of *J. osteosperma*. Ordination reveals four groups: Nevada, San Bernardino Mtns. - Mountain Pass, CA, Utah and, the most differentiated population, Oak Creek Canyon, AZ. The Bridgeport population was not very different in the contoured similarities (Fig. 3), so this may be a feature of the ordination of 4 dimensions into 3 dimensions.

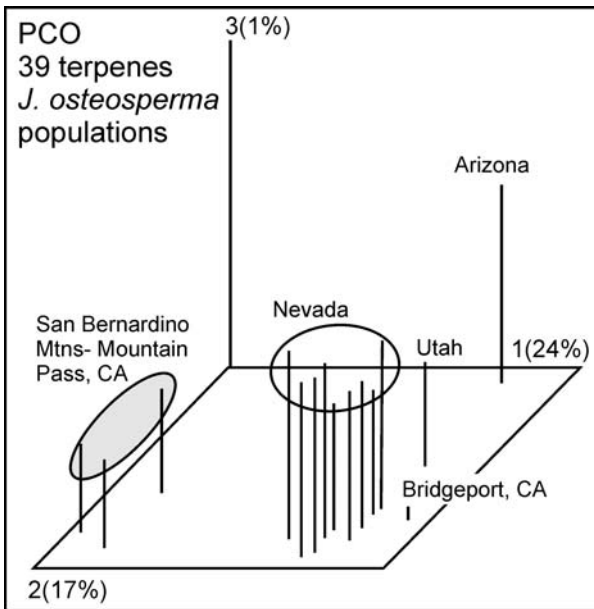


Figure 4. PCO of 13 *J. osteosperma* populations based on 39 terpenes.

Of immediate interest is whether the divergence of *J. osteosperma* in the San Bernardino Mtns.-Mountain Pass is due to introgression from *J. grandis* in the San Bernardino Mtns., where the two species are essentially sympatric in the Basin. Terpenoids have been useful for the detection of hybridization due to their complementary inheritance (Adams 1983, Irving and Adams, 1973). PCO was performed using *J. grandis* from the San Bernardino Mtns. The resulting ordination is shown in Fig. 5. There is no evidence that the San Bernardino Mtns. *J. osteosperma* populations are any more similar to *J. grandis* than the other *J. osteosperma* populations, far removed from the San Bernardino Mtns. (Fig. 5). Thus, the divergence of the San Bernardino Mtns. group does not appear to be due to hybridization with *J. grandis*.

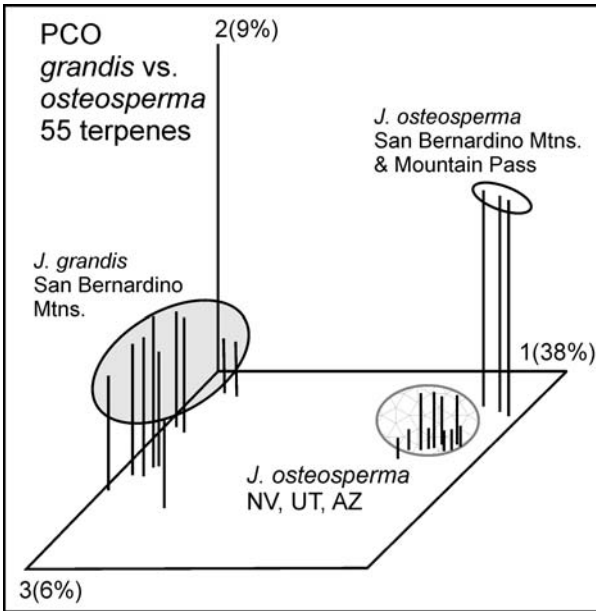


Figure 5. PCO analysis of *J. osteosperma* vs. *J. grandis* individuals from the San Bernardino Mtns.

Juniperus osteosperma grows on the north side of the San Bernardino Mtns. along CA 18 at 1393 m, in a very dry, desert

environment often occupied by *J. californica* (which grows at lower elevation nearby). In fact, trees at this population have been misidentified as *J. californica* in herbaria (pers. obs.). The population of *J. osteosperma* along I15 at Mountain Pass, CA is also near the *J. californica* populations, so it is of interest to compare *J. californica* with *J. osteosperma* so as to assess possible introgression into *J. osteosperma* in the San Bernardino Mtns. - Mountain Pass populations. PCO utilizing *J. californica* from Palmdale and Yucca Valley resulted in a clear separation of the San Bernardino Mtns. - Mountain Pass *J. osteosperma* populations from *J. californica*, with no evidence that the *J. osteosperma* populations are introgressants from *J. californica* (Fig. 6).

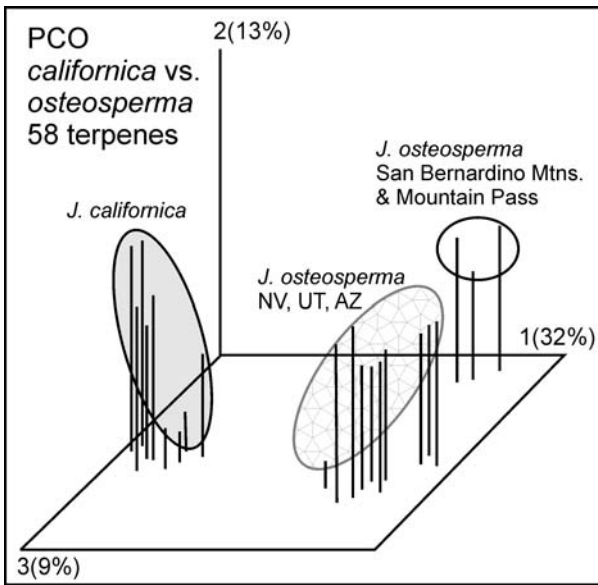


Figure 6. PCO of *J. californica* and *J. osteosperma* based on 58 terpenes.

In summary, geographic variation found in the volatile leaf oils of *J. osteosperma* consists of four major groups: Nevada, San Bernardino Mtns.- Mountain Pass, CA, Thistle, UT and Oak Creek Canyon, AZ. The AZ population may be a Pleistocene relict which

would account for its unusual oil. Life zones descended 300-1100m in the southwestern US during the Pleistocene (Adams 2011), so *J. osteosperma* was likely growing at much lower elevations in Arizona. No evidence of hybridization was found between *J. osteosperma* and *J. grandis* or with *J. californica* in the San Bernardino Mtns. Populations from NW Nevada reported to hybridize with *J. grandis* and *J. occidentalis* (Vasek, 1966; Terry et. al. 2000; Terry, 2010) were not included in the study, but these will be analyzed in a future report.

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Table 1. Leaf essential oil compositions for *J. osteosperma* (McK =McKinney Tanks, NV, UT = Thistle, UT, SB = San Bernardino Mtns., Basin, AZ = Oak Creek Canyon, AZ, *J. occidentalis* (Bend, OR) and *J. grandis* (Meyers, CA). Compounds in boldface appear to separate *J. osteosperma* populations.

KI	Compound	ost McK	ost UT	ost SBM	ost AZ	occ Bnd	gran Mey
921	tricyclene	0.8	0.3	0.3	1.0	1.1	-
924	α -thujene	0.5	0.3	0.5	0.2	1.0	-
932	α-pinene	4.4	1.1	5.6	2.0	5.0	14.0
945	α -fenchene	-	-	t	-	t	1.5
946	camphene	1.1	0.5	0.4	1.0	1.0	-
953	thuja-2,4-diene	t	t	0.2	0.1	t	t
961	verbenene	-	-	-	-	-	2.9
969	sabinene	10.2	8.3	7.5	1.4	12.0	-
974	β -pinene	0.2	0.1	0.2	0.1	0.4	1.3
988	myrcene	1.7	1.0	1.1	0.6	1.3	3.1
1001	δ -2-carene	-	-	t	-	t	1.1
1002	α -phellandrene	0.3	0.1	0.3	t	0.8	1.6
1008	δ -3-carene	-	t	t	t	1.0	27.3
1014	α-terpinene	1.3	0.5	1.6	0.3	1.7	0.4
1020	p-cymene	2.4	1.6	2.8	1.5	10.7	1.4
1024	limonene	2.1	1.6	2.1	2.4	0.9	1.2
1025	β -phellandrene	3.2	1.7	2.0	1.5	3.5	10.6
1044	(E)- β -ocimene	t	t	0.2	t	0.1	t
1054	γ-terpinene	2.1	1.2	2.6	0.6	3.0	0.3
1065	cis-sabinene hydrate	0.8	1.7	1.0	0.3	0.9	-
1078	camphenilone	t	t	t	t	-	-
1086	terpinolene	1.4	0.6	1.2	0.4	1.3	3.7
1090	6,7-epoxy- myrcene	0.1	t	t	t	-	-
1092	96, 109,43,152	-	-	-	-	-	0.9
1095	linalool	t	t	t	t	0.5	t
1098	trans-sabinene hydrate	1.0	2.1	1.4	0.4	0.7	-
1100	55,83,110,156	-	-	-	-	0.3	-
1102	isopentyl- isovalerate	0.2	t	t	-	-	-

KI	Compound	ost	ost	ost	ost	occ	gran
		McK	UT	SBM	AZ	Bnd	Mey
1112	3-me-3-buten- me-butanoate	0.4	t	0.2	t	-	-
1112	trans-thujone	-	-	-	t	t	-
1118	cis-p-menth-2- en-1-ol	0.6	1.1	0.8	0.4	0.7	0.8
1122	α -campholenal	0.3	0.2	0.6	0.4	-	t
1136	trans-p-menth- 2-en-1-ol	-	-	-	-	0.9	0.9
1141	camphor	23.7	19.6	25.6	60.2	2.5	-
1144	neo-isopulegol	-	-	-	-	-	0.5
1145	camphene hydrate	1.5	2.7	1.3	2.0	0.2	t
1154	sabina ketone	0.8	1.4	1.1	0.5	0.4	-
1165	borneol	6.0	4.3	7.2	3.0	2.2	-
1166	coahuilensol	-	-	-	-	0.6	t
1174	terpinen-4-ol	8.3	10.7	12.6	3.2	6.7	0.4
1176	m-cymen-9-ol	-	-	-	-	-	0.4
1179	p-cymen-8-ol	0.5	1.4	1.0	0.5	0.5	0.4
1186	α -terpineol	0.4	0.7	0.5	0.4	0.4	1.2
1195	myrtenol	0.2	0.4	0.3	0.3	-	-
1195	cis-piperitol	0.3	0.4	t	t	0.2	0.4
1204	verbenone	0.2	0.3	0.6	0.8	-	-
1207	trans-piperitol	0.3	0.3	0.7	-	0.3	0.9
1215	trans-carveol	0.6	0.7	1.0	1.0	-	-
1219	coahuilensol, me-ether	0.2	-	0.2	0.4	1.1	0.4
1223	citronellol	t	t	0.7	0.4	8.4	t
1230	<u>43,119,152,194</u>	-	-	-	-	-	3.9
1238	cumin aldehyde	0.3	0.3	0.4	0.1	0.2	-
1239	carvone	0.6	0.8	0.6	0.8	-	t
1249	piperitone	t	-	t	-	0.2	1.2
1254	linalool acetate	-	-	-	-	0.1	-
1255	4Z-decenol	-	-	-	-	-	0.4
1257	me-citronellate	-	-	-	-	-	0.2
1274	neo-isopulegyl acetate	-	-	-	-	-	0.3
1283	α -terpinen-7-al	0.2	-	0.5	-	-	-
1284	bornyl acetate	16.6	19.7	5.5	4.4	9.5	0.4
1285	safrole	-	-	-	-	-	0.3

KI	Compound	ost	ost	ost	ost	occ	gran
		Mck	UT	SBM	AZ	Bnd	Mey
1298	carvacrol	t	0.2	t	t	0.4	0.2
1319	<u>149,69,91,164</u>	0.4	t	0.6	0.4	-	0.8
1322	me-geranate	-	-	-	-	1.0	-
1325	p-mentha-1,4-dien-7-ol	0.5	0.5	1.0	0.1	t	
1332	cis-piperitol acetate	-	-	-	-	-	0.4
1343	trans-piperitol acetate	-	-	-	-	-	0.3
1374	α -copaene	-	-	-	-	1.0	-
1387	β -bourbonene	-	-	-	-	0.2	0.5
1388	<u>79,43,91,180</u>	-	-	-	-	-	0.3
1389	<u>111,81,151,182</u>	-	-	-	-	-	1.0
1429	cis-thujopsene	0.7	-	-	-	0.9	-
1451	trans-muuro-la-3,5-diene	-	-	-	-	0.1	-
1465	cis-muuro-la-4,5-diene	-	-	-	-	0.1	-
1468	pinchotene acetate	0.5	-	0.3	1.0	0.6	-
1475	trans-cadina-1(6),4-diene	-	-	-	-	0.3	-
1478	γ -muurolene	-	-	-	-	0.8	-
1484	germacrene D	-	-	-	-	0.3	0.2
1493	trans-muuro-la-4(14),5-diene	-	-	-	-	0.4	-
1493	epi-cubebol	-	-	t	-	0.4	-
1500	α -muurolene	t	t	-	-	1.1	0.3
1513	γ -cadinene	t	t	t	-	3.7	1.3
1518	epi-cubebol	-	-	-	-	0.4	0.4
1522	δ-cadinene	0.2	0.3	0.2	-	4.1	1.1
1533	trans-cadina-1,4-diene	-	-	-	-	0.1	-
1537	α -cadinene	-	-	-	-	0.4	t
1544	α -calacorene	-	-	-	-	0.3	-
1548	elemol	0.9	0.6	2.5	1.6	-	-
1555	elemicin	-	-	-	-	-	1.5
1574	germacrene-D-4-ol	t	0.2	0.2	-	0.6	0.7

KI	Compound	ost	ost	ost	ost	occ	gran
		McK	UT	SBM	AZ	Bnd	Mey
1582	caryophyllene oxide	t	0.1	0.1	t	-	t
1586	gleenol	-	-	-	-	0.3	-
1607	β -oplophenone	t	t	t	t	0.4	0.4
1608	humulene epoxide II	t	t	t	0.1	-	-
1618	1,10-di-epi-cubenol	-	-	-	-	0.2	t
1627	1-epi-cubenol	-	-	-	-	1.6	t
1630	γ -eudesmol	0.2	t	0.3	0.2	-	-
1638	epi- α -cadinol	t	0.2	0.1	-	1.1	0.7
1638	epi- α -muurolol	t	0.2	0.2	-	1.2	0.7
1644	α -muurolol	-	t	t	-	0.7	t
1649	β -eudesmol	0.2	t	0.4	0.2	-	0.4
1652	α -eudesmol	0.2	0.3	0.3	0.1	-	-
1652	α -cadinol	0.2	0.4	0.5	0.2	1.8	1.6
1670	bulnesol	t	t	0.2	0.1	-	-
1675	cadalene	-	-	-	-	0.3	-
1684	2Z,6Z-farnesal	-	-	0.2	-	-	-
1688	shyobunol	-	-	-	-	-	0.2
1739	oplopanone	t	t	t	t	-	t
1987	manoyl oxide	-	-	-	-	3.2	t
2009	epi-13-manoyl oxide	-	-	-	-	t	-
2056	manool	-	-	-	-	-	t
2055	abietatriene	-	-	-	-	-	t
2298	4-epi-abietal	-	-	-	-	-	t
2312	abieta-7,13-diene-3-one	0.1	t	0.2	0.4	-	-

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified cpds. less than 0.5% are not reported.