Geographic variation in the volatile leaf oil of *Juniperus occidentalis*. II. Analysis from throughout its geographic range

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ABSTRACT

Volatile leaf oils of *J. occidentalis* were analyzed from throughout its range. The major differentiation found was the divergence of the Trinity Alps, California population from other *J. occidentalis* populations in having a high amount of sabinene (20.4%) and only a trace of bornyl acetate. Minor variations in the oil compositions were found, these being chiefly on the margins of the range of the species, except for the Burns, Oregon population. The oil of *J. occidentalis* f. *corbetii*, a shrubby form that occurs about 30 km east of Bend, Oregon , was somewhat distinct in having large amounts of p-cymene (20.0%) and bornyl acetate (24.5%). Published on-line www.phytologia.org *Phytologia 97(4): 265-270 (Oct 1, 2015).* ISSN 030319430.

KEY WORDS: *Juniperus occidentalis, Juniperus occidentalis* forma *corbetii,* Cupressaceae, terpenes, geographic variation.

Juniperus occidentalis, *J. grandis* (=*J. occidentalis* var. *australis*) and *J. osteosperma* are three very closely related junipers in the western United States (Vasek 1966; Adams 2011). Adams and Kauffmann (2010a) and Adams (2012a) reported on the geographic variation in the leaf oils and DNA of *J. grandis, J. occidentalis and J. osteosperma*. Recently, Adams (2012b) examined the leaf oils of *J. occidentalis* f. *corbetii* from east of Bend OR and presented a small study of geographic variation in the leaf oils of *J. occidentalis*. Hybridizations between *J. grandis, J. occidentalis* and *J. osteosperma* have been examined by Vasek (1966) and later by Terry et al. (2000) and Terry (2010) using DNA markers and morphology. Analysis of hybridization using leaf terpenes at Leviathan mine, Nevada (Adams 2013a) and Buffalo Hills, northwestern Nevada were recently published by Adams (2013a,b).

Juniperus occidentalis is a narrowly distributed species, growing largely east of the Cascade Mtns. and thence into nw California, eastern Idaho and northwestern Nevada (Fig. 1).

The purpose this paper is to report on a comprehensive analysis of geographic variation in the leaf essential oils of *J. occidentalis*. The reader is referred to Adams (2013a, b) for a summary of the hybridization between *J. occidentalis* and *J. osteosperma*.

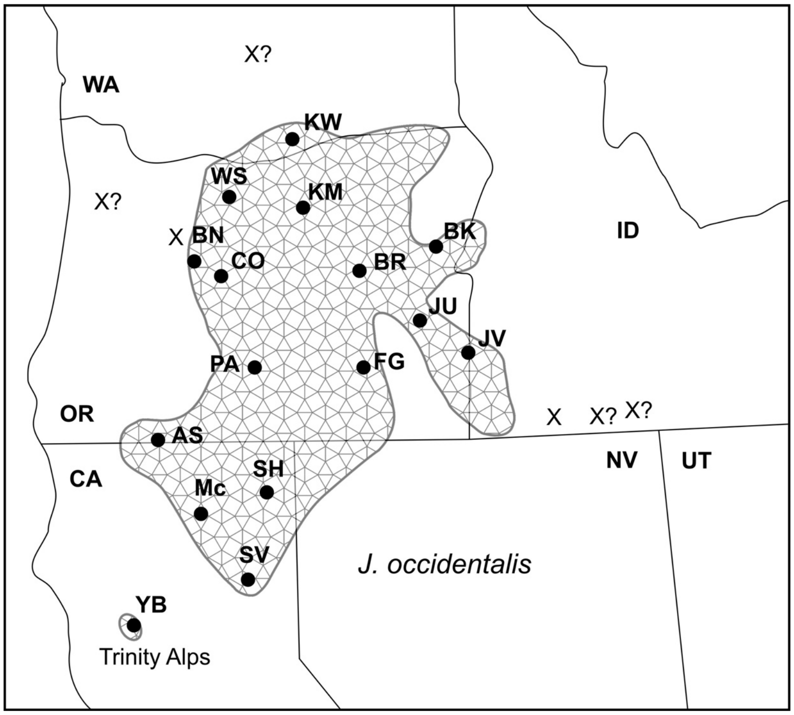

Figure 1. Distribution of *J. occidentalis* modified from Vasek (1966) and Adams (2011). Populations sampled in this study are denoted by shaded circles. Note that the southwestern-most population is at Trinity Alps, CA (Yolla Bolly). X marks disjunct specimens. X? marks questionable reports of *J. occidentalis*.

MATERIALS AND METHODS

Plant material: *J. occidentalis,* **KW** *Adams 11940-11942,* 12 km e of Jct. WA 14 & US 97 on WA 14, 45º 44.392'N, 120º 41.207'W, 170 m, Klickitat Co.; WA, **WS**, *Adams 11943-11945*, 2 km s of jct. US 97 & US 197 on US 97, 38 km ne of Madras, OR; 44º 53.676'N, 120º 56.131'W, 951 m, Wasco Co., OR; **BN** *Adams 11946-11948*, 3 km sw of Bend, OR; on OR 372, 44º 02.390'N, 121º 20.054'W, 1132 m, Deschutes Co., OR; **AS** *Adams 11952-11954,* 14 km e of Jct. OR66 & I5, on OR 66, 42º 08.044'N, 122º 34.130'W, 701 m, Jackson Co., OR east of Ashland; **Mc** *Adams 11957-11959*, on CA 299, 10 km e of McArthur, CA, 41º 05.313'N, 121º 18.921'W, 1091 m, Lassen Co., CA; **TA** *Adams 11995-11998 (Kauffmann A1-A3, B1),* Trinity Alps, Yolla Bolly-Middle Eel Wilderness, 40º 06' 34"N, 122º 57' 59W, 1815- 2000 m, Trinity Co., CA, **SV** *Adams 12342-12346*, 19 km wsw of Susanville, CA, on CA 36, 40º 22.178'N, 120º 50.211' W, 1570 m, Lassen Co., CA, **SH** *Adams 12347-12351*, on US 395, 5 km n of Madeline near Sage Hen Pass, 41º 05.867'N, 120º 28.456' W, 1695 m, Lassen Co., CA, **JU** *Adams 12242-12244*, 3 mi. w Juntura, OR on OR 20, trees, 3 mi w of Juntura, OR on OR 20, 43° 45' 52.61"N; 118° 08' 40.49"W, 953 m (Corbet 2010-1,2,3). **BR** on US 395/20, 9 mi e of Riley, Harney Co., OR, about 17 mi w of Burns, OR. 43° 31' 56.4" N, 119° 19' 22.3" W, 4555 ft, 24 Aug 2012, OR. *Mark Corbet ns*, *Adams 13512-13516*; **PA** on Co Rd. 2-08, 1.5 mi sw of Paisley, Lake Col, OR. 42° 41' 17.7" N, 120° 34' 10.0" W, 4478 ft, 24 Aug 2012, *Mark Corbet ns Adams 13517-13521*, **FG** 0.3 mi w of Frenchglen, OR on OR 205. 42° 49' 34.7" N, 118° 55' 01.2" W, 5064 ft, 24 Aug 2012, Harney Co., OR, *Mark Corbet ns, Adams 13522-13526,* **JV** 2.8 mi sw of Jordan Valley, Owyhee Co., ID, on Trout Creek Rd., thence 0.5 m ne on dirt road. 42° 57' 50.3" N, 117° 00' 01.5" W, 4477 ft, 24 Aug 2012, *Mark Corbet ns, Adams 13527-13531*, **KM**, scattered, with sage, 1 mi s of Kimberly, Grant Co., OR on OR hwy 19. 44° 44' 58.2" N, 119° 38' 10.5" W, 1909 ft. 31 Aug 2012, *Mark Corbet ns, Adams 13537-13541*, **BK** scattered, with sage, 15.5 mi se of Baker, Baker Co., OR on I 84. 44° 38' 59.4" N, 117° 33' 48.2" W, 3350 ft, 31 Aug 2012, *Mark Corbet ns Adams 13542-13546*.

J. occidentalis f. *corbetii* R. P. Adams. **CO** *Adams 11949-11951,* 32 km e of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43º 53.922'N, 120º 59.187'W, 1274 m, Deschutes Co., OR. 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 -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 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.

Data Analysis - 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 volatile leaf oil of *J. occidentalis* is dominated by sabinene, p-cymene and bornyl acetate (Table 1). The leaf oil from the Trinity Alps (Yolla Bolly) population is atypical in having more sabinene (20.4%) and only a trace of bornyl acetate. The *J. o.* f. *corbetii* shrubs east of Bend, OR have large amounts of p-cymene (20.0) and bornyl acetate (24.5%).

To visualize the overall similarities in the leaf oils, a minimum spanning network was constructed (Figure 2). The major differentiation is the divergence of the Trinity Alps (Yolla Bolly) population (Fig. 2). Minor variation is seen among the other populations.

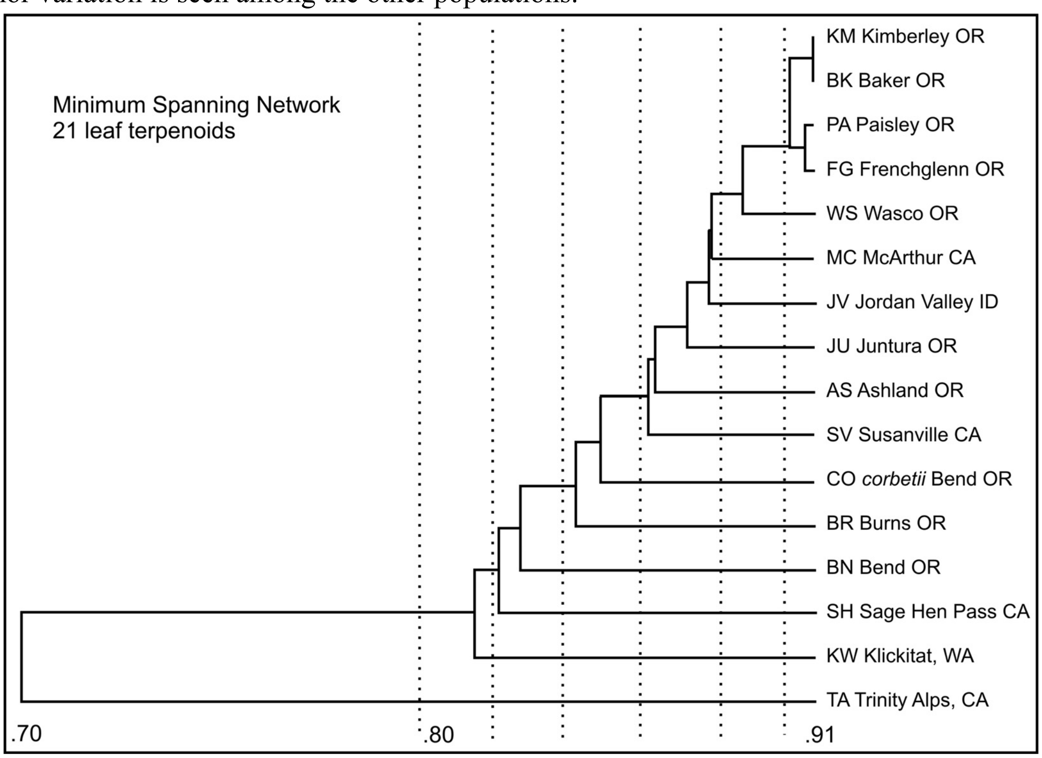

Figure 2. Minimum spanning network based on 21 leaf terpenoids. Notice the divergence of the TA (Trinity Alps, CA) population in its oil. The dotted lines are the contoured levels used in Figure 3.

Contoured clustering shows (Fig. 3) the divergence of the Trinity Alps population, joining last to Burns, OR at an oil similarity of 0.70 (Fig. 3). The leaf oils of the Burns population show some differentiation from adjacent populations BK, KM, JU and FG (Fig. 3, Table 1).

Generally, the leaf oils of *J. occidentalis* are fairly uniform throughout its range in eastern Oregon (Table 1, Fig. 3). Although most of the central populations (KM, BK, PA, FG) are very uniform, there is some differentiation on the periphery of the range: AS, Mc, Sv, SH, BN, and KW.

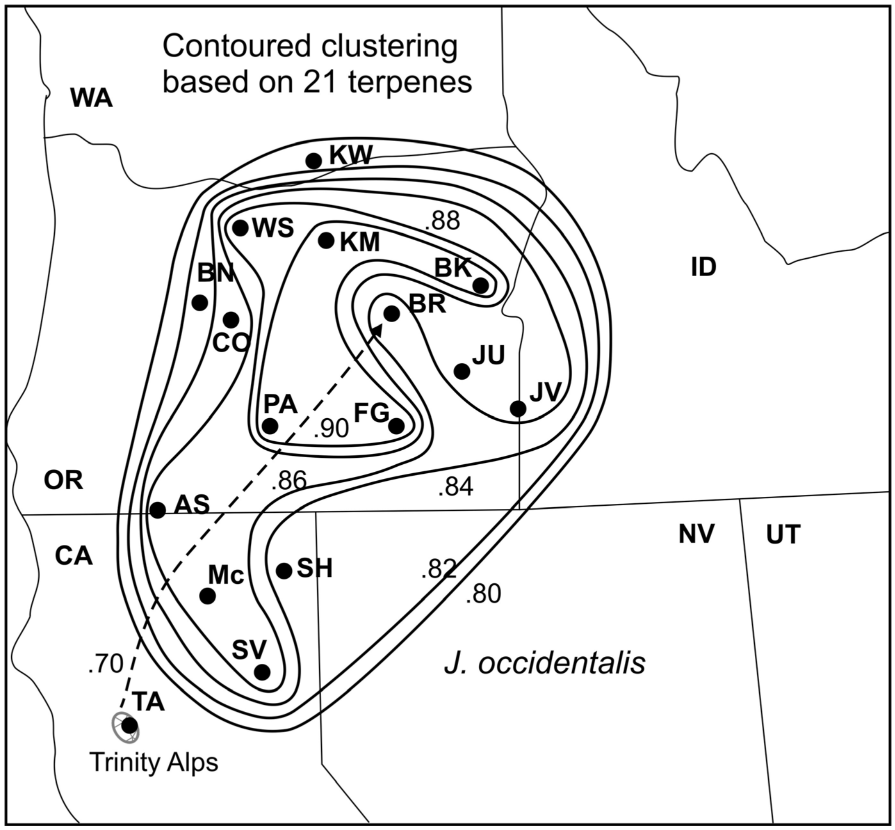

Figure 3. Contoured clustering based on 21 terpenes. See METHODS for population identities.

ACKNOWLEDGEMENTS

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Table 1. Leaf essential oil compositions for populations of *J. occidentalis*, Those 21 compounds used in numerical analyses are in boldface. KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

AI	Compound	Baker	Bend	*corbetii*	Sage Hen	Burns	Trin Alps
921	tricyclene	0.7	1.1	1.7	1.4	0.8	t
924	α-thujene	0.8	1.0	0.9	0.7	1.0	1.8
932	**α-pinene**	**6.2**	**5.0**	**1.8**	**3.6**	**2.8**	**5.1**
945	α-fenchene	0.3	t	t	t	0.1	-
946	camphene	0.6	1.0	1.2	1.1	0.7	0.3
953	thuja-2,4-diene	t	t	-	t	t	-
961	verbenene	-	-	-	t	-	0.7
969	**sabinene**	**11.6**	**12.0**	**7.4**	**8.2**	**16.9**	**20.4**
974	β-pinene	0.3	0.4	0.2	0.4	0.3	0.7
988	**myrcene**	**1.8**	**1.3**	**1.1**	**1.9**	**2.6**	**3.0**
1001	δ-2-carene	t	t	0.6	0.1	t	0.3
1002	α-phellandrene	0.5	0.8	0.5	1.3	1.2	1.2
1008	**δ-3-carene**	**2.9**	**1.0**	**0.6**	**1.2**	**1.4**	**4.4**
1014	**α-terpinene**	**1.3**	**1.7**	**1.5**	**1.4**	**1.8**	**3.2**
1020	**p-cymene**	**14.3**	**10.7**	**20.0**	**7.1**	**10.6**	**5.5**
1024	**limonene**	**0.6**	**0.9**	**0.7**	**1.4**	**1.3**	**0.7**
1025	**β-phellandrene**	**2.5**	**3.5**	**2.0**	**5.9**	**5.0**	**6.7**
1044	(E)-β-ocimene	0.4	0.1	t	0.2	0.2	0.5
1054	**γ-terpinene**	**2.2**	**3.0**	**2.5**	**2.2**	**3.0**	**5.3**
1065	cis-sabinene hydrate	0.6	0.9	0.4	0.6	0.7	1.2
1086	**terpinolene**	**1.5**	**1.3**	**1.4**	**1.3**	**1.7**	**2.4**
1095	**trans-sabinene hydrate**	**2.5**	**0.7**	**t**	**0.4**	**1.1**	**t**
1095	linalool	t	0.5	1.6	0.7	t	1.5
1112	trans-thujone	0.1	t	t	t	t	-
1118	cis-p-menth-2-en-1-ol	0.6	0.7	0.6	0.8	0.7	1.0

AI	Compound	Baker	Bend	*corbetii*	Sage Hen	Burns	Trin Alps
1136	trans-p-menth-2-en-1-ol	0.8	0.9	0.6	0.8	0.7	0.9
1141	**camphor**	**0.2**	**2.5**	**1.3**	**2.8**	**0.5**	**t**
1145	camphene hydrate	0.2	0.2	t	0.4	0.2	-
1154	sabina ketone	0.6	0.4	0.6	0.3	0.4	0.3
1165	**borneol**	**0.4**	**2.2**	**1.9**	**2.3**	**0.5**	**t**
1166	**coahuilensol**	**1.3**	**0.6**	**0.7**	**1.6**	**1.3**	**2.4**
1174	**terpinen-4-ol**	**5.1**	**6.7**	**6.7**	**5.7**	**5.9**	**9.8**
1179	p-cymen-8-ol	1.4	0.5	1.9	1.1	1.0	0.9
1186	α-terpineol	0.4	0.4	0.3	0.3	0.3	0.5
1195	cis-piperitol	0.3	0.2	t	0.3	0.3	0.1
1207	trans-piperitol	0.3	0.3	t	0.4	0.3	0.5
1219	coahuilensol, me-ether	1.4	1.1	0.6	1.3	1.6	2.7
1238	cumin aldehyde	0.4	0.2	0.3	0.1	0.3	0.7
1249	piperitone	0.4	0.2	0.1	0.5	0.3	0.5
1254	linalool acetate	0.5	0.1	0.4	-	0.4	0.1
1284	**bornyl acetate**	**11.3**	**9.5**	**24.5**	**20.3**	**13.3**	**t**
1298	carvacrol	0.5	0.4	0.3	0.4	0.2	0.7
1322	methyl-geranate	2.5	1.0	0.5	2.0	3.6	0.8
1325	p-mentha-1,4-dien-7-ol	0.3	t	0.3	0.3	0.3	0.1
1345	α-cubebene	t	t	t	t	t	t
1374	α-copaene	0.7	1.0	-	0.7	0.5	0.6
1387	β-bourbonene	t	0.2	t	0.1	t	t
1429	cis-thujopsene	-	0.9	-	-	-	-
1451	trans-muurola-3,5-diene	0.1	0.1	t	t	t	0.1
1465	cis-muurola-4,5-diene	t	0.1	t	0.2	t	t
1468	**pinchotene acetate**	**1.2**	**0.6**	**0.6**	**1.1**	**1.8**	**2.0**
1475	trans-cadina-1(6),4-diene	0.1	0.3	t	0.3	t	t
1478	γ-muurolene	0.3	0.8	0.4	0.3	0.3	0.1
1484	germacrene D	0.2	0.3	t	0.2	0.2	t
1493	trans-muurola-4(14),5-diene	0.3	0.4	t	0.4	0.2	0.7
1493	epi-cubebol	0.2	0.4	t	0.4	0.2	0.4
1500	α-muurolene	0.5	1.1	0.5	0.6	0.4	0.6
1513	**γ-cadinene**	**1.9**	**3.7**	**1.4**	**1.8**	**1.1**	**1.8**
1518	epi-cubebol	0.1	0.4	0.4	0.5	0.5	t
1522	**δ-cadinene**	**2.3**	**4.1**	**1.9**	**1.2**	**1.8**	**2.2**
1533	trans-cadina-1,4-diene	0.2	0.1	-	1.1	t	t
1537	α-cadinene	0.2	0.4	-	0.2	0.1	t
1544	α-calacorene	0.1	0.3	-	0.1	0.6	t
1548	elemol	-	-	0.4	t	-	-
1574	germacrene-D-4-ol	0.6	0.6	t	0.4	0.4	0.5
1586	gleenol	0.1	0.3	t	t	t	t
1607	β-oplopenone	0.3	0.4	t	0.3	0.3	0.4
1618	1,10-di-epi-cubenol	-	0.2	t	t	t	t
1627	1-epi-cubenol	1.2	1.6	0.7	1.4	0.7	1.3
1638	epi-α-cadinol	0.7	1.1	0.5	0.6	0.6	0.4
1638	epi-α-muurolol	0.8	1.2	0.5	0.7	0.6	0.6
1644	α-muurolol	0.3	0.7	t	0.3	0.2	t
1649	β-eudesmol	-	-	0.9	t	0.3	-
1652	α-cadinol	1.0	1.8	1.0	1.1	1.2	0.8
1675	cadalene	0.4	0.3	t	0.2	0.2	t
1987	**manoyl oxide**	**1.7**	**3.2**	**3.0**	**2.1**	**1.0**	**1.0**
2009	epi-13-manoyl oxide	t	t	t	t	t	t