# JUNIPERUS OCCIDENTALIS FORMA CORBETII R. P. ADAMS, A NEW SHRUBBY VARIANT: GEOGRAPHIC VARIATION IN LEAF ESSENTIAL OILS

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#### ABSTRACT

The volatile leaf oils of *J. occidentalis* were analyzed from throughout its range. The major differentiation found was the divergence of the Yolla Bolly population and the shrubby form that occurs about 30 km east of Bend, OR. The shrubby taxon is distinct in its habit and terpenes, having large amounts of p-cymene (20.0) and bornyl acetate (24.5%). A new form is named in honor of its discoverer: *Juniperus occidentalis* forma *corbetii* R. P. Adams, *forma nov. Phytologia* 94(1): 22-34 (April 2, 2012).

**KEY WORDS**: Juniperus occidentalis, Juniperus occidentalis forma corbetii R. P. Adams, **forma nov.**, J. grandis, 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). Although Adams and Kauffmann (2010a) and Adams (2012) reported on the compositions of the leaf oil of J. occidentalis, and hybridization with J. grandis, no extensive analysis of geographic variation in the leaf essential oils was reported. Juniperus occidentalis is a narrowly distributed species, growing largely east of the Cascade Mtns. and thence into nw California (Fig. 1). Recently, a shrubby form of J. occidentalis was discovered east of Bend, OR (Fig. 2). Careful field examination revealed that these shrubs are not just damaged (browsed, winter killed, etc.), but differ from the typical J. occidentalis that have a strong central axis. Thus, it seemed opportune to include this unusual population in this study of geographical variation.

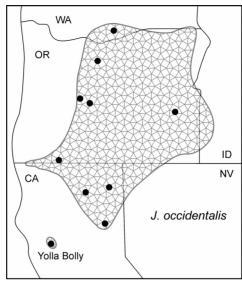


Figure 1. Distribution of *J. occidentalis* modified from Vasek (1966) and Adams (2011) showing sampled areas (dots). Note the southwestern-most population at Yolla Bolly (Trinity Alps, CA).



Figure 2. Mark Corbet with a shrubby form of *J. occidentalis*, 32 km east of Bend, OR (cf. *Adams 11949-11951*).

The purpose this paper is to report on geographic variation in the leaf essential oil of *J. occidentalis*. Analysis of hybridization with *J. osteosperma* in ne California and nw Nevada (Vasek, 1966, Terry, 2010; Terry et al. 2000) is beyond the scope of this paper.

### MATERIALS AND METHODS

Plant material: *J. grandis, Adams 11963-11967*, Jct. US 50 & CA 89, 38° 51.086N, 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.

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 ict. 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 & I5, on OR66, 42° 08.044'N, 122° 34.130'W, 701 m, Jackson Co., OR; Adams 11957-11959, on CA299, 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' 59W, 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, Adams 12242-4, 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). 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.

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, citronellol and bornyl acetate (Table 1). The leaf oil from the Yolla Bolly population is atypical in having more sabinene (20.4%), with a few compounds in common with *J. grandis* from Big Bear (verbenene, unknown 1389, Table 1). The shrubs east of Bend, OR have large amounts of p-cymene (20.0) and bornyl acetate (24.5%).

Principal coordinates analysis using 42 terpenoids resulted in eigenroots that accounted for 22, 15 and 15% of the variance. Ordination of the populations shows coordinate 1 separates the Yolla Bolly population from the other populations (Fig. 3). The shrubs east of Bend are clearly separated (Fig. 3).

Contoured clustering shows (FIg. 4) the sharp differentiation between the typical pyramidal trees at Bend and the shrubs east of Bend and the divergence of the Yolla Bolly (YB) population, joining last at a 0.670 similarity. Small amounts of differentiation is seen on the margins of the central region at Susanville, CA (Sv), Ashland, OR (As) and to a larger degree, the Klickitat, WA (Kw) population (Fig. 4). The oils of *J. occidentalis* appear to be very uniform throughout its range in eastern Oregon (Fig. 4).

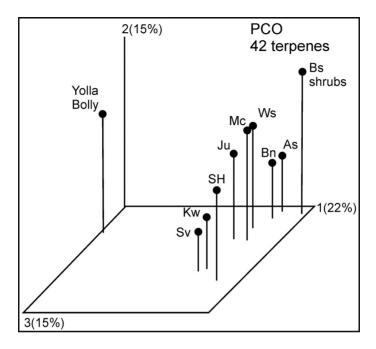


Figure 3. PCO based on 42 terpenes of 10 populations of *J. occidentalis*. Sv = Susanville, CA, Kw = Klickitat, WA, SH = Sage Hen Pass, CA, Ju = Juntura, OR, Mc = McArthur, CA, Ws = Wasco, OR, Bn = Bend, OR, As = Ashland, OR, Bs shrubs = shrubs, east of Bend, OR.

Because hybridization was found in the Beckwourth, CA area (Adams, 2012), it seemed important determine if any of the *J. occidentalis* populations show any evidence of increased similarity to *J. grandis*, suggestive of introgression. PCO ordination between *J. occidentalis* and *J. grandis* shows no intermediate *J. occidentalis* populations (Fig. 5), although the Yolla Bolly population shows some increased similarity to *J. grandis* (as reported by Adams, 2012).

Overall, the leaf essential oils of populations of *J. occidentalis* were found to be rather uniform except for the populations at the extremity of the range, and for the shrubby form east of Bend.

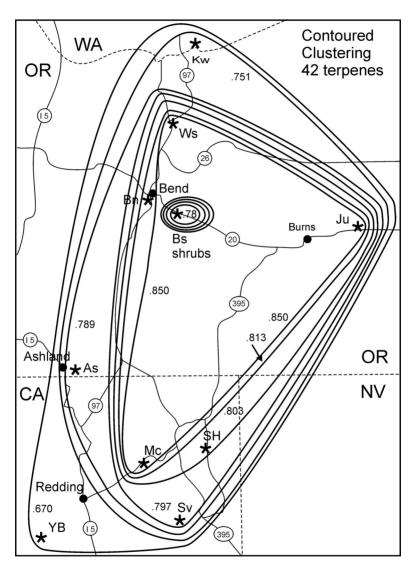


Figure 4. Contoured clustering based on 42 terpenes. See Fig. 3 for population identities.

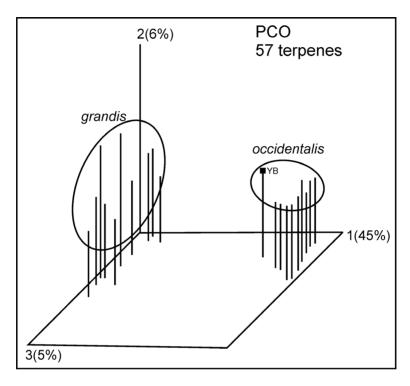


Figure 5. PCO based on 57 terpenes showing clear differentiation between *J. occidentalis* and *J. grandis*. YB is the Yolla Bolly population.

The shrubs of *J. occidentalis*, 32 km east of Bend, OR on hwy 20 appear to form a natural population that is reproducing itself. Due to the apparent differences in their habit from the normal *J. occidentalis* trees and their genetic differences in the expression of terpenoids, the shrubby junipers are worthy of recognition as a new forma:

**Juniperus occidentalis** forma **corbetii** R. P. Adams, **forma nov.** TYPE: United States, Oregon, Deschutes Co., 32 km E of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43° 53.922'N, 120° 59.187'W, 1274 m, OR; 4 Aug 2009, *Robert P. Adams 11949* (HOLOTYPE: BAYLU, PARATYPES: *Robert P. Adams 11950, 11951*, BAYLU).

Junipero occidentali similis sed differt habitu fruticoso et foliis confertim dispositis.

Similar to *Juniperus occidentalis* but differing habit, being a shrub with compact foliage.

The typical variety, with a strong central axis and pyramidal crown, grows on a nearby hillside, whereas f. *corbetii* grows along a dry wash on a mix of lava and sand. No female cones were found in this population.

### **ACKNOWLEDGEMENTS**

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Table 1. Leaf essential oil compositions for three populations of *J. occidentalis*, (Bend, OR, shrubs, e of Bend, OR, and Yolla Bolly, Y Bol) plus *J. grandis* from Big Bear, San Bernardino Mtns., CA. Compounds in boldface appear to separate taxa and were used in numerical analyses. 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. For unknown compounds, four ions are listed, with the largest ion underlined.

		occid	occid.	occid	grandis
KI	Compound	Bend	shrub	Y Bol	Big Bear
921	tricyclene	1.1	1.7	t	0.3
924	lpha-thujene	1.0	0.9	1.8	2.3
932	α-pinene	5.0	1.8	5.1	7.1
945	lpha-fenchene	t	t	-	0.2
946	camphene	1.0	1.2	0.3	0.3
953	thuja-2,4-diene	t	-	-	-
961	verbenene	-	-	0.7	0.3
969	sabinene	12.0	7.4	20.4	24.3
974	β-pinene	0.4	0.2	0.7	0.5
988	myrcene	1.3	1.1	3.0	1.7
1001	δ-2-carene	t	0.6	0.3	0.1
1002	α-phellandrene	0.8	0.5	1.2	0.4
1008	δ-3-carene	1.0	0.6	4.4	2.8
1014	α-terpinene	1.7	1.5	3.2	3.0
1020	p-cymene	10.7	20.0	5.5	6.5
1024	limonene	0.9	0.7	0.7	1.6
1025	β-phellandrene	3.5	2.0	6.7	1.5
1044	(E)-β-ocimene	0.1	t	0.5	0.3
1054	γ-terpinene	3.0	2.5	5.3	4.9
1065	cis-sabinene hydrate	0.9	0.4	1.2	1.9
1086	terpinolene	1.3	1.4	2.4	1.9
1095	trans-sabinene hydrate	0.7	t	t	1.8
1095	linalool	0.5	1.6	1.5	-
1100	55,83,110,156, unknown	0.3	-	-	-
1112	trans-thujone	t	t	-	0.2
1118	cis-p-menth-2-en-1-ol	0.7	0.6	1.0	0.7
1136	trans-p-menth-2-en-1-ol	0.9	0.6	0.9	0.8
1141	camphor	2.5	1.3	t	1.2

		occid	occid.	occid	grandis
KI	Compound	Bend	shrub	Y Bol	Big Bear
1145	camphene hydrate	0.2	t	-	0.2
1154	sabina ketone	0.4	0.6	0.3	0.9
1165	borneol	2.2	1.9	t	0.1
1166	coahuilensol	0.6	0.7	2.4	-
1174	terpinen-4-ol	6.7	6.7	9.8	9.3
1179	p-cymen-8-ol	0.5	1.9	0.9	1.0
1186	lpha-terpineol	0.4	0.3	0.5	0.3
1195	myrtenol	-	-	-	0.2
1195	cis-piperitol	0.2	t	0.1	0.2
1207	trans-piperitol	0.3	t	0.5	0.6
1219	coahuilensol, me-ether	1.1	0.6	2.7	-
1223	citronellol	-	-	-	0.2
1230	trans-chrysanthenyl acetate	-	-	-	0.4
1238	cumin aldehyde	0.2	0.3	0.7	0.3
1249	piperitone	0.2	0.1	0.5	-
1253	trans-sabinene hydrate acetate	-	-	-	0.6
1254	linalool acetate	0.1	0.4	0.1	-
1257	methyl citronellate	-	-	-	0.1
1260	152,123,77,109, C10-OH	-	-	-	0.2
1284	bornyl acetate	9.5	24.5	t	2.2
1298	carvacrol	0.4	0.3	0.7	0.2
1322	methyl-geranate	1.0	0.5	0.8	1.8
1325	p-mentha-1,4-dien-7-ol	t	0.3	0.1	0.7
1345	lpha-cubebene	t	t	t	t
1374	α-copaene	1.0	-	0.6	0.2
1387	β-bourbonene	0.2	t	t	0.3
1388	79,43,91,180, unknown	-	-	0.1	-
1389	<u>111</u> ,81,151,182,	-	-	0.1	0.4
	unknown				
1417	(E)-caryophyllene	-	-	-	0.2
1429	cis-thujopsene	0.9	-	-	-
1430	β-copaene	-	-	-	t
1448	cis-muurola-3,5-diene	-	-	-	0.2
1451	trans-muurola-3,5-diene	0.1	t	0.1	-
1452	lpha-humulene	-	-	-	-
1465	cis-muurola-4,5-diene	0.1	t	t	0.1
1468	pinchotene acetate	0.6	0.6	2.0	-

		occid	occid.	occid	grandis
KI	Compound	Bend	shrub	Y Bol	Big Bear
1471	121,105,180,208,phenol	-	-	-	0.3
1471	dauca-5,8-diene	-	-	-	0.2
1475	trans-cadina-1(6),4- diene	0.3	t	t	-
1478	γ-muurolene	0.8	0.4	0.1	0.2
1484	germacrene D	0.3	t	t	0.3
1491	43,207,161,222, C15-OH	-	-	-	0.3
1493	trans-muurola-4(14),5- diene	0.4	t	0.7	0.2
1493	epi-cubebol	0.4	t	0.4	0.5
1500	α-muurolene	1.1	0.5	0.6	-
1513	γ-cadinene	3.7	1.4	1.8	1.2
1518	epi-cubebol	0.4	0.4	t	1.5
1521	trans-calamenene	-	-	-	2.3
1522	δ-cadinene	4.1	1.9	2.2	-
1533	trans-cadina-1,4-diene	0.1	-	t	0.1
1537	α-cadinene	0.4	-	t	0.2
1544	α-calacorene	0.3	-	t	-
1548	elemol	-	0.4	-	0.9
1559	germacrene B	-		-	0.1
1561	1-nor-bourbonanone	-	-	-	1.1
1574	germacrene-D-4-ol	0.6	t	0.5	-
1582	caryophyllene oxide	-	-	-	0.3
1586	gleenol	0.3	t	t	-
1587	trans-muurol-5-en-4- $lpha$ -ol	-	-	-	t
1607	β-oplopenone	0.4	t	0.4	0.8
1618	1,10-di-epi-cubenol	0.2	t	t	-
1627	1-epi-cubenol	1.6	0.7	1.3	0.5
1630	γ-eudesmol	-	t	-	t
1638	epi-α-cadinol	1.1	0.5	0.4	0.6
1638	epi-α-muurolol	1.2	0.5	0.6	0.6
1644	α-muurolol	0.7	t	t	0.1
1649	β-eudesmol	-	0.9	-	0.2
1652	α-eudesmol	-	-	-	0.6
1652	α-cadinol	1.8	1.0	0.8	0.7
1675	cadalene	0.3	t	t	0.1
1687	43,167,81,238, unknown	-	-	-	0.3
1739	oplopanone	-	-	-	0.2

		occid	occid.	occid	grandis
KI	Compound	Bend	shrub	Y Bol	Big Bear
1987	manoyl oxide	3.2	3.0	1.0	t
0000	epi-13-manoyl oxide				