THE LEAF ESSENTIAL OIL OF *JUNIPERUS MARITIMA* R. P. ADAMS COMPARED WITH *J. HORIZONTALIS*, *J. SCOPULORUM* AND *J. VIRGINIANA* OILS

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ABSTRACT

This is first report on the composition of the leaf essential oil of *J. maritima* R. P. Adams, a new juniper species from the Pacific northwest USA. The volatile leaf oil of *J. maritima* is dominated by elemicin (20.2%), sabinene (20.0%), limonene (11.7%) and 8-α-acetoxyelemol (6.1%) with moderate amounts of safrole (3.8%), pregeijerene B (3.1%) and terpinen-4-ol (1.8%). The leaf oils of *J. horizontalis*, *J. scopulorum* and *J. virginiana* var. *virginiana* were re-analyzed and compared with the oil of *J. maritima*. Each of the four species has a distinct oil composition reflecting their specific status.
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KEY WORDS: *Juniperus maritima, J. horizontalis, J. scopulorum, J. virginiana var. virginiana,* Cupressaceae, essential oil composition, elemicin, sabinene, limonene, 8-α-acetoxyelemol.

Adams (1983) examined geographic variation in leaf terpenoids throughout the range of *Juniperus scopulorum* Sarg. and found that plants from the Puget Sound area of northwestern North America showed considerable differences in their leaf terpenoids compared with typical *J. scopulorum* plants in the Rocky Mountains. Recent DNA sequencing (Schwarzbach et al, in prep.) found that the Puget Sound plants were more related to *J. virginiana* L. than *J. scopulorum*. As part of a continuing study of the genus *Juniperus* (Adams, 2004), additional plants were collected from the Puget Sound area and SNPs (Single Nucleotide Polymorphisms) were examined (Adams, 2007). That study (Adams, 2007) revealed (Fig. 1) that the junipers in the Puget Sound area also differed in their nrDNA SNPs. Based on a combination of SNPs, terpenoids, morphology and ecology, Adams (2007) recognized the junipers of Puget Sound as a new species, *J. maritima* R. P. Adams, the seaside juniper.

Although Adams (1983) reported on multivariate differences in the oils from Puget Sound plants (now *J. maritima*), no information was published on the oil composition. Because *J. maritima* is closely related to *J. horizontalis*, *J. scopulorum* and *J. virginiana*, these leaf oil compositions are included in this report. The leaf oil compositions of *J. horizontalis*, *J. scopulorum* and *J. virginiana* have been recently published (Adams, 2000).

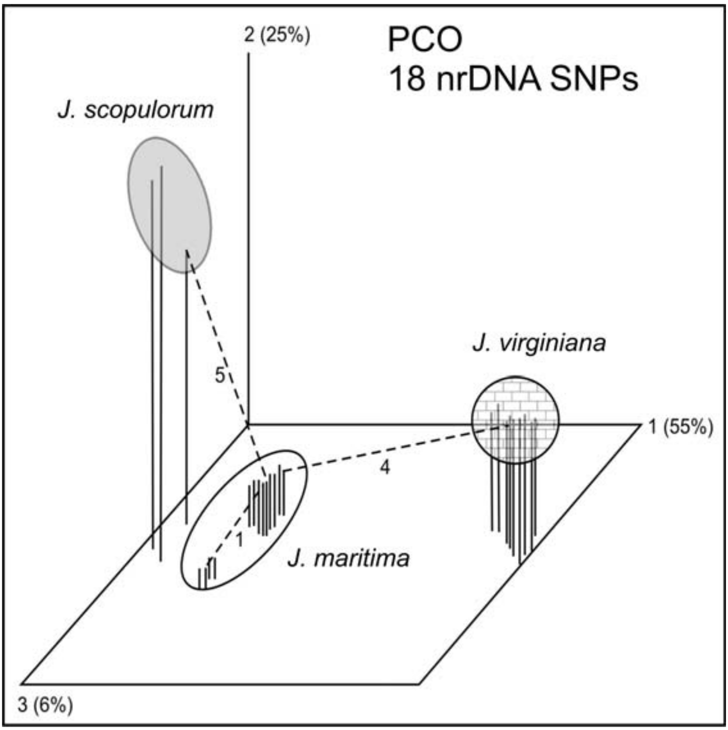

Figure 1. Principal coordinate Ordination (PCO) based on 18 SNPs. Note that *J. maritima* is genetically differentiated from *J. scopulorum* and *J. virginiana*. Modified from Adams (2007).

The purpose of this report is to present analysis of the leaf essential oil of *J. maritima* and compare the oil with the leaf oils of the most closely species: *J. horizontalis*, *J. scopulorum*, and *J. virginiana*.

MATERIAL AND METHODS

Plant material - Specimens used in this study : *J. maritima*: Brentwood Bay, Vancouver Isl., BC, *Adams 11056-58*, Cowichan Bay, Vancouver Isl., BC, Adams *11061-63*, Yellow Point, Vancouver Isl., BC, Adams *11064*, Lesqueti Isl., BC, Adams *11065-66*, Friday Harbor, San Juan Isl., WA, Adams *11067-68*, Whidbey Isl., Cranberry L., WA, Adams *11075*, Fidalgo Isl., Washington State Park, WA, Adams *11076*, Skagit Isl., WA, Adams *11077-78*; *J. horizontalis*: Saskatchewan River bank, Saskatoon, Saskatchewan, *Adams 1651-1660*; *J. scopulorum*: w bank of Animas River, Durango, CO, *Adams 2010-2024*; and *J. virginiana*, 16 km e of Dulles Airport, Washington, DC, *Adams 2409-2423*. Voucher specimens are deposited at 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 are 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 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2006), 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. maritima* is dominated (table 1) by elemicin (20.2%), sabinene (20.0%), limonene (11.7%) and 8-α-acetoxyelemol (6.1%) with moderate amounts of safrole (3.8%), pregeijerene B (3.1%) and terpinen-4-ol (1.8%). Several components are found only in *J. maritima*: isoamyl isovalerate (t), naphthalene (0.5), (2E,4Z)-decadienal (t), α-cubebene (t), α-humulene (t), β-bisabolene (0.3), zonarene (t), $C_{15}OH$ (AI 1586) (0.5) and cedrol (0.1). Most of these unique components are in trace amounts (less than 0.05%), and might be present in the other three juniper species in this study. It is interesting that cedrol is present as it is rare in the leaf oils of *Juniperus* in the western hemisphere (Adams, 2004). It is unusual that the leaf oil of *J. maritima* contains such large quantities of non-terpenoid (phenolic) compounds (elemicin, safrole).

The overall pattern of variation was determined by computing similarity measures among the taxa and subjecting the associational matrix to principal coordinates analysis (PCO). Figure 2 shows the PCO ordination based on the terpenoids. Each of the sticks represents 10-15 individuals: *J. horizontalis* (10); *J. maritima* (15); *J. scopulorum* (15) and *J. virginiana* (15). From this analysis, each oil appears distinct. However, the oil of *J. maritima* appears most similar to the oil of *J. virginiana*. It is interesting that *J. maritima* is separated by 4 SNPs from *J. virginiana* and 5 SNPs from *J. scopulorum* (Fig. 1) similar to the pattern of leaf oils (Fig. 2).

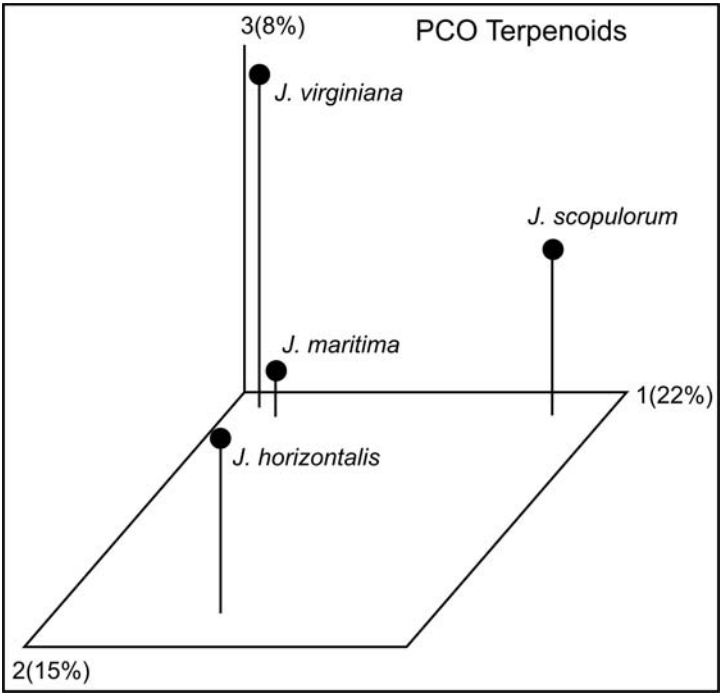

Figure 2. Principal coordinate ordination (PCO) utilizing terpenoids.

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Table I. Compositions of the leaf oils of *J. maritima*, (marit), *J. virginiana* (virg), *J. scopulorum* (scop) and *J. horizontalis* (horiz).

AI	Compound	marit	virg	scop	horiz
921	tricyclene	-	t	t	-
924	α-thujene	0.6	0.2	1.1	0.6
932	**α-pinene**	**0.6**	**1.4**	**4.7**	**1.7**
945	α-fenchene	-	-	t	t
946	camphene	-	0.1	0.1	t
969	**sabinene**	**20.0**	**6.7**	**46.3**	**37.2**
974	β-pinene	t	t	0.2	0.4
988	myrcene	0.9	0.7	1.3	2.8
1001	δ-2-carene	-	t	-	0.1
1002	α -phellandrene	-	-	0.1	t
1008	δ-3-carene	-	0.1	0.1	0.4
1014	α -terpinene	0.7	0.3	1.1	0.6
1020	p-cymene	t	0.1	0.5	0.4
1024	**limonene**	**11.7**	**19.5**	**5.4**	**3.5**
1025	**β-phellandrene**	**-**	**-**	**1.0**	**t**
1044	(E)-β-ocimene	-	t	0.1	0.2
1054	γ-terpinene	1.3	0.4	1.9	1.2
1065	cis-sabinene hydrate	0.5	0.3	1.4	1.3
1086	terpinolene	0.7	0.5	0.8	0.7
1187	2-nonanone	-	-	0.2	-
1095	linalool	0.1	4.0	0.3	0.3
1098	trans-sabinene hydrate	0.2	-	1.0	0.5
1100	n-nonanal	0.1	0.2	-	t
1102	isoamyl-isovalerate	t	-	-	-
1112	trans-thujone (= β-thujone)	-	-	0.1	0.1
1118	cis-p-menth-2-en-1-ol	0.3	0.4	0.4	0.4
1136	trans-p-menth-2-en-1-ol	t	-	0.2	0.2
1141	**camphor**	**-**	**4.0**	**0.2**	**0.2**
1145	**camphene hydrate**	**-**	**0.2**	**0.1**	**-**
1148	citronellal	-	t	-	-
1165	borneol	0.1	0.7	-	-
1167	umbellulone	-	-	-	t
1066	**coahuilensol**	**-**	**0.6**	**-**	**-**
1174	terpinen-4-ol	1.8	1.4	5.8	3.9

1178	naphthalene	0.5	-	-	-
1189	p-cymen-8-ol	-	-	t	-
1186	α-terpineol	0.1	0.2	0.2	0.2
1195	**methyl chavicol**	**0.4**	**t**	**-**	**-**
1195	cis-piperitol	-	-	0.1	t
1207	trans-piperitol	-	t	0.1	0.1
1219	**coahuilensol, methyl ether**	**-**	**0.4**	**-**	**-**
1223	citronellol	0.5	2.4	0.5	-
1249	piperitone	-	0.3	-	-
1253	trans-sabinyl hydrate acetate	-	-	t	-
1255	(4Z)-decenol	t	0.3	0.1	0.2
1257	methyl citronellate	0.2	0.1	t	-
1274	pregeijerene B	3.1	5.7	6.0	-
1285	**safrole**	**3.8**	**10.0**	**t**	**t**
1287	**bornyl acetate**	**t**	**4.0**	**0.7**	**0.5**
1292	(2E,4Z)-decadienal	t	-	-	-
1314	decadienol isomer*	0.1	-	0.1	-
1315	(2E,4E)-decadienal	t	0.2	-	-
1322	methyl geranate	t	t	-	-
1345	α-cubebene	t	-	-	-
1350	citronellyl acetate	-	0.1	-	-
1356	eugenol	-	t	-	-
1379	geranyl acetate	-	t	-	-
1387	β-cubebene	t	-	-	-
1403	**methyl eugenol**	**-**	**3.2**	**0.1**	**-**
1417	(E)-caryophyllene	0.5	t	0.1	-
1442	6,9-guaiadiene	-	-	0.2	0.2
1448	cis-muurola-3,5-diene	-	-	-	0.1
1452	α-humulene	t	-	-	-
1461	cis-cadina-1(6),4-diene	-	-	-	0.1
1468	**pinchotene acetate**	**-**	**0.1**	**-**	**-**
1475	trans-cadina-1(6),4-diene	0.5	-	-	t
1478	γ-muurolene	-	-	0.1	0.3
1480	germacrene D	-	t	-	0.1
1493	**trans-muurola-4(14), 5-diene**	**1.0**	**-**	**-**	**0.2**
1493	epi-cubebol	0.5	-	0.1	0.8
1500	α-muurolene	0.4	0.1	0.1	1.0
1505	**β-bisabolene**	**0.3**	**-**	**-**	**-**

1513	γ-cadinene	0.8	0.2	0.2	2.2
1513	cubebol	0.8	0.1	-	-
1522	δ-cadinene	1.0	0.7	0.3	4.1
1528	zonarene	t	-	-	-
1537	α-cadinene	-	-	t	0.4
1549	**elemol**	**1.0**	**8.7**	**4.3**	**t**
1555	**elemicin**	**20.2**	**1.6**	**-**	**-**
1559	germacrene B	-	-	0.2	-
1574	germacrene D-4-ol	0.9	1.2	0.8	17.7
1586	$C_{15}OH$, 43,207,161,222	0.5	-	-	-
1600	**cedrol**	**0.1**	**-**	**-**	**-**
1607	β-oplopenone	0.1	0.2	0.2	2.4
1627	**1-epi-cubenol**	**0.8**	**-**	**-**	**0.2**
1630	γ-eudesmol	0.1	1.2	0.2	-
1638	epi-α-cadinol	0.6	0.7	0.2	2.0
1638	epi-α-muurolol	0.6	0.7	0.1	1.9
1644	α-muurolol	0.2	t	t	0.5
1649	β-eudesmol	0.2	1.5	0.9	-
1652	α-eudesmol	0.7	2.3	0.6	-
1653	α-cadinol	0.7	1.2	0.5	5.8
1670	bulnesol	0.2	0.8	0.3	-
1685	germacra-4(15),5,10(14)-trien-1-al	-	-	-	0.1
1739	**oplopanone**	**-**	**-**	**-**	**0.2**
1792	**8-α-acetoxyelemol**	**6.1**	**3.6**	**5.9**	**t**
1887	**oplopanonyl acetate**	**-**	**-**	**-**	**0.5**
2055	abietatriene	t	-	-	t
2056	manool	-	t	t	-
2298	4-epi-abietal	0.3	t	t	0.4

AI = Arithmetic Index on DB-5 column. Values less than 0.05% are denoted as traces (t). Unidentified components less than 0.5% are not reported. Those compounds that appear to distinguish taxa are in boldface.