CHEMOSYTEMATICS OF *JUNIPERUS*: EFFECTS OF LEAF DRYING ON ESSENTIAL OIL COMPOSITION III

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ABSTRACT

The essential oils of leaves of *J. virginiana* were collected and analyzed as fresh vs. air dried and stored at ambient conditions (21º C) for up to 25 months before extraction. Changes occurred between months 8 and 25, implying loss due to volatilization and oxygenation. However, for taxonomic analysis involving species closely related to *J. virginiana*, the variations in the oils due to storage were minor. It appears that the oils from dried specimens can be used for studies among species with large differences in the essential oil compositions. Nevertheless, the present study does raise questions about the unexpected changes in leaf oils from specimens stored between 8 and 16 months. *Phytologia 93(1) 372-383 (December 1, 2012).*

KEY WORDS: *Juniperus,* oils from dried leaves, storage tests, chemosystematics.

In a previous study (Adams, 2010), leaves of *Juniperus pinchotii* Sudw. and *J. virginiana* L. were air dried (as herbarium specimens) and the oils analyzed from fresh vs. stored (ambient lab conditions, 21º C) specimens (stored for up to 8 months before extraction). The leaf oils of both species proved to be remarkably stable. For *J. virginiana*, ANOVA of 58 components revealed only 9 significant and 4 highly significant differences among the 7 sample sets. PCO of the samples showed some clustering by length of storage, but with considerable intermixing of samples.

However, in a more recent study on leaves stored for 16 months (Adams, 2011), ANOVA of 58 components revealed 4 significant and 19 highly significant differences among the 8 sample sets, with the major changes occurring between 8 and 16 months storage. PCO of the samples showed the 16 mo. samples to be clearly clustered. In contrast to the previous 8 mo. study (Adams, 2010), unexpected changes in the oils raised concerns about mixing analyses of oils from fresh, recently dried and 16 mo. stored leaves of *Juniperus* for chemosystematic studies

Achak et al. (2008, 2009) compared the leaf essential oils from fresh and air dried (22º C, 16 days) leaves of *J. thurifera* L., *J. phoenicea* L. and *J. oxycedrus* L. and found only small differences.

The purpose of the present study is to report on changes in the composition of the steam distilled leaf oil of *J. virginiana* from specimens stored for 25 months.

MATERIALS AND METHODS

Plant material - *J. virginiana, Adams11768,* cultivated, nw corner of Gruver City Park, Hansford Co. TX, initial bulk collection: 23 Apr 2009. Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh (100 g.) and air dried (10-15 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20º C until analyzed. The extracted leaves were oven dried (48h, 100º C) for the determination of oil yields.

Analyses - 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. For the comparison of oils obtained from leaves stored for various periods, associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967). Principal Components Analysis (PCA) as formulated by Veldman (1967) was performed to examine correlations between components.

RESULTS AND DISCUSSION

Table 1 shows the composition of the leaf oils of *J. virginiana*, and a comparison of components over the 25 month storage period. In contrast to the previous study of 16 mo. (Adams, 2011), the percent oil yield did decline (significantly) in the 25 mo. sample (Table 1). It is unclear why there was no decline during the first 16 mo. of storage. Shanjani et al. (2010) reported that α-pinene (the major and most volatile component) declined from 23.9 to 14.2% when the foliage of *J. excelsa* was air dried. Achak et al. (2008) found oil yields to be greater from fresh than air dried leaves from 2 populations of *J. thurifera* var. *africana*, but with a lower yield in another population. Later, Achak et al. (2009) reported lower oil yields in dried leaves of *J. thurifera* var. *africana* and *J. oxycedrus*, but a much higher yield from dried leaves of *J. phoenicea*.

The compounds (as percent total oil) are remarkably stable during the drying and storage tests for the first 8 months but there are major changes between 8 and 25 months storage tests. In the tests up to 8 months storage, only 9 compounds significantly differed, and only 4 compounds differed highly significantly (Adams, 2010). However, distillation of leaves stored for 25 months revealed 1 significant and 30 highly significant differences (Table 1). Several compounds had large declines in concentration from 8 to 25 month: sabinene (17.6, 10.24),

limonene (14.6, 10.7), β-phellandrene (9.7, 7.1) and germacrene D-4-ol (3.8, 3.6). In contrast, several compounds increased: safrole (9.9, 10.7), methyl eugenol (2.2, 2.6), elemol (5.8, 10.6) and 8-α-acetoxyelemol (10.7, 11.8). Figure 1 (upper) shows the major compounds that declined. Notice that sabinene, limonene, and β-phellandrene show

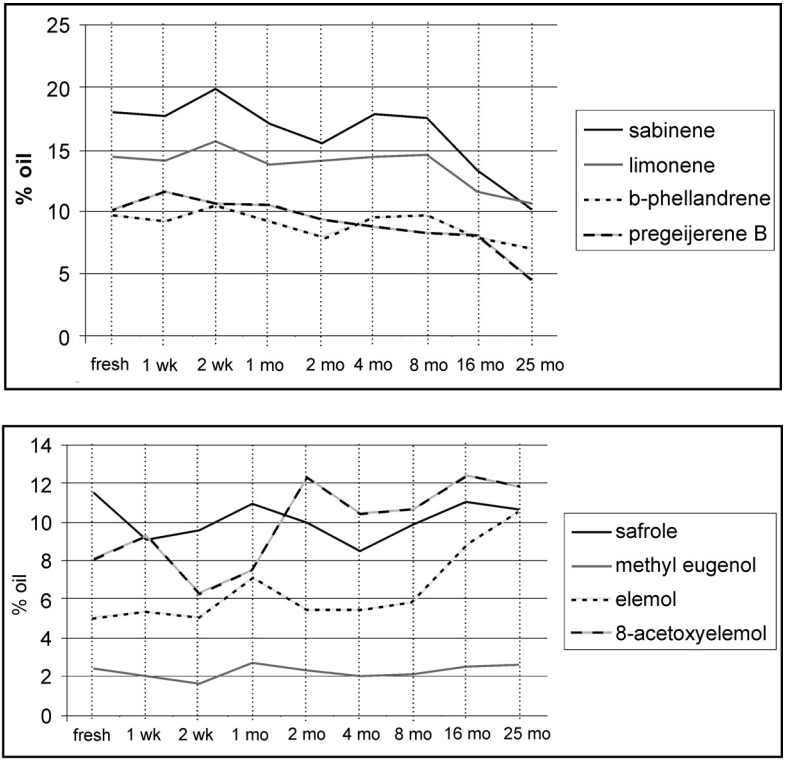

Figure 1. (upper) Changes in concentration (% total oil) for four major components that declined during leaf storage. (lower) Changes in concentration (% total oil) for four major components that increased during leaf storage.

similar patterns. Pregeijerene B shows a gradual decline from 1 month to 25 months.

The patterns for four of the major components that increased during the study are shown in figure 1 (lower). Safrole and methyl eugenol (both from the phenyl propanoid pathway) show similar patterns along with elemol. However, 8-α-acetoxyelemol (dashed line, Fig. 1, lower) increased from fresh to week 1, then declined, then increased to 2 month, then declined, then increased in month 16, and finally decreased in the final, 25 month, sample.

The leaf essential oils in *Juniperus* are stored in leaf glands. In *J. virginiana*, the leaf glands are generally not ruptured and often sunken beneath the waxy cuticle. With the loss of the more volatile monoterpenes and concurrent increase in the sesquiterpenes and diterpenes (Table 1), volatilization seems to be a factor in the changes in composition. The compounds showing the greatest increases (as percent total oil, Fig. 1, lower) are all oxygenated compounds. It seems possible that free radical oxygenation may occurring leading to an increase of these oxygenated compounds.

To estimate the impact of the utilization of oils from fresh versus dried and stored leaves, principal coordinates analysis (PCO) was performed. The PCO (Fig. 2) shows the major trend is the separation of the 16 mo. and 25 mo. samples on axis 1 (33% of the variance among samples). Overall, the samples stored from 1 wk. to 8 mos. seem to form a fairly uniform group.

To determine the utilization of oils from dried *J. virginiana* specimens in a taxonomic study, *J. virginiana* oils were compared with oils of *J. scopulorum* (Durango, CO), *J. blancoi* (Durango, MX), *J. b.* var. *huehuentensis* (Durango, MX) and *J. b.* var. *mucronata* (Maicoba, MX). The resulting PCO ordination (Fig. 3) shows that most of the variation (43%, axis 1) due to the separation of *J. virginiana* from the very closely related *J.* scopulorum and *J. blancoi*. It appears that for taxonomic use, the changes seen in months 16 and 25 are minor as compared to differences in the oils of closely related species.

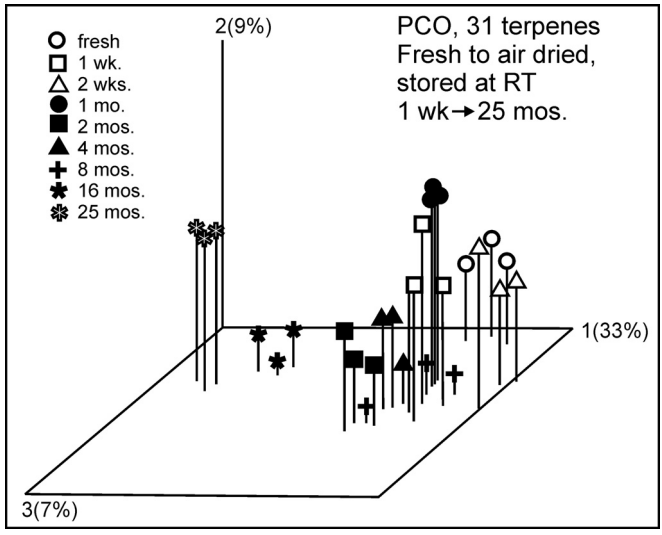

Figure 2. PCO of 9 sample sets ranging from fresh to storage for 25 months at ambient herbarium conditions (air conditioned, 21ºC).

CONCLUSIONS

In this study, ANOVA revealed 1 significant and 30 highly significant differences among the 9 sample sets, with the major changes occurring between 8 and 25 months storage. PCO of the samples showed the 16 and 25 mo. samples to be clearly clustered. In contrast to the previous 8 mo. study (Adams, 2010), unexpected changes in the oils raise concerns about mixing analyses of oils from fresh, recently dried and 16 or 25 mo. stored leaves of *Juniperus* for populational chemosystematic studies. However, for taxonomic analysis involving species closely related to *J. virginiana*, the variation in the oils due to storage appeared to be minor. It appears that the use of oils from dried specimens can be used for studies among species with large differences in the essential oil compositions. Nevertheless, the present study does raise questions about the unexpected changes between 8 and 16 months of herbarium storage. It may be difficult to predict the stability of leaf essential oils in specimens over long periods of storage.

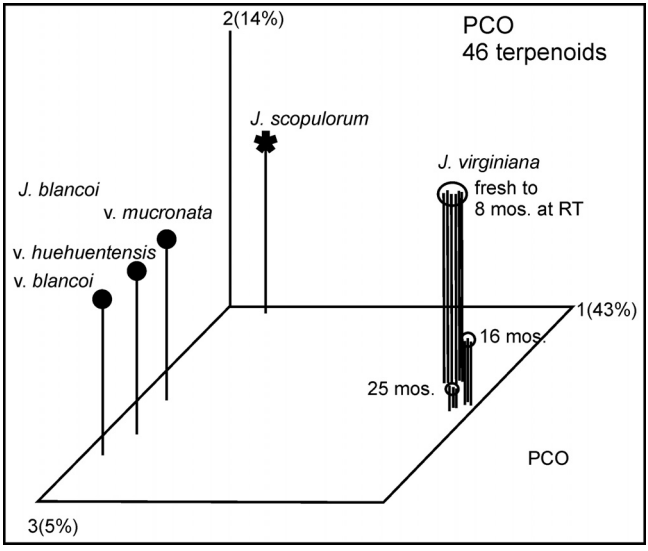

Figure 3. PCO of 9 sample sets of *J. virginiana* plus the oils of *J. scopulorum, J. blancoi, J. b.* var. *huehuentensis* and *J. b.* var. *mucronata*. Note the close clustering of all the *J. virginiana* samples.

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Table 1. Comparison of the composition of leaf oils from fresh leaves of *J. virginiana* vs. leaves dried and stored at 21° C. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non significant, nt = not tested.

KI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	18mo	25mo	F sig
	percent yield	0.55	0.52	0.48	0.51	0.48	0.56	0.53	0.55	0.33	**
924	α-thujene	0.4	0.4	0.5	0.5	0.4	0.4	0.5	0.4	0.3	**
932	α-pinene	0.7	0.7	0.9	0.7	0.5	0.6	0.8	0.5	0.4	**
945	α-fenchene	t	t	t	t	t	t	t	t	t	nt
969	sabinene	18.0	17.7	19.8	17.1	15.5	17.9	17.6	13.3	10.24	**
974	β-pinene	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2	ns
988	myrcene	1.2	0.9	1.1	0.8	0.7	0.7	0.5	0.2	0.2	**
990	74,87,43,115	0.5	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.3	**
1008	3-carene	0.6	0.6	0.6	0.5	0.5	0.7	0.9	0.4	0.4	**
1014	α-terpinene	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.4	ns
1024	limonene	14.4	14.2	15.6	13.8	14.0	14.4	14.6	11.7	10.7	**
1025	β-phellandrene	9.6	9.3	10.4	9.2	7.9	9.5	9.7	8.0	7.1	**
1054	γ-terpinene	0.6	0.5	0.5	0.6	0.5	0.6	0.5	0.6	0.7	*
1065	cis-sabinene hydrate	0.5	0.5	0.5	0.5	0.6	0.6	0.5	0.6	0.8	**
1086	terpinolene	0.8	0.7	0.8	0.7	0.7	0.8	0.7	0.5	0.4	**
1096	trans-sabinene hydrate	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.6	nt
1097	linalool	0.4	0.3	0.6	0.5	0.5	0.7	0.5	1.0	0.6	nt
1100	n-nonanal	t	t	0.2	t	0.2	t	t	t	t	nt
1118	cis-p-menth-2-en-1-ol	t	t	t	t	t	0.2	t	t	t	nt

KI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	25mo	F sig
1136	trans- p-menth-2-en-1-ol	t	t	t	t	t	t	t	t	t	nt
1148	citronellal	0.2	t	t	t	t	t	t	t	0.2	nt
1174	terpinen-4-ol	1.3	0.8	0.8	0.9	1.1	1.2	0.9	1.5	1.5	**
1186	α-terpineol	t	t	t	t	t	t	t	t	0.3	nt
1195	methyl chavicol	0.1	0.2	t	0.2	0.2	0.2	t	t	t	nt
1223	citronellol	0.2	t	t	t	0.2	0.2	t	t	0.2	nt
1261	152,123,81,77, aromatic	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.3	0.4	nt
1274	pregeijerene B	10.2	11.7	10.7	10.6	9.4	8.7	8.3	8.2	4.6	**
1285	safrole	11.6	9.1	9.6	10.9	10.0	8.5	9.9	11.1	10.7	**
1322	methyl geranate	0.1	t	t	t	0.1	0.1	t	t	t	nt
1350	citronellyl acetate	t	t	t	t	t	t	t	t	t	nt
1379	geranyl acetate	t	t	t	t	t	t	t	t	t	nt
1403	methyl eugenol	2.4	2.0	1.6	2.7	2.3	2.0	2.2	2.5	2.6	**
1417	(E)-caryophyllene	t	t	t	t	t	t	t	t	t	nt
1447	43,105,149,178, aromatic	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.6	nt
1465	cis-muurola-4(14),5-diene	t	t	t	t	t	0.2	t	0.2	0.6	nt
1491	epi-cubebol	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	nt
1500	α-muurolene	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.3	0.4	nt
1513	γ-cadinene	0.3	0.4	0.5	0.6	0.5	0.5	0.4	0.5	0.8	**
1522	δ-cadinene	0.8	0.7	0.8	1.0	0.8	0.9	0.9	1.0	1.6	**

KI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	25mo	F sig
1539	α-copaen-11-ol	t	0.3	t	t	t	t	t	t	t	nt
1548	elemol	5.1	5.3	5.1	7.2	5.4	5.5	5.8	8.8	10.6	**
1555	elemicin	0.8	0.8	0.5	0.8	0.9	0.7	1.1	0.7	0.5	**
1565	(3Z)-hexenyl benzoate	0.2	t	0.2	0.2	0.3	0.2	t	t	t	nt
1574	germacrene-D-4-ol	2.8	3.4	3.4	2.6	3.5	3.0	3.8	3.4	3.6	**
1630	γ-eudesmol	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.3	nt
1638	epi-α-cadinol	0.6	0.6	0.5	0.6	0.6	0.6	0.6	0.9	0.8	**
1638	epi-α-muurolol	0.6	0.6	0.5	0.7	0.6	0.6	0.7	0.8	0.8	**
1649	β-eudesmol	0.4	0.5	0.4	0.5	0.2	0.6	0.6	0.7	1.0	**
1652	α-eudesmol	0.6	0.7	0.6	0.6	0.7	0.7	0.8	0.9	1.3	**
1652	α-cadinol	1.0	1.0	0.8	1.0	1.0	1.1	1.2	1.4	1.0	**
1670	bulnesol	0.5	0.4	0.4	0.3	0.5	0.5	0.6	0.5	0.2	**
1688	shyobunol	t	t	t	t	0.2	0.2	t	t	t	nt
1746	8-α-11-elemodiol	t	t	0.2	t	0.3	0.4	0.3	t	t	nt
1761	iso to 8-α-acetoxyelemol	0.2	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	nt
1792	8-α-acetoxy-elemol	8.1	9.3	6.3	7.5	12.3	10.5	10.7	12.4	11.8	**
2054	41,81,137,270,	0.2	0.2	t	0.3	0.3	0.3	0.3	0.4	0.5	**
2087	abietadiene	t	t	t	t	t	t	t	t	t	**
2298	4-epi-abietal	0.4	0.3	0.3	0.2	0.4	0.4	0.3	0.5	0.6	**

KI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	25mo	F sig
2312	abieta-7,13-dien-3-one	t	t	t	t	t	t	t	0.1	0.6	**

KI = Kovats Index (linear) on DB-5 column (see Adams, 2007). Unidentified compounds have the major ions listed. The first ion (underlined) is the base (100%) ion. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.