

Comparison of volatile oils of *Juniperus coahuilensis* in fresh seed cones vs. cones in fresh gray fox scat

Robert P. Adams

Biology Department, Baylor University, Box 97388, Waco, TX
76798, USA robert_adams@baylor.edu

Shirley Powell and A. M. Powell

Herbarium, Sul Ross State University, Box C-64, Alpine, TX 79832 USA ampowell@sulross.edu

ABSTRACT

The composition of volatile oil from *Juniperus coahuilensis* berries (seed cones) in fox scat is very similar to that of berries taken directly from trees. The most notable difference is that the percent yield is significantly higher from scat than intact fresh berries. This may be partially explained by the fact that the scat berry has been partially masticated and thus amenable to steam during distillation, whereas the fresh seed cones have their skin intact, impeding the loss of terpenes in distillation. A second factor is that sugars, starch and protein may have been partially removed during digestion. Thus, the yield from the 'depleted' berry would naturally contain a higher percent volatile oil relative to remaining cellulosic pulp. The gray fox does not extract most of the terpenes from juniper berries. The nearly intact berries, containing most of the terpenes, are excreted in the scat. This undoubtedly results in the loss of some or considerable amounts of nutrients, but the food source is so plentiful, that the loss of nutrients, due to incomplete digestion, may not be significant to the health of the gray fox. Published on-line www.phytologia.org *Phytologia* 98(2): 119-127 (Apr 4, 2016). ISSN 030319430.

KEY WORDS: *Juniperus coahuilensis*, volatile oils, gray fox scat, terpenes, composition.

Based on identification of plant material in gray fox (*Urocyon cinereoargenteus*) scat, White et al. (1999) found that gray fox obtained over half (51.3%) of its diet from *Juniperus osteosperma* berries (seed cones) in eastern Utah. They also reported that gray fox is adept at climbing trees and may have used juniper trees for resting, food source, or as escape cover. Analysis of the berry hull (pulp surrounding the seed) in different seasons, over two years, showed the hull was about 63 - 68% of the whole seed cone. The hulls (pulp) contained 3.8 - 5.2% protein, 15.9 - 28.3% starch and sugars, 25.8 - 26.8% crude fat, and 27.4 - 32.1% ADF (Acid Detergent Fiber). The juniper seeds were not digested by gray fox.

The summer of 2013 presented a remarkable year for the production of a bumper crop of seed cones (berries) of *Juniperus coahuilensis* near Alpine, TX (Fig. 1). Notice the limbs are loaded with berries and leaning. Many branches were so loaded with berries that they drooped to the ground.

Careful observation revealed that in Nov. and Dec. 2013, gray foxes were feeding almost exclusively on the seed cones of *J. coahuilensis* and these seed cones accounted for ca. 90% of the volume in the scat (Fig. 2).



Fig. 1. *J. coahuilensis* with branches loaded with fruits.

Gray foxes were often seen feeding on freshly fallen berries on the ground (Fig. 3), or up in a juniper eating the berries directly from the limbs (Fig. 4).



Fig. 2. Fresh gray fox scat with nearly intact juniper berries (seed cones).



Fig. 3. Gray fox eating *J. coahuilensis* berries on the ground.

In addition to gray fox, mule deer (*Odocoileus hemionus*) ate berries from the ground (Fig. 5) or even resorted to considerable effort to eat berries directly from the trees (Fig. 6). Western blue birds also fed on *J. coahuilensis* berries, both on the ground (Fig. 7) and in trees.

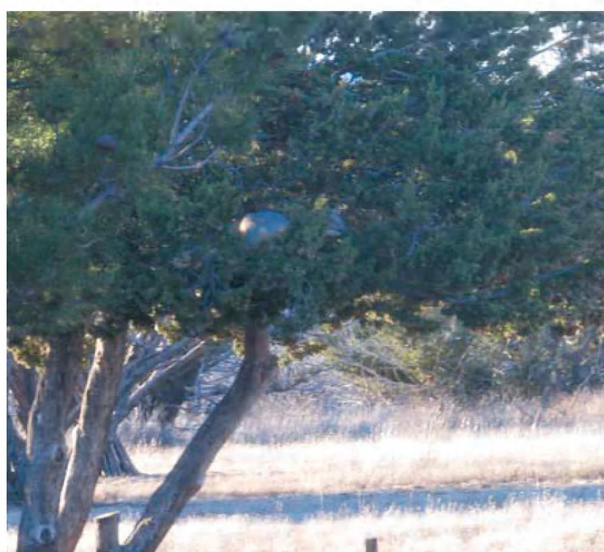


Fig. 4. Gray fox in juniper tree eating berries.



Fig. 5. Mule deer eating berries from the ground.

Cunningham, Kirkendall and Ballard (2006) reported *Juniperus monosperma* berries accounted for 0.0 - 22.5% of the gray fox's diet and from 0.0 to 18.25% of the diet of coyotes in central Arizona. Thacker et al. (2011), by analyzing the terpenes in fecal pellets of greater sage-grouse in Utah, correctly identified the sagebrush species that the sage-grouse was feeding on using crude terpene profiles.

As far as known, there are no reports on the composition of essential oil of *J. coahuilensis* berries present in fresh gray fox scat. The composition of the volatile leaf oil of *J. coahuilensis* has been reported (Adams, 2000, 2014), but there are no reports on the composition of the volatile oil of the berries (seed cones).



Fig. 6. Mule deer expending great effort to reach berries in the *J. coahuilensis* trees.



Fig. 7. Western blue birds eating *J. coahuilensis* berries on the ground.

The changes in terpenes during passage through the gray fox digestive tract are not known. The purpose of the present paper is to compare the volatile oil compositions of fresh *J. coahuilensis* seed cones vs. fresh fox scat in which ca. 90% of the scat consisted of *J. coahuilensis* seed cones.

MATERIALS AND METHODS

Fresh, mature seed cones were collected from *J. coahuilensis* trees in Dec., 2013 at the home of Mike and Shirley Powell, approx. 8 mi se of Alpine, on Tex 118, thence e 2 mi on Mile High Rd., 30° 16.147' N, 103° 33.522' W, 5324 ft (1623 m). Adams 14649-14653 ripe seed cones of *J. coahuilensis*; Adams 14654-14658, fresh fox scat was collected at the small location and frozen. Leaves for volatile oil were collected from *J. coahuilensis*, 85 km north of La Zarca, Durango, Mexico, Adams 6829-6831.

Fresh frozen *J. coahuilensis* seed cones (13-33 g) were co-steam distilled with 2 mg of undecane and 2 mg methyl decanoate (internal standards) for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). In the same manner, individual frozen fox scat (3.88 - 8.74 g) were distilled. The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted scat and leaves were oven dried (48h, 100° C) for the determination of oil yields.

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.

RESULTS AND DISCUSSION

The compositions of the berries in the fox scat are very similar to berries taken directly from trees (Table 1). The most notable difference is that the percent yield is significantly higher from scat than intact fresh berries (Table 2). This may be partially explained by the fact that the berry skin and layer of wax was somewhat disrupted by mastication in the scat (Fig. 8), whereas, fresh seed cones remained intact during distillation. Thus, the pulp was more easily subjected to steam in the scat berries. A second factor is that sugars, starch and protein may have been partially removed during digestion. Thus, the yield from the 'depleted' berry would naturally contain a higher percent volatile oil relative to remaining cellulosic pulp. A third factor might be the enzymatic removal of glucosides attached to terpene-glucosides, leaving free terpenes that easily volatilize during distillation. A fourth factor is that the scat berries and tree berries likely did not come from the same tree. Notice the composition of the berries from tree 14649 is more like that of the scat berries for α -pinene, terpinolene and terpinen-4-ol. It is possible that the scats collected did not come from any of the trees from which berries were collected.

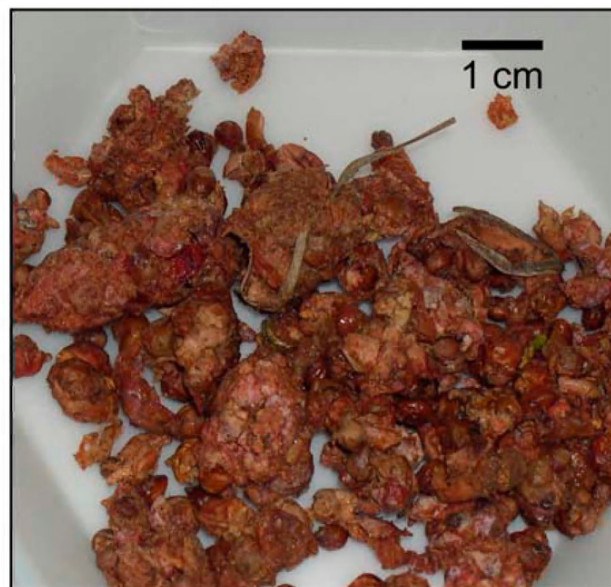


Fig. 8. Fox scat with *J. coahuilensis* berries.

From the current limited study, one can not say which factor was most important (or if there may be additional factors, not considered).

Several terpenes are in larger concentration (Table 2) in the scat: α -pinene, sabinene, and the three diterpenes - abietadiene, 4-epi-abeital, and abieta-7,13-dien-3-one. Two terpenes are larger in the cones: terpinolene and terpinen-4-ol (Table 2).

The volatile berry oils differ in many components, both quantitatively and qualitatively, from the volatile leaf oil of *J. coahuilensis* from La Zarca, Mexico (Table 3). This likely reflects both ontogenetic variation between berries and leaves as well as geographic variation in the leaf oils of *J. coahuilensis* (Figs. 4, 5, Adams, 2000). Although, Adams (2000) found the leaf oil from Alpine, TX to be very similar to that from Ciudad Chihuahua (CM), north of La Zarca.

Although there appear to be no reports on the fate of terpenes in gray fox, there are a few such papers on small mammals. McLean et al. (1993) fed *Eucalyptus radiata* leaves to ringtail possums and found terpene derived metabolites increased in the urine. They concluded possums detoxify dietary terpenes by polyoxygenating the terpenes so the highly polar metabolites will be water soluble and excreted in urine.

Boyle et al. (2000) fed p-cymene to Koala and found no p-cymene or metabolites in the feces, but oxidized metabolites of p-cymene were excreted in urine; a very similar mechanism as found in possum (McLean et al., 1993).

Other methods of detoxifying *Juniperus* terpenes in woodrats (*Neotoma*) are discussed by Adams et al. (2014, 2016).

In conclusion, it appears, in this preliminary study, that gray fox does not extract most of the terpenes from juniper berries. The nearly intact berries, containing most of the terpenes, are excreted in the scat. This undoubtedly results in the loss of some or considerable amounts of nutrients, but the food source is so plentiful, that the loss of nutrients, due to incomplete digestion, may not be significant to the health of the gray fox.

ACKNOWLEDGEMENTS

This research was supported in part with funds from Baylor University.

LITERATURE CITED

- Adams, R. P. 1991. Cedar wood oil - analysis and properties. In *Modern Methods of Plant Analysis: Oils and Waxes*. Edits., H. F. Linskins and J. F. Jackson, pp. 159 - 173, Springer-Verlag, Berlin, Germany.
- Adams, R. P. 2000. The serrate leaf margined *Juniperus* (Section *Sabina*) of the western hemisphere: Systematics and evolution based on leaf essential oils and Random Amplified Polymorphic DNAs (RAPDs). *Biochem. Syst. Ecol.* 28: 975-989.
- Adams, R. P. 2007. Identification of essential oils by gas chromatography/ mass spectrometry, 4th edition. Allured Publ., Carol Stream, IL, USA.
- Adams, R. P. 2014. The junipers of the world: The genus *Juniperus*. 4th ed. Trafford Publ., Victoria, BC, Canada.
- Adams, R. P., M. M. Skopec and J. P. Muir. 2014. Comparison of leaf terpenoids and tannins in *Juniperus monosperma* from woodrat (*Neotoma stephensi*) browsed and non-browsed trees. *Phytologia* 96: 63-70.
- Adams, R. P., M. M. Skopec and J. P. Muir. 2016. Comparison of leaf terpenoids and tannins in *Juniperus osteosperma* from woodrat (*Neotoma stephensi*) browsed and non-browsed trees. *Phytologia* 98: 17-25.
- Cunningham, S. C., L-B. Kirkendall and W. Ballard. 2006. Gray fox and coyote abundance and diet responses after a wildfire in central Arizona. *West. North Am. Natl.* 66: 169-180.
- McLean, S., W. J. Foley, N. W. Davies, S. Brandon, L. Duo and A. J. Blackman. 1993. Metabolic fate of dietary terpenes from *Eucalyptus radiata* in common ringtail possum (*Pseudocheirus peregrinus*). *J. Chem. Ecol.* 19: 1625-1643.
- Boyle, R., S. McLean, W. J. Foley, B. D. Moore, N. W. Davies and S. Brandon. 2000. Fate of the dietary terpene, p-cymene, in the male koala. *J. Chem. Ecol.* 26: 1095-1111.
- Thacker, E. T., D. R. Gardner, T. A. Messmer, M. R. Guttery and D. K. Dahlgren. 2011. Using gas chromatography to determine winter diets of greater sage-grouse in Utah. *J. Wildlife Management* 76: 588-592.
- White, C. G., J. T. Flinders, R. G. Cates, B. H. Blackwell and H. D. Smith. 1999. Dietary use of Utah juniper berries by gray fox in eastern Utah. *USDA Forest Service Proc. RMRS-P-9*: 219-232.

KI	Compound	scat 14654	scat 14655	scat 14656	scat 14657	scat 14658	cones 14649	cones 14650	cones 14651	cones 14653
1582	caryophyllene oxide	0.3	0.3	0.2	0.2	0.3	0.4	0.2	0.3	t
1608	humulene epoxide	0.3	0.2	0.2	0.2	0.2	0.5	0.2	0.2	t
1627	1-epi-cubenol	t	0.2	t	t	t	t	t	t	t
1649	β -eudesmol	t	0.2	t	t	t	t	t	t	t
1652	α -eudesmol	t	0.1	t	t	t	t	t	t	t
1685	germacra-4(15),5,10(14)- trien-1-al	t	t	t	t	t	-	t	t	t
1959	hexadecanoic acid	t	0.2	t	t	t	0.2	0.2	0.7	0.2
2022	cis-abieta-8,12-diene	t	t	t	t	t	-	-	t	-
2055	abietatriene	t	t	t	t	t	-	-	t	-
2087	abietadiene	0.3	0.2	t	0.2	0.4	-	-	t	-
2298	4-epi-abietal	0.2	0.3	t	0.3	0.2	-	-	t	-
2312	abieta-7,13-dien-3-one	0.4	0.4	t	0.4	0.7	-	-	0.2	-
2343	4-epi-abietol	t	t	t	t	t	-	-	t	-
2401	abietol	t	0.2	t	t	0.2	-	-	t	-

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.

Table 2. Statistical analysis of selected components of oils in scat vs. seed cones.

KI	Compound	scat avg	cones avg	P value	significance
	percent yield (% ODW)	1.54	0.39	2×10^{-5}	**
932	α -pinene	12.84	7.22	0.68	ns
969	sabinene	49.64	21.00	1×10^{-5}	**
1086	terpinolene	0.50	1.45	3×10^{-4}	**
1174	terpinen-4-ol	3.58	14.75	2×10^{-3}	**
2087	abietadiene	0.07	0.01	8×10^{-3}	**
2298	4-epi-abietal	0.20	0.02	8×10^{-3}	**
2312	abieta-7,13-dien-3-one	0.39	0.03	1.5×10^{-2}	*

Table 3. Comparison of the volatile oil compositions of fresh seed cones and leaf oil from La Zarca, MX, *Adams 6829* (Adams, 2000). Components with considerable difference between seed cone oils and leaf oil are in bold.

KI	Compound	cones 14649	cones 14650	cones 14651	cones 14653	leaf oil 6829
	percent yield (% ODW)	0.49	0.42	0.26	0.30	1.23
921	tricyclene	t	t	t	t	t
924	α -thujene	1.5	1.6	2.1	1.1	1.6
932	α-pinene	16.7	6.0	3.0	3.2	2.6
946	camphene	0.3	0.3	t	t	t
961	verbenene	0.8	-	-	0.2	-
969	sabinene	21.9	19.9	20.1	22.1	35.5
974	β -pinene	0.9	0.5	0.2	0.3	0.5
988	myrcene	0.5	0.3	0.5	0.8	2.6
1002	α -phellandrene	t	0.2	0.1	0.2	0.3
1014	α -terpinene	1.5	2.9	3.9	2.8	3.6
1020	p-cymene	0.6	1.2	1.1	1.0	0.3
1024	limonene	2.6	1.7	2.4	1.5	1.8
1025	β -phellandrene	1.8	1.1	1.5	1.0	1.7
1026	1,8-cineole	-	-	-	-	0.5
1044	(E)- β -ocimene	t	t	t	t	0.2
1054	γ -terpinene	2.8	5.0	6.7	5.3	5.6
1065	cis-sabinene hydrate	1.6	2.0	1.8	2.0	1.5
1067	cis-linalool oxide (furan-)	-	-	-	-	t
1086	terpinolene	1.0	1.4	1.8	1.6	1.9
1098	trans-sabinene hydrate	2.8	3.1	3.9	2.8	2.2
1099	α -pinene oxide	0.3	0.2	t	t	-
1112	trans-thujone	0.2	0.3	0.3	0.3	t
1118	cis-p-menth-2-en-1-ol	0.7	1.3	1.4	1.6	0.9
1122	α-camphenal	2.0	2.5	1.3	1.3	-
1123	terpene, 67, 81, 109, 156, 168	1.4	1.4	1.3	1.4	-
1135	trans-pinocarveol	2.6	1.7	1.3	1.8	-
1136	trans-p-menth-2-en-1-ol	-	-	-	-	0.6
1137	trans-sabinol	1.1	2.1	1.2	1.7	-
1137	trans-verbenol	5.3	3.1	1.1	1.8	-
1142	camphor	-	-	-	-	0.4
1144	neo-isopulegol	-	-	-	-	0.4
1145	camphene hydrate	-	-	-	-	t
1148	citronellal	-	-	-	-	4.1
1154	sabina ketone	1.9	4.0	3.7	3.9	-
1155	iso-isopulegol	-	-	-	-	0.1
1160	pinocarvone	0.6	0.6	0.2	0.4	-
1165	borneol	-	-	-	-	t
1166	p-mentha-1,5-dien-8-ol	0.7	0.9	0.3	0.6	-
1166	coahuilensol	-	-	-	-	t
1169	terpene, 92, 81, 134, 152	0.9	1.8	1.6	1.7	-
1174	terpinen-4-ol	6.8	13.9	18.1	20.2	12.4
1181	thuj-3-en-10-al	0.5	1.1	1.1	1.2	-
1186	α -terpineol	0.5	0.6	0.7	0.8	0.5
1195	cis-piperitol	-	-	-	-	0.2
1195	myrtanal	2.3	3.7	3.1	4.0	-
1196	myrtanol	1.0	0.7	0.3	0.1	t
1204	verbenone	1.7	1.2	0.9	1.1	-
1207	trans-piperitol	-	-	-	-	0.3
1215	trans-carveol	1.2	1.0	0.6	0.7	-
1223	citronellol	1.2	0.3	0.5	0.3	4.9
1235	cis-chrysanthenyl acetate	t	-	-	-	-

KI	Compound	cones 14649	cones 14650	cones 14651	cones 14653	leaf oil 6829
1238	cumin aldehyde	0.3	0.6	0.4	0.4	-
1239	carvone	0.4	0.5	0.5	0.4	-
1269	perilla aldehyde	0.3	0.6	0.4	0.4	-
1274	pregeijerene B	-	-	-	-	0.4
1284	bornyl acetate	0.5	1.0	0.7	0.7	t
1289	p-cymen-7-ol	0.6	0.4	0.2	0.6	-
1325	p-mentha-1,4,dien-7-ol	0.9	1.5	1.6	1.4	-
1387	β -cubebene	-	-	-	-	t
1389	β -elemene	-	-	-	-	0.1
1400	tetradecane	t	t	t	t	-
1417	(E)-caryophyllene	t	t	t	t	t
1448	cis-muurolo-3,5-diene	-	-	-	-	0.2
1452	α -humulene	-	-	-	-	t
1468	pinchotene acetate	-	-	-	-	t
1475	trans-cadina-1(6),4-diene	-	-	-	-	0.2
1480	germacrene D	t	t	t	t	-
1489	β -selinene	-	-	-	-	t
1493	trans-muurolo-4(14),5-diene	-	-	-	-	0.2
1496	valencene	-	-	-	-	0.2
1500	α -muurolene	-	-	-	-	t
1513	γ -cadinene	t	t	t	t	0.2
1522	δ -cadinene	t	t	t	t	0.1
1545	selina-3,7(11)-diene	-	-	-	-	t
1548	elemol	0.4	0.2	0.2	0.1	5.8
1574	germacrene D-4-ol	t	t	t	t	t
1582	caryophyllene oxide	0.4	0.2	0.3	t	-
1608	humulene epoxide	0.5	0.2	0.2	t	-
1627	1-epi-cubenol	t	t	t	t	0.5
1630	γ -eudesmol	-	-	-	-	1.0
1649	β -eudesmol	t	t	t	t	1.2
1652	α -eudesmol	t	t	t	t	1.3
1670	bulnesol	-	-	-	-	0.5
1685	germacra-4(15),5,10(14)-trien-1-al	-	t	t	t	-
1746	8-α-11-elemodiol	-	-	-	-	0.3
1792	8-α-acetoxylemol	-	-	-	-	0.7
1959	hexadecanoic acid	0.2	0.2	0.7	0.2	-
1987	manoyl oxide	-	-	-	-	t
2022	cis-abieta-8,12-diene	-	-	t	-	-
2055	abietatriene	-	-	t	-	t
2087	abietadiene	-	-	t	-	t
2298	4-epi-abietal	-	-	t	-	t
2312	abieta-7,13-dien-3-one	-	-	0.2	-	t
2313	abietal	-	-	-	-	t
2331	trans-ferruginol	-	-	-	-	t
2343	4-epi-abietol	-	-	t	-	-
2401	abietol	-	-	t	-	-



Adams, Robert P., Powell, Shirley, and Powell, A M. 2016. "Comparison of volatile oils of *Juniperus coahuilensis* in fresh seed cones vs. cones in fresh grey fox scat." *Phytologia* 98(2), 119–127.

View This Item Online: <https://www.biodiversitylibrary.org/item/216734>

Permalink: <https://www.biodiversitylibrary.org/partpdf/219021>

Holding Institution

Phytologia

Sponsored by

IMLS LG-70-15-0138-15

Copyright & Reuse

Copyright Status: In copyright. Digitized with the permission of the rights holder.

Rights Holder: Phytologia

License: <http://creativecommons.org/licenses/by-nc-sa/4.0/>

Rights: <https://biodiversitylibrary.org/permissions>

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at <https://www.biodiversitylibrary.org>.