First comprehensive report on the composition of the leaf volatile terpenoids of *Pinus arizonica* Engelm. and *P. ponderosa* var. *brachyptera* (Engelm.) Lemmon

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ABSTRACT

The first comprehensive report on the composition of the volatile leaf terpenoids of *Pinus arizonica* and *P. ponderosa* var. *brachyptera* from Arizona is presented. The leaf oil of *P. arizonica* contains major amounts of α-pinene (19.4%), germacrene D (19.0%), (E)-caryphyllene (10.1%) and β-phellandrene (9.0%) with moderate amounts of β-pinene (5.0%) and limonene (4.4%). The leaf oil of *P. p*. var. *brachyptera* has considerable α-pinene (15.2%), germacrene D (13.6%), β-pinene (11.2%), (E)-caryphyllene (9.7%) and δ-3-carene (9.2%), with modest amounts of β-phellandrene (2.4%), limonene (2.3%),terpinolene (2.1%), camphene(2.1%), myrcene(2.1%), bornyl acetate(2.0%) and (Z)-β-ocimene(1.8%). The oil of *P. arizonica* contains seven unique compounds: longifolene, unknown sesquiterpene (KI 1494), ethyl dodecanoate, ethyl tetradecanoate, iso-abienol, abienol and unknown diterpene (KI 2341). The oil of *P. p*. var. *brachyptera* has eight unique compounds: δ-3-carene, 2-undecanone, β-longipinene, β-himachalene, α-cuprenene, unknown sesquiterpene aldehyde (KI 1756), putative (2E)-tridecenol, and phenyl ethyl octanoate. Published on-line **www.phytologia.org** *Phytologia 97(1): 45-50 (Jan 2, 2015)*. ISSN 030319430.

KEY WORDS: *Pinus arizonica*, *Pinus ponderosa* var. *brachyptera*, volatile leaf oil, terpenes, composition.

Smith (1977) reported extensively on five major monoterpenes in the xylem resin and found five types of wood oils in ponderosa pine (Table 1). Notice that the combination of the five monoterpenes characterizes each of the five types. However, types IV and V are not very distinct. Type V (Chiricahua,

Table 1. Average normalized monoterpene composition in xylem resin from naturally growing trees. (data from Smith, 1977, Table 4) arranged by the five oil types (I, II, III, IV, V) of Smith (1977).

compound	San Bernardino Type: I	Eldorado, CA II	Pierce, WA III	Coconino, AZ IV	Coronado, AZ V (*P. arizonica*)
α-pinene	9.1	6.1	4.8	50.4	69.9
β-pinene	46.2	28.3	11.8	4.2	6.9
δ-3-carene	4.2	34.5	59.5	26.9	12.0
myrcene	13.7	13.1	13.6	4.3	1.4
limonene	24.8	14.9	6.5	11.2	4.6

AZ) is currently treated as *P. arizonica* and type IV (Coconino, AZ) is in the range of *P. ponderosa* var. *brachyptera*. Potter et al. (2013) did not find many differences in mitochondria haplotypes, although it is unclear if they sampled typical *P. arizonica*. Gernandt et al. (2009, fig. 2) also found only a few inconsistent cpDNA differences between *P. arizonica* and *P. ponderosa* (*P. scopulorum*).

Smith (1977) did not find differences in the xylem monoterpenes between the northwestern ponderosa pine (*P. p.* var. *ponderosa*) and the northeastern populations (*P. p.* var. *scopulorum*). However, von Rudloff and Lapp (1992), using leaf essential oils, found var. *ponderosa* and var. *scopulorum* to clearly differ in leaf oil composition. Potter et al. (2013) confirmed these varieties in their mitochondrial DNA study.

Although there are scores of studies on the xylem monoterpenes (see review in Smith, 1977), there are very few studies on the volatile leaf oil composition of *P. ponderosa*. The earliest report was by Schorger (1919) that the leaf oil was composed of α-pinene (2%), β-pinene (75%), limonene (6%), borneol (7%), bornyl acetate (2%), and 'green oil' (3%). In the modern era of gas chromatography/ mass spectrometry, Zavarin et al. (1971) reported on the variation in the leaf oil with season and needle age. Cobb et al. (1972) reported the effects of pollution on the volatile leaf oils. Von Rudloff (1975) gave a bar histogram of 12 components from *P. ponderosa* (var. *ponderosa*) from British Columbia.

The first comprehensive analysis of the volatile leaf oil of *P. ponderosa* (var. *ponderosa*), was by Adams and Edmunds (1989) from 20 trees near Dryden, WA. The only major study on geographical variation in the volatile leaf oils of *P. ponderosa* was by von Rudloff and Lapp (1992). They examined the leaf oils from 37 populations west of the Continental Divide and 5 populations east of the Divide in MT using 12 terpenoids plus estragole and 5 consolidated characters (composed of difficult to resolve, isomers). They found the terpenoids clearly distinguished the western (var. *ponderosa*) from the eastern (var. *scopulorum*) populations. However, there appear to be intergradation between the two varieties in the terpene patterns near the Continental Divide. In addition, von Rudloff and Lapp (1992) analyzed one tree from Grand Canyon, AZ (presumably *P. ponderosa* var. *brachyptera*) and reported the oil was dominated by α-pinene (37.0%), β-pinene (18.4%), δ-3-carene (6.9%), limonene (6.5%),

The purpose of the present paper is to make the first comprehensive report on the leaf volatile oil of *P. arizonica* from near the type locality in far southern Arizona and compare it with the leaf oil of *P. ponderosa* var. *brachyptera* from northern Arizona.

MATERIALS AND METHODS

Leaf samples were collected from *Pinus arizonica:* Coronado National Forest, Santa Catalina Mountains, Bear Canyon, 0.2 mi WNW Cypress picnic area, 32.3732° N, 110.6975° W, elev. 5900 ft. Pima Co., AZ, *G. M. Ferguson 3679-3687,* lab acc. *Robert P. Adams 14439-14447*; Coronado National Forest, Santa Catalina Mountains, Bear Canyon, 0.1 mi E Cypress picnic area, same tree visited previously with David Gernandt in 2007, his DNA analysis. 32.3729° N, 110.6923° W, elev. 5870 ft., Pima Co., AZ, *G. M. Ferguson 3688,* lab acc. *Robert P. Adams 14448*.

Pinus ponderosa var. *brachyptera*: 19 mi. east of Camp Verde on AZ Hwy 260 at Salmon Lake, Coconino National Forest. 34° 30' 38.5" N, 111° 31' 28.1" W. elev. 6,300 ft, Coconino, Co., AZ, *David Thornburg 1419 (1-10)*, lab acc. *Robert P. Adams 14428-14437.* Voucher specimens are deposited in the herbarium, Baylor University.

Fresh, frozen 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.

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 the Adams 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 leaf oil of *P. arizonica* contains major amounts (Table 2) of α-pinene (19.4%), germacrene D (19.0%), (E)-caryphyllene (10.1%) and β-phellandrene (9.0%) with moderate amounts of β-pinene (5.0%) and limonene (4.4%). The leaf oil of *P. p.* var. *brachyptera* has considerable α-pinene (15.2%), germacrene D (13.6%), β-pinene (11.2%), (E)-caryphyllene (9.7%) and δ-3-carene (9.2%), with modest amounts of β-phellandrene (2.4%), limonene (2.3%),terpinolene (2.1%), camphene (2.1%), myrcene (2.1%), bornyl acetate (2.0%) and (Z)-β-ocimene (1.8%). The oil of *P. arizonica* contains seven unique compounds: longifolene, unknown sesquiterpene (KI 1494), ethyl dodecanoate, ethyl tetradecanoate, iso-abienol, abienol and unknown diterpene (KI 2341). The oil of *P. p.* var. *brachyptera* has eight unique compounds: δ-3-carene, 2-undecanone, β-longipinene, β-himachalene, α-cuprenene, unknown sesquiterpene aldehyde (KI 1756), putative (2E)-tridecenol, and phenyl ethyl octanoate.

Pinus arizonica individual 14443 is unusual in having larger amounts of limonene (11.0%) and β-phellandrene (22.2%) but low in α-pinene (7.5%). Tree 14442 is high in α-pinene (31.9%) and low in limonene (0.4%) and β-phellandrene (0.8%). However, the unique components that separate the two taxa are still present (or absent) in these unusual tree oils (Table 2).

Likewise, *P. p.* var. *brachyptera* has trees with unusual oils: 14430 is low in monoterpenes and high in linalyl acetate (8.5%), germacrene D (21.5%) and (E)-caryphyllene (11.7%); trees 14433 (and 14436, not shown) are also low in monoterpenes, but high in bornyl acetate (10.6%), (E)-caryphyllene (13.9%) and germacrene D (14.5%). Just as with *P. arizonica*, these trees, with unusual oils, still have the unique components that separate the two taxa or the compounds are absent in their oils as in the average (Table 2).

Overall the leaf oils differ about as expected between conifer species. In any case, the differences in the morphology clearly distinguish these taxa.

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Table 2. Comparison of leaf oil compositions of *Pinus arizonica and P. ponderosa* var. *brachyptera.* Compounds in bold face appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. KI is the Kovat's Index using a linear calculation on DB-5 column.

		P. arizonica			*P. ponderosa brachyptera*		
KI	compound	*14443*	*14442*	average	average	*14430(1)*	*14433(2)*
846	(E)-hexenal	0.6	0.1	0.3	0.1	t	0.4
850	(3Z)-hexenol	0.4	0.1	0.3	t	t	0.3
863	n-hexenol	0.1	t	t	t	t	t
921	tricyclene	0.1	0.2	0.1	0.3	t	0.5
924	α-thujene	t	-	t	0.2	t	0.2
932	α-pinene	7.5	31.9	19.4	15.2	7.9	7.2
946	camphene	0.6	0.5	1.1	2.1	0.6	1.8
969	sabinene	0.4	t	0.1	0.2	0.2	0.2
974	**β-pinene**	**3.5**	**0.5**	**5.0**	**11.2**	**1.3**	**6.8**
988	myrcene	1.7	0.7	1.4	2.1	2.5	1.8
1002	α-phellandrene	1.0	t	0.4	0.2	t	0.1
1008	**δ-3-carene**	**-**	**-**	**-**	**9.2**	**4.3**	**7.5**
1014	α-terpinene	0.3	0.2	0.8	1.0	0.8	0.5
1020	p-cymene	0.1	t	t	0.3	0.2	0.2
1024	**limonene**	**11.0**	**0.4**	**4.4**	**2.3**	**1.7**	**0.9**
1025	**β-phellandrene**	**22.2**	**0.8**	**9.0**	**2.4**	**1.8**	**1.1**
1032	**(Z)-β-ocimene**	**1.7**	**0.6**	**0.4**	**1.8**	**1.6**	**1.0**
1044	(E)-β-ocimene	0.2	0.8	1.6	0.7	0.2	0.1
1054	γ-terpinene	0.2	0.1	0.2	0.5	0.4	0.4
1086	terpinolene	0.4	0.6	0.8	2.1	1.6	1.4
1095	linalool	0.4	0.1	0.1	1.2	0.9	3.7
1122	α-campholenal	0.1	0.1	0.1	t	t	0.1
1135	trans-pinocarveol	0.3	0.1	0.1	0.1	t	0.2
1137	cis-verbenol	0.1	0.1	0.1	-	t	-
1166	p-mentha-1,5-dien-8-ol	t	0.1	t	0.2	t	0.8
1174	terpinen-4-ol	0.2	t	t	0.1	t	0.2
1186	**α-terpineol**	**0.3**	**0.2**	**0.2**	**0.7**	**0.4**	**0.7**
1254	linalyl acetate	2.2	0.8	1.1	1.2	8.5	2.8
1287	bornyl acetate	0.8	0.4	0.6	2.0	0.3	10.6
1298	**trans-pinocarvyl acetate**	t	t	**t**	**0.4**	t	0.3
1345	α-terpinyl acetate	0.1	0.4	t	0.2	0.8	1.0
1345	α-cubebene	0.1	0.1	t	0.1	0.1	0.2
1365	**2-undecanone**	**-**	**-**	**-**	**0.1**	**0.3**	**0.2**
1373	α-ylangene	0.1	t	t	t	t	t
1374	α-copaene	0.1	0.2	0.2	0.3	0.4	0.4
1379	geranyl acetate	t	t	t	-	t	-
1387	β-bourbonene	t	0.1	t	-	0.2	-
1387	β-cubebene	t	0.1	t	t	0.1	0.2
1400	**β-longipinene**	**-**	**-**	**-**	**t**	**0.4**	**0.9**
1407	**longifolene**	**2.2**	**t**	**0.3**	**-**	**-**	**-**
1417	(E)-caryophyllene	7.1	13.4	10.1	9.7	11.7	13.9
1430	β-copaene	0.1	0.2	0.2	t	0.1	0.1
1448	cis-muurola-3,5-diene	t	t	t	t	t	t
1451	trans-muurola-3,5-diene	t	t	t	t	t	t
1452	α-humulene	1.1	2.2	1.6	1.6	1.9	2.2
1461	cis-cadina-1(6),4-diene	t	t	t	t	t	t
1465	cis-muurola-4(14),5-diene	t	t	t	t	t	t
1475	γ-muurolene	0.7	2.2	2.0	0.5	2.2	0.7
1478	germacrene D	12.7	23.2	19.0	13.6	21.5	14.5
1475	trans-cadina-1(6),4-diene	t	t	t	t	0.1	0.1
1493	trans-muurola-4(14),5-diene	t	t	t	t	0.1	0.3
1494	**sesquiterpene,<u>161</u>,119,105, 204**	**0.5**	**0.5**	**0.5**	**-**	**-**	**-**
1500	**β-himachalene**	**-**	**-**	**-**	**1.1**	**1.6**	**1.0**
1500	α-muurolene	0.9	0.9	1.0	0.8	1.3	0.8
1505	**α-cuprenene**	**-**	**-**	**-**	**0.2**	**0.4**	**0.1**
1511	δ-amorphene	-	-	0.1	-	-	-
1513	γ-cadinene	1.5	1.1	1.3	1.4	1.8	1.1

		P. arizonica			*P. ponderosa brachyptera*		
KI	compound	*14443*	*14442*	average	average	*14430(1)*	*14433(2)*
1522	δ-cadinene	3.3	2.3	3.0	2.4	3.4	1.8
1533	trans-cadina-1,4-diene	0.1	0.1	t	t	0.2	0.1
1537	α-cadinene	0.2	0.1	0.2	0.1	0.2	0.1
1561	(E)-nerolidol	t	0.2	t	0.9	0.8	0.7
1574	germacrene-D-4-ol	5.5	3.5	3.9	2.7	5.0	0.4
1582	caryophyllene oxide	0.3	0.3	0.3	0.2	0.2	0.3
1594	**ethyl dodecanoate**	**0.7**	**0.3**	**0.5**	**-**	**-**	**-**
1638	epi-α-cadinol	0.8	0.4	0.6	0.6	1.0	0.1
1640	epi-α-muurolol	0.8	0.5	0.7	0.6	0.9	0.2
1644	α-muurolol	0.3	0.2	0.2	0.2	0.4	0.1
1652	α-cadinol	2.3	1.3	1.8	1.7	2.7	0.5
1756	**hydrocarbon-aldehyde <u>55</u>, 43, 69, 121**	**-**	**-**	**-**	**0.8**	**0.6**	**0.7**
1772	**(2E-tridecenol?)**	**-**	**-**	**-**	**0.7**	**0.5**	**0.7**
1795	**ethyl tetradecanoate**	**0.2**	**0.2**	**0.1**	**-**	**-**	**-**
1846	**phenyl ethyl octanoate**	**-**	**-**	**-**	**0.5**	**1.9**	**0.1**
1987	manool oxide	0.1	0.3	0.5	0.3	t	t
2105	**iso-abienol**	**-**	**-**	**0.9**	**-**	**-**	**-**
2149	**abienol**	**0.1**	**0.3**	**0.5**	**-**	**-**	**-**
2341	**diterpene,<u>55</u>,81,239,286**	**0.9**	**0.2**	**1.0**	**-**	**-**	**-**