The leaf essential oils of *Picea chihuahuana* Martinez and *P. martinezii* Patterson *(Pinaceae)*

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ABSTRACT

The first comprehensive analysis of the volatile leaf terpenoids of *Picea chihuahuana* and *P. martinezii* is reported. The volatile leaf oil of *P. chihuahuana* is dominated by bornyl acetate (47.9%) with moderate amounts of α-pinene (4.7), camphene (6.3), β-pinene (5.2), camphene (6.2), limonene (7.4) and β-phellandrene (7.3%). The leaf oil of *P. martinezii* is dominated by bornyl acetate (26.4%), α-pinene (16.6) and myrcene (15.1%), with moderate amounts of camphene (5.6) and β-pinene (9.7%). For such closely related species (Patterson and Harrod, 1994), the oils are quite differentiated. Components unique in *P. chihuahuana* oil are: α-phellandrene, cis- and trans-p-menth-2-en-1-ol, cis- and trans-piperitol, piperitone, cis- and trans-piperitol acetate, methyl isopimarate, methyl levopimarate and methyl abietate. Unique compounds in *P. martinezii* oil include: α-muurolene, δ-cadinene, caryophyllene oxide, humulene epoxide II, epi-α-cadinol, epi-α-muurolol, α-muurolol, α-cadinol, germacra-4(15),5,10(14)-triene-1-al and neo-abienal. Published on-line **www.phytologia.org** *Phytologia 96(4): 260-263 (Oct 1, 2014).* ISSN 030319430

KEY WORDS: *Picea chihuahuana*, *P. martinezii,* terpene composition, volatile leaf oils, Pinaceae.

Taylor, Patterson and Harrod (1994) used a combination of morphology, phenolics and terpenoids to examine Mexican species of *Picea* . Of interest to the present study, was the observation that *Picea chihuahuana* and *P. martinezii* were shown to be distinct species by their morphology (Fig. 2), phenolics (Fig. 5) and terpenoids (Fig. 6), re-confirming their previous study (Taylor and Patterson, 1980). Taylor, Patterson and Harrod (1994) listed α-pinene, camphene, β-pinene, myrcene, γ-terpinene, piperitone, citronellol, geraniol, camphor, and bornyl acetate as present in six species of *Picea* examined, but did not give any composition data.

Recently, Jaramillo-Correa et al. (2006) examined mt and cp DNA markers among *P. chihuahuana* and *P. martinezii* and concluded that they are not conspecific, but distinct species.

Aside from the qualitative report of Taylor, Patterson and Harrod (1994), no reports have been published on the composition of the leaf volatile oils of *Picea chihuahuana* or *P. martinezii.* The purpose of the present paper is to give the first detailed report on the composition of the volatile oils of these species.

MATERIALS AND METHODS

Leaf samples were collected from *Picea chihuahuana* (*Adams 14329-14331*) from trees cultivated at Medford city park, Medford, OR (grown from seed collected on Rio Oteros, near Creel, Chihuahua, Mexico) and from *P. martinezii* (*Adams 14326-14328*) cultivated at Frank Callahan's land, Central Point, OR, grown from seed collected by Frank Callahan in Nuevo Leon, Mexico. 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 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 leaf oils of *P. chihuahuana* and *P. martinezii* were clear in color and the yields (w/DW) very small: 0.34% and 0.25%, respectively. The volatile leaf oil of *P. chihuahuana* is dominated by bornyl acetate (47.9%, Table 1) with moderate amounts of α-pinene (4.7), camphene (6.3), β-pinene (5.2), camphene (6.2), limonene (7.4) and β-phellandrene (7.3%). The leaf oil of *P. martinezii* is dominated by bornyl acetate (26.4%, Table 1), α-pinene (16.6) and myrcene (15.1%) with moderate amounts of camphene (5.6) and β-pinene (9.7%). For such closely related species (Patterson and Harrod, 1994), the oils are quite different (Table 1). There are several unique oil components in *P. chihuahuana*: α-phellandrene, cis- and trans-p-menth-2-en-1-ol, cis- and trans-piperitol, piperitone, cis- and trans-piperitol acetate, methyl isopimarate, methyl levopimarate and methyl abietate. Unique oil compounds in *P. martinezii* include: α-muurolene, δ-cadinene, caryophyllene oxide, humulene epoxide II, epi-α-cadinol, epi-α-muurolol, α-muurolol, α-cadinol, germacra-4(15),5,10(14)-triene-1-al and neo-abienal (Table 1).

The leaf volatile terpenoids data support the conclusions of Patterson and Harrod (1994) and Jaramillo-Correa et al. (2006) that *P. chihuahuana* and *P. martinezii* are distinct species.

It might be noted that *P. chihuahuana*, in cultivation in Medford, OR (Fig. 1), is a robust, fast growing tree that will likely be widely cultivated for its glaucous foliage, shape and hardiness.

Figure 1. *Picea chihuahuana* , seed cone (insert) in cultivation at the Medford City Park, OR with Frank Callahan.

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Table 1. Comparison of leaf oil compositions of *Picea chihuahuana* and *P. martinezii*. 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.

KI	compound	*chihuahuana*	*martinezii*
846	(E)-hexenal	0.2	0.3
921	tricyclene	0.4	0.4
932	**α-pinene**	**4.7**	**16.6**
946	camphene	6.3	5.6
969	sabinene	t	t
974	**β-pinene**	**5.2**	**9.7**
988	**myrcene**	**3.2**	**15.1**
997	ethyl hexanoate	0.2	-
1002	**α-phellandrene**	**0.7**	-
1008	δ-3-carene	t	-
1014	α-terpinene	0.1	-
1020	p-cymene	t	t
1024	**limonene**	**7.4**	**1.6**
1025	**β-phellandrene**	**7.3**	**2.4**
1032	(Z)-β-ocimene	t	-
1054	γ-terpinene	t	t
1086	terpinolene	1.2	1.2
1095	linalool	0.3	0.2
1118	**cis-p-menth-2-en-1-ol**	**0.9**	-
1122	α-campholenal	0.1	0.3
1136	**trans-p-menth-2-en-1-ol**	**0.7**	-
1136	trans-pinocarveol	-	0.1
1141	camphor	t	0.1
1145	camphene hydrate	0.5	0.2
1165	**borneol**	**1.7**	**0.5**
1174	terpinen-4-ol	0.1	0.2
1186	α-terpineol	1.1	1.1
1190	methyl salicylate	-	t
1195	**cis-piperitol**	**0.3**	-
KI	compound	*chihuahuana*	*martinezii*
1207	**trans-piperitol**	**0.6**	-

KI	compound	*chihuahuana*	*martinezii*
1218	endo-fenchyl acetate	t	-
1223	citronellol	t	-
1249	**piperitone**	**0.6**	**-**
1287	**bornyl acetate**	**47.9**	**26.4**
1298	trans-pinocarvyl acetate	0.4	0.3
1324	myrtenyl acetate	0.1	-
1332	**cis-piperitol acetate**	**0.1**	**-**
1343	**trans-piperitol acetate**	**0.2**	**-**
1350	α-longipinene	0.1	-
1379	geranyl acetate	-	0.1
1396	duvalene acetate	t	0.1
1417	**(E)-caryophyllene**	**0.3**	**2.1**
1454	(E)-β-farnesene	t	-
1469	n-dodecanol	t	-
1480	**germacrene D**	**t**	**1.1**
1500	**α-muurolene**	**-**	**0.5**
1522	**δ-cadinene**	**-**	**0.2**
1565	dodecanoic acid	t	-
1582	**caryophyllene oxide**	**-**	**0.3**
1608	**humulene epoxide II**	**-**	**0.3**
1638	**epi-α-cadinol**	**-**	**0.2**
1640	**epi-α-muurolol**	**-**	**0.2**
1644	**α-muurolol**	**-**	**0.1**
1652	**α-cadinol**	**-**	**0.8**
1685	**germacra-4(15),5,10(14)-triene-1-al**	**-**	**0.3**
1959	hexadecanoic acid	0.2	0.5
1987	iso-pimara-7,15-diene	0.3	0.2
2182	hexadecanoic acid, butyl ester	t	-
2222	abietal isomer	0.3	0.9
2298	**methyl isopimarate**	**0.2**	**-**
2300	tricosane	0.2	0.4
2306	**methyl levopimarate**	**0.4**	**-**
2313	abietal	0.5	0.6
2375	**neo-abienal?**	**-**	**0.4**
2385	**methyl abietate**	**0.1**	**-**