Comparison of the volatile leaf and wood oils of the subspecies of *Pinus torreyana*: two isolated, narrow endemics in California

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ABSTRACT

The composition of the volatile leaf oils of *Pinus torreyana* subsp. *torreyana*, Del Mar, CA and subsp. *insularis* from Santa Rosa Island are reported. The leaf oil of subsp. *torreyana* is dominated by thunbergol (30.6%), iso-cembrene (25.5%), and cembrene (20.0%) with moderate amounts of limonene (6.9%) and trans-α-bergamotene (2.4%). In contrast, the leaf oil of subsp. *insularis* is dominated by a very large amount of thunbergol (67.1%), with moderate amounts of iso-cembrene (6.8%), cembrene (6.4%), limonene (3.6%) and β-phellandrene (3.6%). Trans-α-bergamotene and (E)-β-ocimene are unique to subsp. *torreyana*, whereas; β-phellandrene, trans-calamenene, 1-epi-cubenol, epi-α-muurolol and manoyl oxide are unique to subsp. *insularis*. The wood oils (turpentines) differed in the two subsp. (Zavarin et al., 1967), but shared very few compounds with the leaf oils. In short, either leaf or wood oils are sufficient to separate the subspecies. The differences in the oil compositions between the subspecies seems to indicate either a considerable period of isolation between the populations and/or genetic drift in the very small populations of subsp. *torreyana* and *insularis*. Published on-line **www.phytologia.org** *Phytologia 95(2): 188- 191 (May 1, 2013).*

KEY WORDS: *Pinus torreyana, var. insularis,* leaf oils, wood oils, alkanes, terpenes, diterpenes, evolution.

Pinus torreyana C. Parry ex Carr. (Torrey pine) is in subsection *Sabinianae* with *P. sabiniana* Doug. and *P. coulteri* D. Don. (Haller, 1986). *Pinus sabiniana* Doug. and *P. coulteri* are very widespread, but, in contrast, *P. torreyana* has one of the most limited ranges of any *Pinus* species (Haller, 1986). It is has only two disjunct populations: in and around Del Mar, CA and 280 km northwest on Santa Rosa Island. Haller (1986) recognized the Santa Rosa elements as a new subspecies (*P. torreyana* subsp. *insularis* Haller). The new taxon differed in crown shape, needle color, cone width and shape, cone scale apex and seed width and color (Table 3, Haller, 1986). Haller noted that Zavarin et al. (1967) reported differences in the turpentine composition between plants from Del Mar and Santa Rosa Island.

The oleoresin oils (wood oils) of *Pinus* normally contain monoterpenes (turpentine) and non-volatile diterpene resin acids (rosin) (1996). However, the turpentine of *P. jeffreyi* Grev. & Balif. (Jeffrey pine) and *P. sabiniana* Dougl. (gray or digger pine) is composed of 95-99% n-heptane (with small amounts of undecane and other alkanes) and only less than a few percent monoterpenes (Mirov, 1948; Smith, 1967). Mirov (1961) reported 5% undecane in *P. torreyana* Perry and less than 0.1% heptane and 5% heptane in *P. coulteri* D. Don. Smith (1982) also reported varying amounts of heptane in the wood of *P. rudis* (0 - 32%) and *P. pseudostrobus* (0 - 47%). Smith (2000) published a massive recompilation of data from xylem monoterpenes in *Pinus*, but still reported that only the wood oils of *P. jeffreyi* and *P. sabiniana* contained high levels n-heptane (95-99%) in the monoterpene fraction.

Ekundayo (1988) reviewed the volatile constituents of *Pinus* needle oils for 33 species. He reported the compositions (from the literature) for mono- and sesquiterpenes, but not for di-terpenes. No data were presented for *Pinus torreyana*. Kurose et al. (2007) analyzed leaf oils from nine *Pinus* species, but not for *Pinus torreyana.*

Savage, Hamilton and Croteau (1996) appear to have been the first to compare the very volatile (monoterpenes and lower alkanes) of both wood and leaves of *P. jeffreyi*. They did not detect heptane in the needle oil, but did find a progressive increase from the phloem (current 0.8, basal, 33.1%), to xylem (current, 35.4, sapwood, 80.6, heartwood, 95.2%).

Recently, Adams and Wright (2012) compared the compositions of the leaf and wood oils for *P. jeffreyi* and *P. sabiniana*. They reported that in each species the leaf oil compositions were almost completely different from the wood oils.

The purpose of this study is to report on the first analyses of the leaf oils from *P. torreyana* subsp. *torreyana* and subsp. *insularis* and to compare their leaf oils with published data for wood oils.

MATERIALS AND METHODS

Plant Specimens: *Pinus torreyana* subsp. *torreyana*, *Adams 13652-13654*, Del Mar (City), CA, *P. torreyana* subsp. *insularis, Adams* 13736-13737, Univ. of California Botanic Garden, Berkeley, CA, ex. Santa Rosa Island, e end of Torrey pine grove, Acc. 78.0073. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Essential oil extraction: Fresh leaves were collected at 1.5 m above ground, on the south-facing side of each tree. Fresh leaves (200 g) were cut into 2 cm lengths to promote oil volatilization and steam distilled for 2 h using a circulatory Clevenger-type apparatus with a layer of diethyl ether as an oil trap (Adams, 1991). The oil samples were concentrated (diethyl ether removed) with nitrogen and the samples stored at -20ºC until analyzed.

GC and GC/MS analysis: 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). The oils were run at both 60-246ºC/3ºC/min and at 40 ºC, isothermal, 4 min, then 3ºC/min to 246ºC in order to resolve heptane and diethyl ether. 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 and the NIST database. 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 without FID response factors.

RESULTS

The leaf oil of subsp. *torreyana* is dominated (Table 1) by thunbergol (30.6%), iso-cembrene (25.5%), and cembrene (20.0%) with moderate amounts of limonene (6.9%), and trans-α-bergamotene (2.4%). In contrast, the leaf oil of subsp. *insularis* is dominated (Table 1) by very large amount of thunbergol (67.1%), with moderate amounts of iso-cembrene (6.8%), cembrene (6.4%), limonene (3.6%), β-phellandrene (3.6%). Trans-α-bergamotene and (E)-β-ocimene were unique to subsp. *torreyana*, whereas β-phellandrene, trans-calamenene, 1-epi-cubenol, epi-α-muurolol and manoyl oxide were unique to subsp. *insularis*.

The wood oils (turpentine) differ between the subsp. (Zavarin et al., 1967), but share very few compounds with the leaf oils (Table 1). This is very similar to the situation for the compositions of the leaf and wood oils for *P. jeffreyi* and *P. sabiniana* (Adams and Wright, 2012), where these pines also share few components in the leaf and wood oils. It appears there is a different set of genes turned on in the leaves than in the wood for a few pine species (e. g., *P. jeffreyi*, *P. sabiniana,* and *P. torreyana*), whereas nearly all *Pinus* species have very similar leaf and wood oils (Smith, 2000). It is interesting that in the Cupressaceae (particularly *Juniperus*), all of the ca. 75 species (Adams and Schwarzbach, 2013) appear to have leaf and wood oils that share few terpenes (Adams, 1991). The role of chemical defenses may be quite different between leaves and wood (Keeling and Bohlmann, 2006). This could account for the large differences between leaf and wood oil compositions.

Finally, it should be noted that data from either the leaf or wood oils are sufficient to separate subsp. *torreyana* from subsp. *insularis*. The differences in the oil compositions between the two taxa seem to indicate a considerable period of isolation between the populations, this resulting in genetic drift in the very small populations concerned.

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Table 1. Leaf and wood oil compositions (%) for *Pinus torreyana* subsp. *torreyana* from Del Mar, CA and subsp. *insularis* from Santa Rosa Island. Wood oil compositions are from Zavarin et al. (1967).

KI*	Compound	*torreyana* leaf oil	*insularis* leaf oil	*torreyana* wood oil	*insularis* wood oil
700	n-heptane	-	-	2.2	3.6
900	n-nonane	-	-	1.7	2.4
932	α-pinene	0.1	0.1	2.7	2.8
974	β-pinene	-	-	0.2	0.1
988	myrcene	0.3	0.3	2.9	1.7
1002	α-phellandrene	-	t	-	-
1020	p-cymene	-	0.2	-	-
1024	**limonene**	**6.9**	**3.6**	**84.2**	**73.4**
1025	**β-phellandrene**	**-**	**3.6**	**0.1**	**8.7**
1026	**1,8-cineole**	**-**	**-**	**0.9**	**-**
1032	(Z)-β-ocimene	t	t	-	-
1044	**(E)-β-ocimene**	**1.9**	**-**	**-**	**-**
1063	n-octanol	t	t	-	-
1086	terpinolene	t	-	-	-
1095	linalool	0.1	0.1	-	-
1100	undecane	-	-	6.1	7.4
1100	nonanal	0.1	-	-	-
1118	cis-p-menth-2-en-1-ol	-	t	-	-
1136	trans-p-menth-2-en-1-ol	-	t	-	-
1141	camphor	t	t	-	-
1148	citronellal	-	t	-	-
1174	terpinen-4-ol	t	0.2	-	-
1186	α-terpineol	t	t	-	-
1201	**decanal**	**-**	**-**	**0.4**	**-**
1223	citronellol	t	0.2	-	-
1260	2-decenal	-	t	-	-
1284	bornyl acetate	t	0.9	-	-
1298	carvacrol	-	0.1	-	-
1322	methyl geranate	-	0.3	-	-
1400	tetradecane	-	-	t	-
1407	**longifolene**	**-**	**-**	**0.4**	**2.8**
1417	(E)-caryophyllene	1.2	1.0	-	-
1432	**trans-α-bergamotene**	**2.4**	**-**	**-**	**-**
1452	α-humulene	0.2	0.1	-	-
1454	(E)-β-farnesene	0.2	0.1	-	-
1484	germacrene D	-	0.1	-	-
1491	(E)-methyl isoeugenol	0.1	-	-	-
1505	**(E,E)-α-farnesene**	**0.2**	**t**	**-**	**-**
1521	**trans-calamenene**	**-**	**0.2**	**-**	**-**
1561	E-nerolidol	1.5	0.7	-	-
1574	germacrene-D-4-ol	-	t	-	-
1627	1-epi-cubenol	-	0.2	-	-
1638	epi-α-muurolol	-	0.3	-	-
1722	(2Z,6E)-farnesol	1.5	1.8	-	-
1814	hexadecanal	1.3	0.2	-	-
1937	**cembrene**	**20.0**	**6.4**	**-**	**-**
1943[12]	**iso-cembrene**	**25.2**	**6.8**	**-**	**-**
1965	(3Z)-cembrene A	1.0	0.7	-	-
1987	**manoyl oxide**	**-**	**0.4**	**-**	**-**
2048	**thunbergol (isocembrol)**	**30.6**	**67.1**	**-**	**-**

KI* = Kovats Index (linear) on DB-5 column from Adams (2007). Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.