First comprehensive report on the composition of the leaf volatile terpenoids of *Pinus contorta* vars. *contorta, latifolia* and *murrayana*

Robert P. Adams
Biology Department, Baylor University, Waco, TX 76798 robert_adams@baylor.edu

Thomas A. Fairhall
953 U Street, Port Townsend WA 98368

Gay Hunter
Museum Curator, Olympic National Park, 600 East Park Ave., Port Angeles, WA 98362

and

Chauncey Parker
11643 Norse Ave., Truckee, CA 96161

ABSTRACT

The first comprehensive report on the compositions of the volatile leaf terpenoids of *Pinus contorta* var. *contorta,* var. *latifolia and* var. *murrayana* are presented. The volatile leaf oils of vars. *contorta* and *latifolia* were nearly identical, dominated by β-phellandrene (54.6, 45.1%), with moderate amounts of β-pinene (8.7, 10.3%), α-phellandrene (4.9, 1.7%), α-pinene (3.7, 3.4%), myrcene (1.9, 2.1%), δ-3-carene (0.9, 11.5%), α-terpinene (3.7, 1.6%) and terpinolene (3.7, 2.0%). With the exception of δ-3-carene (0.9, 11.5%), they differ only in minor compounds: camphor (0.2, trace), methyl chavicol (none, 0.4%), (E)-anethole (none, 0.5%),dodecanoic acid (0.6, trace), and sandaracopimarinal (0.3, trace). The oil of var. *murrayana* is considerably different from that of vars. *contorta* and *latifolia*. The volatile leaf oil of var. *murrayana* is dominated by β-pinene (38.1%) and β-phellandrene (18.2%) with moderate amounts of α-pinene (5.2%), myrcene (1.8%) and δ-3-carene (5.9%) and 7 unique components: citronellal, citronellol, geraniol, methyl eugenol, a C_{10} -diene acetate, elemicin, and sandaracopimarinol. The major components (as percent total oil) were very variable for all three taxa. Published on-line **www.phytologia.org** *Phytologia 97(1): 76-81 (Jan 2, 2015).* ISSN 030319430.

KEY WORDS: *Pinus contorta*, var. *contorta,* var. *latifolia,* var. *murrayana,* volatile leaf oil, terpenes, composition.

Smith (1967) made an early study on the wood resin monoterpenes of lodgepole pine (*Pinus contorta*) and reported the major components were β-phellandrene (53.1-78.8%) and δ-3-carene (5.6-28.6%). Zavarin, Critchfield and Snajberk (1969) examined the inheritance of turpentine composition in *P. contorta* x *P. banksiana* hybrids. Anderson, Riffer and Wong (1969) reported the major compound in *P. contorta* wood oil was β-phellandrene (71%).

However, the first report on the volatile leaf oils of *P. contorta* (var. *latifolia*) was by Pauly and von Rudloff (1971). They found the volatile leaf oil from a population near Banff, Alberta, was dominated by β-phellandrene, 37.3% (31.0-47.5%) and β-pinene, 24.2% (8.4-43%), with moderate amounts of α-pinene, 5.4% (2.8-8.9%), myrcene, 3.5% (2.0-10.5%), δ-3-carene, 4.8% (0.1-21.5%) and α-terpineol, 4.4% (2.1-4.4%). They noted that the variation among *P. contorta* individuals was very large (compared to other conifer oils they had examined). Von Rudloff, Lapp and McMinn (1985) studied variation in the leaf oils from young and old lodgepole pine from different moisture regimes, Prince George, BC. In addition to the typical terpene patterns previously seen in lodgepole, they found some

old trees without resin canals, that had a new terpene profile and very low oil yield (0.1%). No terpene differences were found in young trees from upland or bog sites or from dry and wet sites.

Von Rudloff and Lapp (1987) surveyed the leaf oils of P. *contorta* vars. *contorta* and *latifolia*, *murrayana* and *bolanderi* from 111 different population sites in British Columbia, Alberta and the northwestern United States. They found hybridization between vars. *contorta* and *latifolia* in western BC and on Vancouver Island, BC. The oils from populations of putative *P. c*. var. *murrayana* from northern California and Oregon were found to be similar to that of var. *contorta*; although in this area, the two varieties seem to intergrade.

At the same time, Croteau, et al. (1987) began research on the biochemistry of oleoresins with an emphasis on the bark beetle induction of the synthesis of defense chemicals (terpenes). This has led to numerous papers on the effects of bark beetles on resin production (see Ott et al., 2011; Wallis, et al. 2011). Recently, the search for the gene sequence of terpene synthase (TPS) genes has exploded by the work of Bohlmann's lab (see review by Keeling and Bohlmann, 2006, and Foster et al. 2013).

Because the early work by von Rudloff's lab did not report comprehensive oil analyses, it seems an appropriate time to re-analyze the leaf volatile oils of *Pinus contorta* var. *contorta,* var. *latifolia and* var. *murrayana* using modern GC/MS/computer technology (Adams, 2007).

MATERIALS AND METHODS

Leaf samples were collected from *Pinus contorta* var. *contorta*: Fort Worden State Park, Port Townsend, WA, on sand. 48° 08' 27" N; 122° 45' 38" W. elev. 25 ft, 20 Nov 2104, Jefferson Co., WA, Coll. *Tom Fairhall 1-5,* Lab Acc. *Robert P. Adams, 14477-14481*.
Pinus contorta var. *latifolia*: Deer Ridge, Olympic Mountains, 47° 56' 45.5" N; 123° 12' 58.4" W. elev. 4291 ft, 20 Nov 2104, Jefferson Co., WA, Coll. *Tom Fairhall & Gay Hunter 1,* Lab Acc. *Robert P. Adams 14482*; Deer Trail Ridge, Olympic Mountains, with *J. jackii*, 47° 56' 43.2" N; 123° 13' 53.5" W. elev. 4885 ft, 20 Nov 2104, Jefferson, Co., WA, Coll. *Tom Fairhall & Gay Hunter 2-5*, Lab Acc. *Robert P. Adams 14483-14486*.
Pinus contorta var. *murrayana*: abundant, south side of Donner Pass Road (Lincoln Hwy), 0.25 air mi. east of Donner Pass, 39° 19' 03.24" N; 120° 19' 17.64" W. elev. 6863 ft, 28 Sep 2014, Placer Co., CA, Coll. *Chauncey Parker BA 1-10*, Lab Acc. *Robert P. Adams 14450-14459*.
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 composition of the leaf oils are given in table 1. The volatile leaf oils of vars. *contorta* and *latifolia* were nearly identical, dominated by β-phellandrene (54.6, 45.1%), with moderate amounts of β-pinene (8.7, 10.3%), α-phellandrene (4.9, 1.7%), α-pinene (3.7, 3.4%), myrcene (1.9, 2.1%), δ-3-carene (0.9, 11.5%), α-terpinene (3.7, 1.6%) and terpinolene (3.7, 2.0%). With the exception of δ-3-carene (0.9, 11.5%), they differ only in minor compounds: camphor (0.2, trace), methyl chavicol (none, 0.4%), (E)-anethole (none, 0.5%), dodecanoic acid (0.6, trace), and sandaracopimarinal (0.3, trace). The oil of var. *murrayana* is considerably different from that of vars. *contorta* and *latifolia*. The volatile leaf oil of var. *murrayana* is dominated by β-pinene (38.1%) and β-phellandrene (18.2%) with moderate amounts of α-pinene(5.2%), myrcene(1.8%) and δ-3-carene(5.9%) and 7 unique components: citronellal, citronellol, geraniol, methyl eugenol, a C_{10} -diene acetate, elemicin, and sandaracopimarinol.

The major components (as percent total oil) were very variable for all three taxa. (Table 1). For vars. *contorta*, *latifolia,* and *murrayana* the ranges were: β-pinene (1.4-21.3%), (0.7-26.5%) and (27.1-46.2%), δ-3-carene (0.4-1.2%), 6.1-19.4%) and (2.8-11.0%), and β-phellandrene (39.2-61.5%), 32.5%-58.5%) and (15.9-21.9%). This agrees with the observations of Pauly and von Rudloff (1971) on *P. contorta* leaf oils.

In contrast to von Rudloff and Lapp (1987) who found the oils from populations of putative *P. c*. var. *murrayana* from northern California and Oregon to be similar to that of var. *contorta*, we found our oil from *P. c*. var. *murrayana* to be quite different from *P. c*. vars. *contorta* and *latifolia*. This may be because our samples came from Donner Pass, CA (west of Reno, NV). A site that is far to the south of the von Rudloff and Lapp (1987) samples in northern California and Oregon. Their var. *murrayana* samples may be the product of hybridization with vars. *contorta* and *latifolia*. Our var. *murrayana* population is quite disjunct from vars. *contorta* and *latifolia* with no opportunity for hybridization and appear to be typical of the variety.

Von Rudloff, Lapp and McMinn (1985) reported finding some old *P. contorta* trees with very low oil yields (0.1%). Their leaves did not contain resin canals. In this study, we found one *P. contorta* var. *contorta* tree (*14479*) with an oil yield of 0.1% and it does not appear to have resin canals. We also found one *P. c*. var. *latifolia* (*14485*) with an unusually high yield (3.45%). The 2 hr. steam distillation is estimated to remove about 30-35% of the oil. Thus, this tree is storing about 10% (dry wt) of oil in its leaves. This is about 5 times the amount of any other tree sampled and 30 times the oil in tree *14479*. .It appears *Pinus contorta* appears to be a good species for the study of TPS genes controlling the synthesis of essential oil production.

ACKNOWLEDGEMENTS

Thanks to Ernst von Rudloff for mentoring RPA in his lab on a sabbatical (1976) in Saskatoon, Saskatachewan. I have tried to follow his example of meticulous research. This research supported with funds from Baylor University.

LITERATURE CITED

Adams, R. P. 1991. Cedarwood oil - Analysis and properties. pp. 159-173. in: Modern Methods of Plant Analysis, New Series: Oil and Waxes. H.-F. Linskens and J. F. Jackson, eds. Springler- Verlag, Berlin.

Adams, R. P. 2007. Identification of essential oil components by gas chromatography/ mass spectrometry. 4th ed. Allured Publ., Carol Stream, IL.

Croteau, R., S. Gurkewitz, M. A. Johnson and H. J. Fisk. 1987. Biochemistry of oleoresinosis. Monoterpene and diterpene biosynthesis in lodgepole pine saplings infected with *Ceratocystis clavigera* or treated with carbohydrate elicitors. Plant Physiology 85: 1123-1128.

Foster, A. J., D. E. Hall, L. Mortimer, S. Abercromby, R. Gries, G. Gries, J. Bohlmann, J. Russell and J. Mattsson. 2013. Identification of genes in *Thuja plicata* foliar terpenoid defenses. Plant Physiol. 161: 1993-2004.

Keeling, C. I. and J. Bohlmann. 2006. Tanksley Review: Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. New Phytologist 170: 657-675.

Ott, D. S., A. D. Yanchuk, D. P. W. Huber and K. F. Wallin. 2011. Genetic variation of Lodgepole pine, *Pinus contorta* var. *latifolia*, chemical and physical defenses that affect mountain pine beetle, *Dendroctonus ponderosae*, Attack and tree mortality. J. Chem. Ecol. 37: 1002-1012.

Pauly, G. and E. von Rudloff. 1971. Chemosystematic studies in the genus Pinus. Leaf oil of *Pinus contorta* var. *latifolia*. Can. J. Bot. 49: 1201-1210.

Schorger, A. W. 1919. Contribution to the chemistry of American conifers. Wisc. Acad. Sci., Arts. and Letters. 19: 728-766.

Smith, R. H. 1971. Variations in the monoterpene composition of the wood resin of Jeffery, Washoe, Coulter, and lodgepole pines. Forest Sci. 13:246-252.

von Rudloff, E. 1975. volatile leaf oil analysis in chemosystematic studies of North American conifers. Biochem. Syst. Ecol. 2: 131-167.

von Rudloff, E. and M. S. Lapp. 1992. Chemosystematic studies in the genus *Pinus*. VII. The leaf oil terpene composition of ponderosa pine, *Pinus ponderosa*. Can. J. Bot. 70: 374-378.

von Rudloff, E., M. S. Lapp and R. G. McMinn. 1985. Chemosystematic studies in the genus Pinus. V. Variation in the leaf oil terpene composition of young and old lodgepole pine trees from different moisture regimes near Prince George, British Columbia. Can. J. Bot. 15: 801-808.

von Rudloff, E. and M. S. Lapp. 1987. Chemosystematic studies in the genus Pinus. VI. General survey of the leaf oil terpene composition of lodgepole pine. Can. J. For. Res. 17: 1013-1025.

Wallis, C. M., D. P. W. Huber and K. J. Lewis. 2011. Ecosystem, location and climate effects on foliar secondary metabolites of lodgepole pine populations from central British Columbia. J. Chem. Ecol. 37: 607-621.

Zavarin, E., W. B. Critchfield and K. Snajberk. 1969. Turpentine composition of *Pinus contorta* x *Pinus banksiana* hybrids and hybrid derivatives. Can. J. Bot. 47: 1443-1453.

Table 2. Comparison of leaf oil compositions of *Pinus contorta* var. *contorta,* var. *latifolia and* var. *murrayana.* 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	*contorta*	*latifolia*	murrayana
846	(E)-hexenal	0.4	0.1	t
850	(3Z)-hexenol	1.3	0.3	0.2
921	tricyclene	0.1	0.1	t
924	α-thujene	0.2	0.2	0.1
932	α-pinene	3.7(2.4-4.3)	3.4(1.9-5.3)	5.2(2.8-6.6)
946	camphene	1.2(0.7-2.7)	0.7(0.4-1.3)	1.2(0.7-2.2)
969	sabinene	0.9	0.7	0.1
974	**β-pinene**	**8.7(1.4-21.3**	**10.3(0.7-26.5)**	**38.1(27.1-46.2)**
988	myrcene	1.9(1.5-2.1)	2.1(1.8-2.40	1.8(1.3-2.4)
1002	**α-phellandrene**	**4.9(1.4-4.0)**	**1.7(1.5-4.6)**	**0.7(0.2-0.9)**
1008	**δ-3-carene**	**0.9(0.4-1.2)**	**11.5(6.1-19.4)**	**5.9(2.8-11.0)**
1014	α-terpinene	3.7(2.1-8.9)	1.6(1.1-6.9)	0.7(0.5-2.7)
1020	p-cymene	t	t	0.1
1025	**β-phellandrene**	**54.6(39.2-61.50**	**45.1(32.6-58.5)**	**18.2(15.9-21.9)**
1032	(Z)-β-ocimene	0.8	2.4	2.7
1044	(E)-β-ocimene	t	0.2	0.1
1054	γ-terpinene	0.8(0.6-1.7)	0.5(0.5-2.7)	0.3(0.3-1.4)
1065	cis-sabinene hydrate	t	t	t
1086	terpinolene	3.7(3.4-5.5)	2.0(1.1-3.8)	1.6(0.6-3.8)
1095	linalool	0.1	0.2	0.4
1100	n-nonanal	t	t	t
1118	cis-p-menth-2-en-1-ol	0.1	0.1	0.1
1122	α-campholenal	t	t	t
1135	nopinone	t	t	t
1136	trans-p-menth-2-en-1-ol	-	0.1	0.1
1141	camphor	0.2	t	t
1145	camphene hydrate	t	t	t
1148	**citronellal**	**-**	**-**	**0.1**
1158	trans-pinocamphone	t	t	-
1160	pinocarvone	t	0.1	-
1165	borneol	t	0.1	0.3
1174	terpinen-4-ol	0.3	0.2	0.2
1179	p-cymen-8-ol	0.3	0.1	t
1186	α-terpineol	0.2	0.3	1.6
1195	**methyl chavicol**	**-**	**0.4**	**0.3**
1195	myrtanal	t	-	-
1195	myrtanol	t	-	-
1207	trans-piperitol	t	t	-
1223	citronellol	-	-	t
1232	thymol, methyl ether	-	t	-
1238	cumin aldehyde	t	-	-
1249	geraniol	-	-	t
1249	piperitone	t	t	-
1282	**(E)-anethole**	**-**	**0.5**	**0.2**
1284	**bornyl acetate**	**0.6(0.1-2.0)**	**0.2(0.1-0.4)**	**t**
1289	thymol	t	-	-
1293	2-undecanone	t	0.1	0.2
1315	(2E,4E)-decadienal	t	-	-
1335	δ-elemene	t	t	-
1345	α-cubebene	t	t	-
1350	citronellyl acetate	t	t	-
1374	α-copaene	-	t	t
1379	**geranyl acetate**	**t**	**t**	**0.5**
1389	β-elemene	t	t	t
1396	duvalene acetate	t	-	-
1400	cis-sibirene	t	t	-
1403	methyl eugenol	-	-	t
1417	(E)-caryophyllene	t	t	0.2
1439	aromadendrene	t	t	-

KI	compound	*contorta*	*latifolia*	murrayana
1442	**6,9-guaiadiene**	**1.7(0.4-4.0)**	**0.6(0.2-0.9)**	**-**
1449	trans-sibirene	t	t	-
1454	(E)-β-farnesene	-	t	-
1467	**C10-diene, acetate, 43,54,67,196**	**-**	**-**	**0.5(0.3-1.5)**
1475	trans-cadina-1(6),4-diene	-	t	-
1478	γ-muurolene	t	0.2	0.2
1480	germacrene D	0.2	0.4	0.6
1489	β-selinene	0.1	0.1	t
1493	epi-cubebol	-	0.3	-
1500	bicyclogermacrene	0.8	0.6	0.8
1500	α-muurolene	t	0.4	0.3
1513	γ-cadinene	0.5	1.4	1.0
1522	δ-cadinene	0.8(0.4-2.0)	2.3(2.2-4.4)	2.1(0.4-3.4)
1537	α-cadinene	t	0.1	0.1
1555	elemicin	-	-	0.2
1561	(E)-nerolidol	-	-	0.1
1565	**dodecanoic acid**	**0.6**	**t**	**-**
1574	**germacrene-D-4-ol**	**0.3**	**1.5**	**2.4(0.5-4.0)**
1577	**spathulenol**	**-**	**-**	**0.2**
1590	globulol	t	t	-
1627	1-epi-cubenol	-	0.1	-
1638	epi-α-cadinol	0.1	0.4	0.5
1640	epi-α-muurolol	0.1	0.4	0.5
1644	α-muurolol	t	0.1	0.2
1652	**α-cadinol**	**0.1**	**0.8**	**1.4**
1671	tetradecanol	t	-	-
1722	(2Z,6E)-farnesol	0.2	0.2	0.4
1758	tetradecanoic acid	0.1	-	-
1814	hexadecanol	t	-	-
1944	pimara-8(14),15-diene	t	-	0.2
1987	iso-pimara-7,15-diene	t	t	0.2
2056	manool	0.1	0.5(0.1-1.8)	0.6(0.1, 1.6)
2087	abietadiene	t	t	-
2149	abienol	t	t	-
2184	sandaracopimarinal	0.3	t	1.0
2221	diterpene, 51,187,257,286	0.4	0.2	0.8
2237	diterpene, *43*,91,*133*,286	0.3	0.4	0.6
2269	**sandaracopimarinol**	**-**	**-**	**0.2**
2274	dehydro abietal	t	t	-
2313	abietal	t	t	-