CHEMOSYTEMATICS OF DOUGLAS FIR *(PSEUDOTSUGA MENZIESII)*: EFFECTS OF LEAF DRYING ON ESSENTIAL OIL COMPOSITION

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ABSTRACT

A comparison of the essential oil from fresh and air-dried leaves of Douglas fir (*Pseudotsuga menziesi*i) revealed relatively minor changes in the oil composition (on a percent total oil basis), except for terpinen-4-ol, which declined from 12.2% to 7.8 and 8.6% when dried at 21ºC and 42ºC (Table 1). Citronellyl and geranyl acetates appear to increase with drying and storage for the first week, then are stable. It seems that careful air drying of Douglas fir leaves can result in the conservation of the terpenoid profile in the composition. This appears to be a solution to the problem of the transport of fresh materials across international borders. *Phytologia 94(1):133-138 (April 2, 2012).*

KEY WORDS: *Pseudotsuga menziesii*, Douglas fir, oils from dried leaves, chemosystematics.

Recently, Adams (2010, 2011) reported the effects of air-drying leaves on the essential oil composition of *Juniperus pinchotii* Sudw. and *J. virginiana* L. He found that gently air-drying (21-42ºC) had generally small effects on the oil compositions in these *Juniperus* species, similar to the results reported for *J. thurifera* (Achak et al., 2008, 2009).

Our lab is currently involved in a study of Douglas fir (*Pseudotsuga menziesii*) from throughout its range. The transport of fresh materials from Mexico to our lab has presented considerable difficulties with government customs agents. However, herbarium vouchers are generally (in the author's experience) permitted without too much difficulty. Part of the ease of importing herbarium specimens is because specimens are frozen to kill insects, then air dried. In order to facilitate this project, a small study was undertaken to evaluate the effects of leaf-drying on the essential oils from leaves of Douglas fir.

The purpose of this study was to determine if the changes in oil composition upon air-drying the leaves of Douglas fir would preclude their use in chemosystematics.

MATERIALS AND METHODS

Plant material - *Pseudotsuga menziesii*, *Adams 12918*, Olympic National Forest, Port Angeles, WA, 48º 02' 48.1" N, 123º 25' 04.08" W, 525 m. Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Fresh (200 g.) and air dried (100 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20º C until analyzed. The extracted leaves were oven-dried (48h, 100º 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

Table 1 shows the composition of the leaf oil of *Pseudotsuga menziesii,* and a comparison of components, over the 5 month storage period. The compounds are remarkably stable during the drying and storage tests. The major components (α-pinene, sabinene, β-pinene, γ-terpinene, terpinolene) show small changes, except for terpinen-4-ol, which declined from 12.2% to 7.8 and 8.6% when dried at 21ºC and 42ºC, then increased after one and 2 months (Table 1). Citronellyl and geranyl acetates appear to increase with drying and storage for the first week, then they are stable.

Comparing the storage tests oils, with var. *menziesii* (var. *menz*, Table 1) and var. *glauca* (var. glauc, Table 1) shows that oils from both fresh and dried leaves of coastal Douglas fir (var. *menziesii*, Olympic Natl. Forest) are clearly in the published ranges of von Rudloff (1973).

Three kinds of variation seem apparent (Fig. 1): α-pinene, β-pinene and sabinene; γ-terpinene and terpinolene; and terpinen-4-ol. The decline of terpinen-4-ol in leaves air-dried (24 h, at 21ºC and 42ºC), and similar decline in the 5 month sample (Table 1), is not understood and deserves additional study.

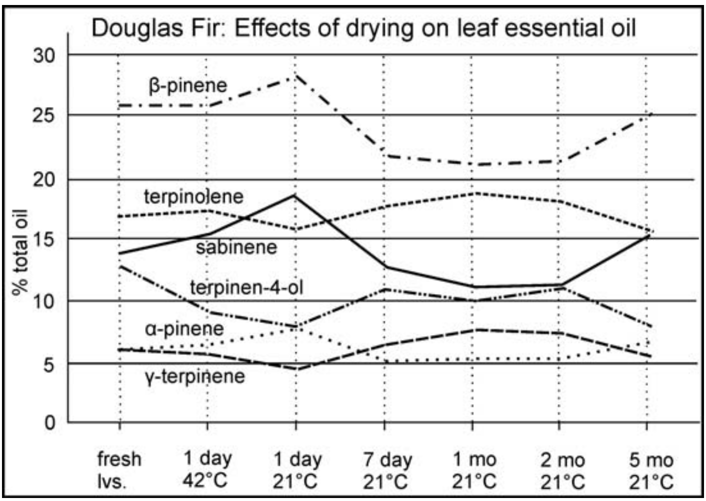

Figure 1. Variation in the major terpenoids of Douglas fir with leaf drying.

In conclusion, it appears that careful air-drying of Douglas fir leaves can result in the conservation of the terpenoid profile in the composition. This appears to be a solution to the problem of transport of fresh materials across international borders.

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Table 1. Comparison of leaf oil compositions for *Pseudotsuga menziesii* var. *menziesii* (Olympic National Forest, WA): fresh leaves, air dried 42ºC then distilled, air dried at 21ºC, then stored at 21ºC for 1 day, 1 month, 2 months and 5 months. var. *menz.* = coastal type (var. *menziesii*), von Rudloff (1973), var. *glauc* = Rocky Mtn. type (var. *glauca*), von Rudloff (1973).

KI	compound	fresh lvs.	21ºC 24h	42ºC 24h	21ºC 1 mo	21ºC 2 mo	21ºC 5 mo	var. *menz.*	var. *glauc*
884	santene	-	-	-	-	-	-	-	3-5
921	tricyclene	t	t	t	t	t	t		2.4-4
924	α-thujene	0.4	0.6	0.7	0.6	0.6	0.8	-	
932	α-pinene	6.2	7.6	6.5	4.8	4.8	6.4	7-15	15-20
946	camphene	0.4	0.4	0.4	0.3	0.3	0.4	0-0.2	20-30
969	sabinene	14.0	18.6	15.4	11.2	11.2	15.5	2-15	0.1-0.5
974	β-pinene	25.8	28.3	25.9	21.2	21.3	25.2	25-30	5-10
988	myrcene	1.3	1.5	1.5	1.2	1.2	1.3		
1002	α-phellandrene	0.2	0.2	0.3	0.5	0.4	0.3		
1008	δ-3-carene	0.5	0.7	0.8	0.5	0.4	0.3		
1012	1,4-cineole	t	t	t	t	t	t		
1014	α-terpinene	3.4	2.7	3.3	4.3	4.2	3.5	2-5	0-0.3
1020	p-cymene	0.1	0.1	0.2	0.3	0.2	0.4		
1024	limonene	0.8	0.8	1.0	1.0	1.0	0.9	0.5-1.5	5-10
1025	β-phellandrene	1.7	1.8	2.0	2.0	2.0	1.9		
1054	γ-terpinene	5.8	4.5	5.6	7.4	7.2	5.6	3-8	0.1-1
1065	cis-sabinene hydrate	0.4	0.3	0.4	0.4	0.4	0.4		
1086	terpinolene	16.8	15.8	17.3	18.5	18.0	15.6	5-20	0.5-3
1098	trans-sabinene hydrate	0.3	0.2	0.4	0.2	0.2	0.3		
1098	linalool	0.2	0.1	0.1	0.3	0.3	0.2		
1118	endo-fenchol	t	t	t	t	t	t		
1118	cis-p-menth-2-en-1-ol	0.7	0.5	0.6	0.6	0.7	0.6		
1130	1-terpineol	t	0.1	0.1	0.1	0.2	0.1		
1136	trans-p-menth-2-en-1-ol	0.5	0.4	0.4	0.6	0.7	0.5		
1145	camphene hydrate	t	t	t	t	t	0.1		
1148	citronellal	0.6	0.5	0.3	0.7	0.8	0.5		
1165	borneol	t	t	t	t	t	t		
1174	terpinen-4-ol	12.2	7.8	8.6	10.2	11.4	8.2	5-15	0.5-3
1186	α-terpineol	1.9	0.9	1.0	1.1	1.3	0.3	1-3	0.2-1
1195	cis-piperitol	t	t	t	0.2	0.1	t		
1207	trans-piperitol	0.2	0.1	0.2	0.2	0.2	0.2		
1223	citronellol	0.5	0.4	0.3	0.8	0.5	0.8	1-5	0.1-1
1287	bornyl acetate	0.1	0.1	0.1	0.2	0.2	0.1	0-0.3	20-30
1350	citronellyl acetate	1.9	1.9	2.9	2.5	2.6	2.6	2-4	0.1-2
1379	geranyl acetate	1.9	1.7	2.3	2.4	2.7	2.1	1-3	0.1-1

KI	compound	fresh lvs.	21ºC 24h	42ºC 24h	21ºC 1 mo	21ºC 2 mo	21ºC 5 mo	var. *menz.*	var. *glauc*
1452	α-humulene	0.1	0.1	0.1	0.3	0.3	0.2		
1483	α-amorphene	t	t	t	t	t	t		
1483	germacrene D	t	t	t	t	t	t		
1638	epi-α-cadinol	t	t	t	t	t	t		
1638	epi-α-muurolol	t	t	t	t	t	t		
1652	α-cadinol	0.1	0.1	t	0.1	0.1	0.2		
2300	tricosane (C23)	0.1	0.1	0.1	t	t	0.1		