Comparison the leaf essential oils of the cultivated fastigiate (strict) growth forms of *Cupressus sempervirens* in California and Turkey

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ABSTRACT

The volatile leaf oils of *Cupressus sempervirens* fastigiate (strict) cultivars from California, USA, and Turkey were analyzed and compared. The volatile leaf oils of cultivated fastigiate growth forms are dominated by α-pinene (27.4 - 65.7%), δ-3-carene (0.2 - 30.1%) and manoyl oxide (0.1 - 9.1%), with moderate amounts of myrcene (2.1 - 2.6%), terpinolene (1.4 - 4.7%), α-terpinyl acetate (1.1 - 4.7%), iso-pimara-7,15-diene (0.3, 1.2%), isoabienol (0.9, 6.0%), and trans-totarol (0.8 - 5.5%). Cedrol was found to be very variable, ranging from none to 7.6%. Cultivars 'Glauca', 'Stricta', 'Tiny Tower', and 'Totem' were each distinct in one or a few components of their oils, but otherwise nearly identical qualitatively and quantitatively. The slight variation in oils (in both California and Turkey cultivars) indicative that there have been multiple selections for the strict or fastigiate habit throughout history. No general suite of compounds distinguished California and Turkey strict cultivars. In spite of the slight differences in the leaf volatile oils, three commercial California cultivars ('Glauca', 'Tiny Tower', and 'Totem') are quite different in their foliage. Published on-line www.phytologia.org *Phytologia 99(2): 89-94 (May 9, 2017).* ISSN 030319430.

KEY WORDS: *Cupressus sempervirens,* 'Glauca', 'Stricta', 'Tiny Tower', 'Totem', terpenoids.

C*upressus sempervirens* L.; referred to as Mediterranean, common, Italian, cemetery, graveyard, or Tuscan cypress; ranges naturally from the eastern Mediterranean, Crete, Cyprus, eastern Aegean Islands, Iran, Israel, Jordan, Lebanon, Syria, Turkey, and possibly Libya (Sękiewicz et al. 2016). Despite having fastigiate (strict) and horizontal forms in the Old World that have been variously taxonomically treated, Farjon (2010) concluded that the widely cultivated fastigiate forms or "cultigens" common throughout the Mediterranean are not a taxonomic variety. The species has been widely cultivated within and outside its range throughout the warm temperate world (More and White 2002). The fastigiate or 'Stricta' form or cultivar is widely planted throughout the world.

The volatile leaf essential oils of *Cupressus sempervirens* have been analyzed based mostly on locally cultivated fastigiate trees. The report by Ulukanli et al. (2014) is typical reporting the major components being: α-pinene (35.6%), trans-pinocarveol (5.22%), α-phellandrene-8-ol (4.56%), β-pinene (3.1%), limonene (2.8%), borneol 2.3%) and camphene (2.2%). Chanegriha, et al. (1977) reported on the leaf oils of *C. sempervirens* from Algeria, likely from a cultivated plant, as having α-pinene (44.9%), δ-3-carene (10.6%), limonene (4.5%), terpinolene (2.7%), terpin-4-ol (1.9%), α-terpinyl acetate (12.0%) and manoyl acetate (1.5%). Floreani et al. (1981) reported the essential oil of cv. 'Stricta' (Argentina) contained α-pinene (50.1%), camphene (1.4%), β-pinene (4.1%), δ-3-carene (30.5%), limonene (3.5%), terpinolene (1.3%) and α-terpineol (1.6%). Other reports are by Adams et al. (1997), Amri et al. (2013), Pauly et al. (1983), Floreani et al. (1982) and Gamero et al. (1978)

Recently, Adams et al. (2017) reported on the leaf essential oils from *Cupressus sempervirens* from both fastigiate and horizontal forms. They found the oils did not differ sufficiently between the two forms to justify the recognition of varieties.

The purpose of the present paper is to report on variation the volatile leaf oils of additional commercial cultivars of the fastigiate forms from California, USA, and compare their oils with those of cultivated trees of the fastigiate forms from Montenegro and Turkey.

MATERIALS AND METHODS

Plant materials:
See Adams et al. (2017) for collection information concerning samples of cv. 'Stricta' from Turkey (*Adams 14597, 14647, 14648, 14675*).
cultivated, Carlsbad, CA, approx. 33° 06' 56.6" N, 117° 18' 39.3" W, 151ft, 17 July 2015, San Diego Co.:
Coll. *Jim A. Bartel 1631,* cv. 'Stricta', Lab Acc. *Robert P. Adams 14591,* oil type I, planted ca. 1985,
Coll. *Jim A. Bartel 1632,* cv. 'Glauca', Lab Acc. *Robert P. Adams 14592,* oil type II, planted ca. 2005,
Coll. *Jim A. Bartel 1633,* cv. 'Stricta' or 'Glauca', Lab Acc. *Robert P. Adams 14593,* oil type I, planted ca. 2000,
Coll. *Jim A. Bartel 1634,* cv. 'Glauca', Lab Acc. *Robert P. Adams 14594,* oil type II, planted ca. 1980,
Coll. *Jim A. Bartel 1635,* cv. 'Glauca', Lab Acc. *Robert P. Adams 14595,* oil type I, planted ca. 2010,
C. sempervirens cv. 'Tiny Tower,' cult. Carlsbad, CA Dec 8, 2016, Coll. *Jim A. Bartel 1637,* Lab Acc. *Robert P. Adams 15057*, planted ca. 2013.
C. sempervirens cv. 'Glauca', cult. Carlsbad, CA, Dec 8, 2016, Coll. *Jim A. Bartel 1638,* Lab Acc. *Robert P. Adams 15058,* planted ca. 2010.
C. sempervirens cv. 'Totem', purchased in 1 gal. container, Green Thumb Super Garden Center, Carlsbad, CA, Dec 8, 2016, Lab Acc. *Robert P. Adams 15059*
All specimens are deposited in the BAYLU herbarium.

Isolation of Oils - Fresh 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.

Chemical Analyses - 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 5 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 volatile leaf oils of cv. 'Stricta' are dominated by α-pinene (27.4 - 65.7%), δ-3-carene (0.2 - 30.1%) and manoyl oxide (0.1 - 9.1%), with moderate amounts of myrcene (2.1 - 2.6%), terpinolene (1.4

- 4.7%), α-terpinyl acetate (1.1 - 4.7%), iso-pimara-7,15-diene (0.3, 1.2%), isoabienol (0.9, 6.0%), and trans-totarol (0.8 - 5.5%) (Table 1). Cedrol was found to be most variable ranging from none to 7.6%.

The oils compositions of samples of *C. sempervirens* cv. 'Stricta' or 'Glauca' growing near San Diego, CA, USA proved to very uniform, but two oil types (Type I, II), are seen (Table 1) by their differences in: linalool (0.4%, trace); germacrene D (0.6, 1.6%) and abienol (2.3, 0.7%). Otherwise, the oils are nearly identical. As a group, Types I and II are distinguished from the other California cultivars by their higher amounts of manoyl oxide (7.0 - 9.1% vs. 0.1 - 3.3% in 'Totem', 'Glauca', and 'Tiny Tower,' Table 1).

The oil of cv. 'Totem' is distinguished (Table 1) by high amounts of α-thujene (0.5%), sabinene (5.4%), karahanaenone (0.3%), unknown terpene alcohol (KI1264, 0.2%) and isoabienol (6.0%). In addition, cv. 'Totem' has low amounts of iso-pimara-8(14), 15-diene (0.1%), manoyl oxide (0.1%), and iso-pimara-7,15-diene (0.1%).

The oil of cv. 'Glauca' has no very distinguishing components, except karahanaenone (0.3%) (which it has in common with cv. 'Totem' (Table 1).

The oil of cv. 'Tiny Tower' is distinct by its linalool (2.5%) and camphor (0.3%, Table 1).

Nothing in the leaf oils of the cultivars from Turkey separate them from cultivars in California (Table 1). However, the 'Stricta' Turkey *14597* sample was unusual in having a high concentration of α-pinene (65.7%), and very low concentrations of δ-3-carene (0.2%), linalool (trace), α-cedrene (none), β-cedrene (0.1%), cedrol (trace) and abietadiene (trace).

Jacobson (1996) noted that the introduction of the fastigiate Italian cypress into North America is unknown, but George Washington planted one at Mt. Vernon in 1786. It seems very probable that Italian cypress was introduced into Mexico by the Spaniards much earlier, as it is universally planted at churches and cemeteries in Mexico. Jacobson (1996) lists the introductions of known cultivars as:
cv. 'Glauca Stricta' ≤ 1934; cv. 'Stricta' date uncertain; cv. 'Swane's Golden' ≤1977-78 by Swane Bros. Nursery, Australia; cv. 'Totem' ≤1992, ex Duncan & Davies nursery, NZ; cv. 'Variegata' ≤1930s likely from England ca. 1848.

The commonly cultivated Italian cypress around San Diego, CA appears to be cv. 'Glauca Stricta.' In spite of the slight differences in the leaf volatile oils, the three commercial cultivars ('Tiny Tower,' 'Glauca' and 'Totem') are quite different in their foliage (Fig. 1).

Figure 1. Fresh foliage of cultivars 'Tiny Tower,' 'Glauca' and 'Totem'. Note the variation in color and leaf shapes.

ACKNOWLEDGEMENTS

Thanks to Amy TeBeest for lab assistance. This research was supported in part with funds from Baylor University, project BU0324512.

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Table 1. Leaf essential oil compositions for *Cupressus sempervirens* taxa. Compounds in bold show large differences between samples. The oil of Type 1 (14591) was the same as for samples 14593, 14595. The oil of Type 2 (14592) was the same as for sample 14594.

		strict cultivars from California, USA					strict cultivars from Turkey			
KI	compound	Stricta Type 1 14591	Stricta Type 2 14592	cv. Totem 14059	cv. Glauca 15058	cv. Tiny Tower 15057	Stricta Istan. 14674	Stricta Turk. 14647	Stricta Turk. 14648	Stricta Turk. 14597
921	tricyclene	0.1	0.1	0.1	0.1	0.1	0.1	t	0.1	0.1
924	α-thujene	0.1	t	0.5 H	0.1	0.1	0.5 H	0.1	t	t
932	α-pinene	41.2	38.2	27.8	32.5	39.6	34.4	28.5	35.2	65.7 H
945	α-fenchene	0.6	0.5	1.0	0.6	0.7	0.6	0.8	0.9	0.1
946	camphene	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3
969	sabinene	0.7	0.7	5.4 H	0.6	1.0	3.4 H	0.4	0.4	1.0
974	β-pinene	1.1	1.1	1.2	1.0	1.1	1.1	1.1	0.9	1.9
988	myrcene	2.2	2.1	2.8	2.2	2.3	2.4	2.3	2.3	2.6
1002	α-phellandrene	t	t	t	t	t	t	t	t	t
1008	δ-3-carene	17.1	15.8	24.7	18.3	16.5	17.3	30.1	25.7	0.2 L
1014	α-terpinene	0.2	0.1	0.3	0.2	0.2	0.2	0.1	0.1	t
1020	p-cymene	t	t	t	t	t	0.1	0.1	t	t
1023	sylvestrene	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.3	t
1024	limonene	1.8	2.3	1.4	2.2	2.1	1.0	1.0	0.8	1.4
1025	β-phellandrene	1.3	1.4	1.0	1.5	1.5	0.9	1.0	0.7	1.3
1044	(E)-β-ocimene	0.1	0.2	t	0.1	t	0.1	0.1	0.1	0.1
1054	γ-terpinene	0.3	0.3	0.7	0.3	0.4	0.4	0.2	0.3	0.2
1067	linalool oxide	t	t	0.2	0.1	0.1	t	t	t	t
1086	terpinolene	3.9	4.2	4.7	3.6	3.2	3.2	3.2	4.4	1.4
1099	linalool	0.4	t	0.6	0.5	2.5 H	0.3	0.6	t	t
1118	cis-p-mentha-2-en-1-ol	t	t	0.1	t	0.1	t	0.1	0.1	t
1123	α-camphenal	t	t	-	t	0.1	t	0.1	t	t
1136	trans-p-mentha-2-en-1-ol	t	t	0.1	t	0.1	t	t	t	t
1141	camphor	-	t	-	-	0.3 H	t	t	t	t
1154	karahanaenone	-	-	0.3 H	0.3 H	-	t	t	t	t
1067	umbellulone	-	-	0.5 H	-	-	t	t	t	t
1174	terpinen-4-ol	0.9	0.6	1.8 H	1.0	1.0	0.6	0.6	0.3	0.2
1179	p-cymen-8-ol	t	t	t	t	t	t	0.1	t	t
1186	α-terpineol	0.2	0.2	0.1	0.1	0.1	t	0.2	0.1	t
1204	verbenone	-	-	t	t	t	t	0.2	0.1	t
1241	carvacrol, methyl ether	t	0.1	0.1	0.1	0.1	0.1	0.2	1.0	t
1264	terpene alcohol, FW152	t	-	0.2 H	t	t	t	t	t	t
1287	bornyl acetate	0.2	0.1	0.2	0.2	0.2	0.4	0.2	0.1	t
1295	terpinyl acetate, FW196	-	-	0.2	0.2	0.2	t	t	t	t
1315	<2E,4E->decadienal	0.1	t	0.1	0.2	0.2	t	t	t	t
1334	linalool propionate	0.5	0.4	0.9	0.9	0.9	0.4	1.3	0.7	t
1346	α-terpinyl acetate	2.1	2.1	3.4	4.7	4.7	1.5	4.4	2.8	1.1
1410	α-cedrene	0.1	0.1	NONE	0.1	0.1	0.1	t	NONE	NONE
1411	2-epi-β-funebrene	0.1	0.1	NONE	0.1	0.1	0.1	t	NONE	NONE
1417	(E)-caryophyllene	0.2	0.2	0.1	0.1	0.1	0.2	0.3	t	0.1
1419	β-cedrene	0.1	0.1	NONE	0.1	0.1	0.2	0.3	t	0.1
1448	cis-muurola-3,5-diene	t	0.1	0.1	t	t	0.1	t	0.6	0.2
1452	α-humulene	t	0.1	0.2	t	t	0.2	0.3	t	0.2
1465	cis-muurola-4(14),5-diene	0.2	0.3	0.2	t	t	0.3	0.2	1.5	0.5
1478	γ-muurolene	-	-	-	t	t	t	0.1	t	0.2
1480	germacrene D	0.6	1.6	1.1	0.3	0.3	4.1	1.2	0.6	3.5
1499	epi-zonarene	-	-	-	-	-	t	t	0.6	t
1500	α-muurolene	t	t	-	t	t	t	t	t	0.3
1513	γ-cadinene	t	t	-	t	t	t	t	t	t
1521	trans-calamenene	t	t	-	t	t	0.1	0.1	0.3	0.2
1522	δ-cadinene	t	t	-	t	t	0.1	0.2	0.2	0.2
1550	cis-muurola-5-en-4-β-ol	t	t	0.1	t	t	t	t	t	t
1559	cis-muurola-5-en-4-α-ol	t	0.1	0.1	0.1	0.1	t	t	t	t
1600	cedrol	3.5	5.2	NONE	7.6	7.6	6.2	1.6	NONE	t L
1652	α-cadinol	0.2	0.4	0.3	0.1	0.1	0.4	0.7	1.3	1.3
1685	germacra-4(15),5,10(14)-trien-1-al	0.1	0.2	0.1	0.1	0.1	0.1	1.0	0.3	0.3

		strict cultivars from California, USA					strict cultivars from Turkey			
KI	compound	*Stricta* Type 1 14591	*Stricta* Type 2 14592	cv. Totem 14059	cv. Glauca 15058	cv. Tiny Tower 15057	Stricta Istan. 14674	Stricta Turk. 14647	Stricta Turk. 14648	Stricta Turk. 14597
1930	diterpene, FW272	t	t	0.3 H	t	t	t	t	t	t
1958	iso-pimara-8(14),15-diene	0.5	0.6	0.1 L	0.3	0.3	0.4	0.6	1.2	0.4
1987	manoyl oxide	7.0 H	9.1 H	0.1L	3.3	3.3	0.2	1.3	0.7	2.0
1987	iso-pimara-7,15-diene	1.9	2.7	0.1 L	3.2	3.2	0.2	1.4	0.4	1.3
2055	abietatriene	1.3	1.5	1.7	1.1	1.1	0.5	0.9	1.6	2.5
2087	abietadiene	t	t	0.1	t	t	3.0 H	t	5.4 H	t
2105	isoabienol	1.8	1.8	6.0 H	1.5	1.5	1.4	0.9	0.9	1.2
2149	abienol	2.3	0.7	1.2	1.7	1.7	3.2	0.4	0.8	0.2
2179	abienol isomer	0.2	0.2	0.3	0.4	0.4	t	t	t	t
2269	sandaracopimarinol	0.1	0.1	0.1	0.2	0.2	0.2	t	0.2	t
2282	sempervirol	0.1	0.1	0.3	0.2	0.2	t	t	t	t
2314	trans-totarol	3.0	2.7	3.5	3.0	3.0	5.5 H	1.9	1.4	0.8
2331	trans-ferruginol	0.4	0.4	0.7	0.4	0.4	0.7	0.4	0.2	t

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