Comparison of leaf essential oils of fastigiate (strict) and horizontal forms of *Cupressus sempervirens* from Cyprus, Montenegro, Turkey, and United States.

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX
76798, USA,
Robert_Adams@baylor.edu

Tuğrul Mataraci
Eskidji Müz. AŞ., Sanayi Cad. Vadi Sokak No:2, Tarabya, İstanbul, Turkey

Salih Gücel
Environmental Research Institute, Near East University, North Nicosia, Cyprus

and

Jim A. Bartel
San Diego Botanic Garden, P. O. Box 230005, Encinitas, CA 92023

ABSTRACT

The volatile leaf oils of the horizontal form of *C. sempervirens* from natural populations in Cyprus and Turkey were very uniform and dominated by α-pinene (36.2, 26.0%), myrcene (2.4, 2.4%), δ-3-carene (18.3, 16.0%), terpinolene (3.2, 3.8%), α-terpinyl acetate (4.7, 3.5%), cedrol (4.4, 3.3%), manoyl oxide (0.7, 3.8%), iso-pimara-7,15-diene (0.4, 2.6%), isoabienol (2.4, 4.0%), and trans-totarol (1.5, 5.7%). Overall, the major terpenes compositions were very uniform for the sampled natural populations (Cyprus, Turkey) and fastigiate (strict) forms from California and Istanbul. But they were very variable for the oils from other fastigiate forms (Turkey and Montenegro). The fastigiate forms of *Cupressus sempervirens* from California and Istanbul (14674) have oils that are similar to natural populations. Variation in the composition of oils from cultivated fastigiate forms in Turkey and Montenegro suggests that these cultivars arose from multiple selections of fastigiate (strict) trees, rather than cloning and widespread cultivation. The volatile leaf oil composition does not support the recognition of the two growth forms of *C. sempervirens* as distinct taxa. Published on-line www.phytologia.org *Phytologia 99(1): 48-53 (Jan 19, 2017).* ISSN 030319430.
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Cupressus sempervirens L. ranges naturally from the eastern Mediterranean, Crete, Cyprus, eastern Aegean Islands, Iran, Israel, Jordan, Lebanon, Syria, Turkey, and possibly Libya (Sękiewicz et al. 2016). The species has been widely cultivated within and outside its range throughout the warm temperate world (More and White 2002). Farjon (2005, 2010) noted that *C. sempervirens* has traditionally been separated into two "elements": pyramidal trees with horizontal branches (horizontal form) (= *C. horizontalis* Mill.) and fastigiate trees with strict branching (fastigiate form) (= *C. fastigiata* DC.). The fastigiate trees are often called Italian, cemetery, graveyard, and Tuscan cypress in the Old World, while in the New World, the widely cultivated fastigiate cultivars are called Italian and cemetery cypress. Farjon (2005) concluded that the fastigiate (strict) form of *C. sempervirens,* widely cultivated all over the Mediterranean and beyond, was selected many centuries ago from natural populations, which likely were largely horizontal.

The volatile leaf essential oils of *Cupressus sempervirens* (both horizontal and fastigiate forms) 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 (cv. *stricta*?) 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)

This paper compares the volatile leaf oil of the horizontal form of *C. sempervirens* from natural populations in Cyprus and Turkey to that of cultivated fastigiate trees from Montenegro, Turkey, and California, USA.

MATERIALS AND METHODS

Plant materials:

***Cupressus sempervirens* L. (horizontal form):**

Cyprus: 35° 16' 34.58" N, 33° 23' 14.12" E, 361 m, 3 June 2015, Salih Gucel ns, Lab Acc. *Robert P. Adams 4560-14564,*

Turkey: pyramidal trees, branches horizontal,

Vicinity of Beskonak village, Serik, Antalya, 37° 17' N, 31° 18' E, elev. 180 m, 23 May 2015, Coll. Tuğrul *Mataraci, 2015-14,* Lab Acc: *Robert P. Adams 14565,*

In Köprülü Kanyon National Park, on the road of Ancient city of Selge, Beskonak village, Serik, Antalya, 37° 21' N, 31° 53' E, elev. 708 m, 23 May 2015, Coll. *Tuğrul Mataraci*, *2015-15*, Lab. Acc: *Robert P. Adams 14566.*

In Köprülü Kanyon National Park, on the road of Ancient city of Selge, Beskonak village, Serik, Antalya, 37° 22' N, 31° 13' E, elev. 817 m, 23 May 2015, Coll. *Tuğrul Mataraci*, *2015-16*, Lab. Acc: *Robert P. Adams 14567.*

In Köprülü Kanyon National Park, on the road of Ancient city of Selge, Beskonak village, Serik, Antalya, 37° 21' N, 31° 14' E, elev. 764 m, 23 May 2015, Coll. *Tuğrul Mataraci*, *2015-17*, Lab. Acc: *Robert P. Adams 14568*

***Cupressus sempervirens* (fastigiate form):**

Montenegro:

fastigiate (strict), columnar tree in maquis, appearing natural but likely an escaped cultivar, Komunal Budva, Petrovac, between coasts of Lucica and Buljarica, forest rd, ca. 42° 12' N, 18° 57' E, 30 m, 24 Aug 2015, Coll. *Tuğrul Mataraci, 2015-28*, Lab Acc: *Robert P. Adams 14672*,

cultivated, strict, columnar trees, in the park, Komunal Budva, Petrovac, Sv, Stefab coast, 42° 12' N, 18° 57' E, 2 m, 24 Aug 2015, Coll. *Tuğrul Mataraci, 2015-29*, Lab Acc: *Robert P. Adams 14673*.

Turkey:

cultivated, Ayvalik- Town cemetery, Balikesir Province, living hedge around the cemetery, up to 20m tall, strict habit, 39° 17' N, 26° 41' E, ca. 50 m, 18 July 2015, Coll. *Tuğrul Mataraci, 2015-24*, Lab Acc: *Robert P. Adams 14597*,

cultivated in park, Istanbul, Beyoğlu-Halicioğlu jct., strict habit, 41° 29' N, 28° 56' E , 34 m, 12 Aug 2015, Coll. *Tuğrul Mataraci, 2015-25*, Lab Acc: *Robert P. Adams 14647*,

cultivated on the highway between Izmit-Kocaeli, strict habit, 40° 46' N, 29° 39' E, 26m, 16 Aug 2015, Coll. *Tuğrul Mataraci, 2015-26*, Lab Acc: *Robert P. Adams 14648*,

cultivated, Emirgan Park, Istanbul,strict, columnar trees, 41° 11' N, 29° 05' E, 84 m, 6 Sept 2015, Coll. *Tuğrul* Mataraci, 2015-30, Lab Acc: *Robert P. Adams 14674,*

United States:
cultivated, Carlsbad, CA, approx. 33° 06' 56.6" N, 117° 18' 39.3" W., 151 ft, 17 July 2015, San Diego Co., Coll. *Jim A. Bartel, 1631-1635*, Lab Acc. *Robert P. Adams 14591-14595*. Dates trees *1631-1635* planted: 1985, 2005, 2000, 1980, 2010,
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 the horizontal form of *C. sempervirens* from natural populations in Cyprus and Turkey were very uniform and dominated (Table 1) by α-pinene (36.2, 26.0%), myrcene (2.4, 2.4%), δ-3-carene (18.3, 16.0%), terpinolene (3.2, 3.8%), α-terpinyl acetate (4.7, 3.5%), cedrol (4.4, 3.3%), manoyl oxide (0.7, 3.8%), iso-pimara-7,15-diene (0.4, 2.6%), isoabienol (2.4, 4.0%), and trans-totarol (1.5, 5.7%).

The oils compositions of samples of *C. sempervirens* cv. 'Glauca Stricta' from near San Diego, CA, USA proved to very uniform, suggesting that these are likely clones. The average values of the components show its oil to be quite similar to the horizontal form of *C. sempervirens* from natural populations from Cyprus and Turkey (Table 1.) In contrast, the oils of the fastigiate forms from Turkey and Montenegro were quite variable (Table 1). Interestingly the oils from a cultivated tree and the 'wild' (escaped cultivar?) fastigiate tree in Montenegro had quite different oils (Table 1).

Jacobson (1996) elaborated on the introduction and cultivation of *Cupressus sempervirens* cultivars into the United States. He notes the introduction of the Italian cypress (cv. 'Stricta') 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.'

It is interesting that three components characteristic of cedarwood oil (α-cedrene, β-cedrene, cedrol) are present in the leaf oils from Cyprus, Turkey, 'Stricta' from California, and 14674 and 14647 from Turkey, but only a trace or absent from the other oils from fastigiate trees (Table 2). Overall, the major terpenes compositions are very uniform for the horizontal form from natural populations (Cyprus, Turkey) and cultivated fastigiate trees in California and Istanbul, but very variable (Table 2) for the other cultivated fastigiate tree oils (Turkey and Montenegro).

α-pinene varies from 19.9% to 65.7% among the *stricta* oils (Table 2). In fact, the *stricta* Turkey 14597 is most unusual in having a high concentration of α-pinene, but very low concentrations of δ-3-carene (0.2%), linalool (trace), α-cedrene (none), β-cedrene (0.1%), cedrol (trace) and abietadiene (trace).

Cultivated fastigiate *Cupressus sempervirens* trees from California and Istanbul (14674) both have oils that are very similar to from natural populations from Cyprus and Turkey (Tables 1, 2). Variation in the composition of oils from cultivated trees in Turkey and Montenegro suggests that these cultivars arose from multiple selections of fastigiate trees, rather than cloning and subsequent widespread cultivation. The volatile leaf oil composition does not support the recognition of the two growth forms of *C. sempervirens* as distinct taxa. Similarly Farjon (2010) considered that the cultivated fastigiate form was not a taxonomic variety but a cultigen.

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Table 1. Leaf essential oil compositions for *Cupressus sempervirens*. Compounds in bold show large differences between samples. Table abbreviations: horiz. = horizontal form, fast. = fastigiate form, Turk. = Turkey, Calif. = California, Istan. = Istanbul, Mont. = Montenegro. Cyprus 15030 is the average of 5 samples (14560-14564); Turkey 15031 is the average of 4 samples (14564-14568); California is the average of 5 samples (14591-14595). In these three cases, because little variation existed among the samples, average oils are presented. All the other samples (Table 1) were collected from individual trees. Mont. c 14673 is from a cultivated tree in Montenegro, whereas, Mont. ec 14672 is from an escaped cultivar (?) tree in Montenegro.

KI	compound	horiz. Cyprus 15030	horiz. Turk. 15031	fast. Calif. 15032	fast. Istan. 14674	fast. Turk. 14647	fast. Turk. 14648	fast. Turk. 14597	fast. Mont. c 14673	fast. Mont. ec 14672
921	tricyclene	0.01	0.1	0.1	0.1	t	0.1	0.1	t	0.1
924	α-thujene	0.02	0.1	0.1	0.5	0.1	t	t	0.4	1.8
932	**α-pinene**	**36.2**	**26.0**	**39.1**	**34.4**	**28.5**	**35.2**	**65.7**	**19.9**	**29.6**
945	α-fenchene	0.6	0.4	0.6	0.6	0.8	0.9	0.1	0.9	0.3
946	camphene	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.1	0.2
969	sabinene	0.5	0.6	0.7	3.4	0.4	0.4	1.0	1.2	3.6
974	β-pinene	1.2	1.1	1.1	1.1	1.1	0.9	1.9	1.3	1.4
988	**myrcene**	**2.4**	**2.4**	**2.2**	**2.4**	**2.3**	**2.3**	**2.6**	**2.7**	**3.9**
1002	α-phellandrene	t	t	t	t	t	t	t	t	t
1008	**δ-3-carene**	**18.3**	**16.0**	**16.8**	**17.3**	**30.1**	**25.7**	**0.2**	**30.7**	**12.2**
1014	α-terpinene	0.2	0.2	0.1	0.2	0.1	0.1	t	0.2	0.5
1020	p-cymene	0.2	0.2	t	0.1	0.1	t	t	t	0.5
1023	sylvestrene	0.2	0.2	0.2	0.2	0.3	0.3	t	0.4	t
1024	limonene	1.4	1.2	2.2	1.0	1.0	0.8	1.4	1.7	2.3
1025	β-phellandrene	0.9	1.2	1.5	0.9	1.0	0.7	1.3	1.7	2.4
1044	(E)-β-ocimene	t	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1054	γ-terpinene	0.4	0.3	0.3	0.4	0.2	0.3	0.2	0.4	1.3
1067	linalool oxide	t	0.1	t	t	t	t	t	t	0.1
1082	m-cymenene	t	t	t	t	t	t	t	t	t
1086	**terpinolene**	**3.2**	**3.8**	**4.1**	**3.2**	**3.2**	**4.4**	**1.4**	**4.8**	**1.3**
1099	**linalool**	**1.5**	**0.6**	**0.2**	**0.3**	**0.6**	**t**	**t**	**0.4**	**1.1**
1122	methyl octanoate	t	t	t	t	t	0.1	t	t	t
1123	α-camphenal	0.3	0.1	t	t	0.1	t	t	t	0.1
1133	cis-p-mentha-2,8-dien-1-ol	0.2	t	t	t	0.1	0.1	t	t	t
1135	trans-pinocarveol	0.2	t	t	t	t	0.1	t	t	t
1141	camphor	0.2	t	t	t	0.1	0.1	t	t	t
1154	karahanaenone	0.8	0.1	t	t	t	t	t	t	t
1159	p-mentha-1,5-diene-8-ol, isomer	0.3	0.1	t	t	0.2	0.1	t	t	t
1160	pinocarvone	0.2	t	t	t	t	t	t	t	t
1166	p-mentha-1,5-diene-8-ol	t	t	t	t	t	t	t	t	t
1067	umbellulone	0.1	0.3	t	t	t	t	t	t	t
1174	terpinen-4-ol	1.6	1.3	0.7	0.6	0.6	0.3	0.2	0.5	1.4
1176	m-cymen-8-ol	0.1	0.5	t	0.2	0.1	t	0.2	0.2	0.2
1179	p-cymen-8-ol	0.2	0.1	t	t	0.1	t	t	t	t
1186	α-terpineol	0.3	0.2	0.2	t	0.2	0.1	t	0.1	0.1
1204	myrtenol	t	0.2	t	t	t	t	t	t	t
1204	verbenone	0.3	0.1	t	t	0.2	0.1	t	t	t
1241	carvacrol, methyl ether	t	0.2	0.1	0.1	0.2	1.0	t	0.4	0.5
1254	linalool acetate	t	t	t	t	t	t	t	t	t
1287	bornyl acetate	0.2	0.2	0.1	0.4	0.2	0.1	t	0.8	1.5
1315	<2E,4E->decadienal	t	0.5	0.1	t	t	t	t	t	t
1323	methyl decanoate	t	t	t	t	t	t	t	t	t
1334	linalool propionate	0.6	0.7	0.4	0.4	1.3	0.7	t	1.0	0.4
1346	**α-terpinyl acetate**	**4.7**	**3.5**	**2.0**	**1.5**	**4.4**	**2.8**	**1.1**	**2.7**	**2.4**
1345	α-cubebene	t	t	t	t	t	t	t	t	t
1374	α-ylangene	t	t	t	t	t	t	t	t	t
1400	tetradecane	t	0.1	t	t	t	0.1	t	t	0.1
1410	**α-cedrene**	**0.3**	**0.1**	**0.1**	**0.1**	**t**	**-**	**-**	**t**	**-**
1411	2-epi-β-funebrene	t	0.1	0.1	0.1	t	-	-	t	-
1417	(E)-caryophyllene	0.1	0.2	0.1	0.2	0.3	t	0.1	0.4	0.8
1419	**β-cedrene**	**0.3**	**0.3**	**0.1**	**0.2**	**0.3**	**t**	**0.1**	**0.4**	**t**

KI	compound	horiz. Cyprus 15030	horiz. Turk. 15031	fast. Calif. 15032	fast. Istan. 14674	fast. Turk. 14647	fast. Turk. 14648	fast. Turk. 14597	fast. Mont c 14673	fast. Mont w 14672
1448	cis-muurola-3,5-diene	0.3	0.3	0.1	0.1	t	0.6	0.2	0.4	0.3
1452	α-humulene	0.2	0.5	0.1	0.2	0.3	t	0.2	0.3	0.4
1465	cis-muurola-4(14),5-diene	0.8	0.7	0.2	0.3	0.2	1.5	0.5	0.8	0.9
1478	γ-muurolene	0.2	0.1	t	t	0.1	t	0.2	0.2	0.5
1480	germacrene D	2.1	2.6	0.7	4.1	1.2	0.6	3.5	3.4	3.4
1499	epi-zonarene	0.2	0.2	t	t	t	0.6	t	0.2	0.3
1500	α-muurolene	0.1	0.1	t	t	t	t	0.3	0.1	0.1
1513	γ-cadinene	0.1	t	t	t	t	t	t	0.1	0.2
1521	trans-calamenene	0.3	0.2	t	0.1	0.1	0.3	0.2	0.2	0.4
1522	δ-cadinene	0.3	0.2	t	0.1	0.2	0.2	0.2	0.2	0.3
1600	**cedrol**	**4.4**	**3.1**	**4.5**	**6.2**	**1.6**	**-**	**t**	**t**	**0.1**
1652	α-cadinol	0.6	0.7	0.2	0.4	0.7	1.3	1.3	0.8	1.0
1685	germacra-4(15),5,10(14)-trien-1-al	0.2	0.3	0.1	0.1	1.0	0.3	0.3	0.4	0.5
1921	methyl hexadecanoate	0.2	0.2	t	t	t	t	t	t	0.1
1958	iso-pimara-8(14),15-diene	0.5	0.7	0.5	0.4	0.6	1.2	0.4	1.5	0.8
1987	**manoyl oxide**	**0.7**	**3.8**	**8.5**	**0.2**	**1.3**	**0.7**	**2.0**	**1.6**	**2.2**
1987	**iso-pimara-7,15-diene**	**0.4**	**2.6**	**1.7**	**0.2**	**1.4**	**0.4**	**1.3**	**1.5**	**1.5**
2055	abietatriene	1.5	3.4	1.4	0.5	0.9	1.6	2.5	1.2	1.1
2087	**abietadiene**	**0.6**	**t**	**0.1**	**3.0**	**t**	**5.4**	**t**	**4.2**	**t**
2103	6-octadecanoic acid, methyl ester	0.4	t	t	t	t	0.2	t	t	0.5
2105	**isoabienol**	**2.4**	**4.0**	**1.7**	**1.4**	**0.9**	**0.9**	**1.2**	**2.2**	**4.7**
2149	abienol	0.4	1.3	1.0	3.2	0.4	0.8	0.2	1.1	0.9
2269	sandaracopimarinol	-	0.2	0.1	0.2	t	0.2	t	t	0.1
2282	sempervirol	t	0.4	0.1	t	t	t	t	t	0.1
2314	**trans-totarol**	**1.5**	**5.7**	**3.1**	**5.5**	**1.9**	**1.4**	**0.8**	**3.8**	**4.2**
2331	trans-ferruginol	0.2	0.7	0.4	0.7	0.4	0.2	t	0.5	0.6

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.

Table 2. Comparison of the leaf oil compositions for the most variable compounds among samples.

KI	compound	horiz. Cyprus 15030	horiz. Turk. 15031	fast. Calif. 15032	fast. Istan. 14674	fast. Turk. 14647	fast. Turk. 14648	fast. Turk. 14597	fast. Mont c 14673	fast. Mont w 14672
932	**α-pinene**	**36.2**	**26.0**	**39.1**	**34.4**	**28.5**	**35.2**	**65.7**	**19.9**	**29.6**
1008	**δ-3-carene**	**18.3**	**16.0**	**16.8**	**17.3**	**30.1**	**25.7**	**0.2**	**30.7**	**12.2**
1086	**terpinolene**	**3.2**	**3.8**	**4.1**	**3.2**	**3.2**	**4.4**	**1.4**	**4.8**	**1.3**
1099	**linalool**	**1.5**	**0.6**	**0.2**	**0.3**	**0.6**	**t**	**t**	**0.4**	**1.1**
1410	**α-cedrene**	**0.3**	**0.1**	**0.1**	**0.1**	**t**	**-**	**-**	**t**	**-**
1419	**β-cedrene**	**0.3**	**0.3**	**0.1**	**0.2**	**0.3**	**t**	**0.1**	**0.4**	**t**
1600	**cedrol**	**4.4**	**3.1**	**4.5**	**6.2**	**1.6**	**-**	**t**	**t**	**0.1**
1987	**manoyl oxide**	**0.7**	**3.8**	**5.2**	**0.2**	**1.3**	**0.7**	**2.0**	**1.6**	**2.2**
1987	**iso-pimara-7,15-diene**	**0.4**	**2.6**	**5.2**	**0.2**	**1.4**	**0.4**	**1.3**	**1.5**	**1.5**
2087	**abietadiene**	**0.6**	**t**	**0.1**	**3.0**	**t**	**5.4**	**t**	**4.2**	**t**
2105	**isoabienol**	**2.4**	**4.0**	**1.7**	**1.4**	**0.9**	**0.9**	**1.2**	**2.2**	**4.7**
2314	**trans-totarol**	**1.5**	**5.7**	**3.1**	**5.5**	**1.9**	**1.4**	**0.8**	**3.8**	**4.2**