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Full Length Research Paper

The effects of rootstock on the volatile flavor components of page mandarin [(*Citrus reticulata* var dancy × *Citrus paradisi* var dancan) × *Citrus clemantina*] flower and leaf

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The effects of rootstock on the volatile flavor components of page mandarin flower and leaf were investigated in this study. Flower flavor components were extracted by using ultrasound(US) water bath apparatus and eluted by n-pentane: diethylether(1:2) solvent and then analyzed by GC-FID and GC-MS. Leaf flavor components were extracted by using water distillation method and eluted by using n-hexane solvent and then analyzed by GC-FID and GC-MS. Fifty-two flower components and, sixty-nine leaf components including: aldehydes, alcohols, esters, ketones, monoterpenes, sesquiterpenes and other components were identified and quantified. The major flavor components were linalool, limonene, ocimene, α-pinene, sabinene and myrcene. The flower and leaf oil from plants grown on Swingle Citrumelo and Yuzu showed the highest content of aldehydes. Since the aldehyde content of citrus oil is considered one of the more important indicators of high quality, rootstock apparently has a profound influence on Page mandarin flower and leaf oil quality.

Key words: Page mandarin flower, page mandarin leaf, flavor components, rootstock, ultrasound, water-distillation.

INTRODUCTION

Oil glands are more or less conspicuous in all parts of the citrus flower except the stamens. The oil glands are situated, as in the calyx, just under the epidermis of the outside of the petal. Typical citrus oil glands are present even in the very young leaves (Webber and Batchelor, 1948). These valuable essential oils are composed of many compounds including: terpenes, sesquiterpenes, aldehydes, alcohols, esters and sterols. They may also be described as mixtures of hydrocarbons, oxygenated compounds and nonvolatile residue. Branched aldehydes and alcohols are important flavor compounds in many food products. Flower and leaf oils of citrus can for example be used commercially in medicines, perfumes, cosmetics (Salem, 2003). The quality of an essential oil may be calculated from the quantity of oxygenated compounds present in the oil (Salem, 2003). The quantity of oxygenated compounds present in the oil is variable and depends upon a number of factors including: Rootstock (Babazadeh et al., 2009; Babazadeh, 2009), harvesting time (Toncer et al., 2010), variety (Scora and Torrisi, 1966) and climate (Scora and Torrisi, 1966).

Various studies have shown that the rootstock used may influence the quantity of oxygenated compounds present in the oil. Babazadeh et al. (2009) showed that rootstocks can influence the aldehydes and alcohols content in page mandarin peel and juice and also Hendrickson et al. (1970) showed that rootstocks can influence on the aldehyde content of orange rind oil. Verzera et al. (2003) found that the quality of bergamot peel oil was determined by a high amount of alcohols and esters, which in turn is related to the type of rootstock used. Usai et al. (1994) showed that rootstock could have an influence on the content of aldehydes, alcohols and esters present in marsh seedless grapefruit peel oil. The quality of a honey can be calculated from the amount of oxygenated components (Alissandrakis et al., 2003; Alistair et al., 1993) and various rootstocks used may

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Table 1. Common and botanical names for citrus taxa used as scions and rootstocks (Fotouhi and Fattahi, 2007).

Bakraei (Rootstock)	*C.reticulata* × *C. aurantifolia*
Changsha (Rootstock)	*C.reticulata* Blanco
Citrumelo 'Swingle' (Rootstock)	*C. paradisi* var dancan × *P. trifoliata* (L.) Raf .
Cleopatra mandarin (Rootstock)	*C. reticulata* Blanco (*C. reshni* Hort.ex.Tan)
Page (scion)	[(*C.reticulata* var dancy × *C. paradisi* var dancan) × *C. Clemantina*]
Sour orange (Rootstock)	*C. aurantium* (L.)
Trifoliate orange (Rootstock)	*Poncirus trifoliata* (L.) Raf .
Troyer citrange (Rootstock)	*C. sinensis* (L). osbeck× *P. trifoliata* (L.) Raf .
Yuzu (Rootstock)	*C. junos* Sieb. ex Tan. (C.Ichangensis swing. × *C.reticulata* Blanco)

influence the quality of volatile flavor components present in the honey. It had been recognized previously that oxygenated compounds are important factor in deceiving and attracting the pollinators. These results may have consequences for yield in agricultural (Kite et al., 1991; Andrews et al., 2007). Endogenous hormones particularly cytokinins, regulated by the rootstock, are positively related with essential oil (Stoeva and Iliev, 1997; Zlatev et al., 1978; El-Keltawi and Croteau, 1987). In addition to plant hormones, differences in nutrient uptake by the rootstock may be another factor affecting essential oil (El-Sawi and Mohamed, 2002; Zehtab-Salmasi et al., 2008). The present study reports the effects of rootstocks on the oxygenated compounds of Page mandarin flower and leaf oil with the aim of determining whether the quantity of oxygenated compounds was influenced by the rootstocks.

MATERIALS AND METHODS

Rootstocks

In 1989, rootstocks were planted at 8 · 4 m^2 with three replication at Ramsar research station [latitude 36° 54' N, Longitude 50° 40' E ; Caspian Sea climate, average rainfall 970 mm per year and average temperature 16.25°C; soil was classified as loam-clay, pH range 6.9 to 7.0). The following rootstocks were investigated (Table 1).

Preparation of flower and leaf sample

In the first week of June 2007, for each rootstock about 500 g of leaves and at least 50 g of flowers were collected from many parts of the same trees, located in Ramsar research station, early in the morning (6 to 8 am) and only in dry weather.

Flower extraction technique

The methodology, used in this study, was described by Allissandrakis et al. (2003). In order to obtain the volatile compounds from the flowers of each of the eight citrus rootstocks, 50 g of fresh flowers were placed in a 2000-ml spherical flask, along with 300 ml of n-pentane: diethylether (1:2). The flask was covered and then placed in an ultrasound (US) water bath apparatus for 20 min. Ultrasonic extractions were performed with an ultrasound cleaning bath-Fisatom Scientific-FS14H(Frequency of 40 KHz, nominal power 90 W and 24 ×14×10 cm internal dimensions water bath). The temperature of the US water bath was held constant at 25°C. The extract was subsequently filtered through MgSeO4 monohydrate. The extract was finally concentrated with a gentle stream of nitrogen to 1 ml, placed in a vial and sealed. It was kept in the freezer at $^{-}$4°C until the GC-MS analysis.

Leaf extraction technique

In order to obtain the volatile compounds from the leaf of the eight citrus rootstocks, 500 g of fresh leaves were subjected to hydrodistillation for 3 h using a Clavenger-type apparatus. N-Hexane was used to isolate the oil layer from the aqueous phase. The hexane layer was dried over anhydrous sodium sulphate and stored at -4°C until used.

GC and GC-MS

An Agilent 6890 N gas chromatograph equipped with a DB-5 (30 m · 0.25 mm i.d; film thickness = 0.25 ∝m) fused silica capillary column (J&W Scientific) and a flame ionization detector (FID) was used. The column temperature was programmed from 50°C (2 min) to 188°C (20 min) at a rate of 3°C/min. The injector and detector temperatures were 220°C and helium was used as the carrier gas at a flow rate of 0.8 ml/min and a linear velocity of 22 cm/s. The linear retention indices (LRIs) were calculated for all volatile components using a homologous series of n-alkanes (C9-C16) under the same GC conditions. The weight percent of each peak was calculated according to the response factor to the FID. Some standards such as citronellal, decanal, β -sinensal, α -sinensal, linalool, terpinene-4-ol, α -terpineol, (E) nerolidol, linalyl acetate, geranyl acetate, cis-jasmone, sabinene and limonene were acquired from the Sigma-Aldrich. Gas chromatography- mass spectrometry was used to identify the volatile components. The analysis was carried out with a Varian Saturn 2000R. 3800 GC linked with a Varian Saturn 2000R MS.

The oven condition, injector and detector temperatures, and column (DB-5) were the same as those previously given for the Agilent 6890 N GC. Helium was the carrier gas at a flow rate of 1.1 ml/min and a linear velocity of 38.7 cm/s. Injection volume was 1 ∝l.

Identification of components

Components were identified by comparing their LRIs and matching their mass spectra with those of reference compounds in the data system of the Wiley library and NIST Mass Spectral Search program (Chem. SW . Inc; NIST 98 version database) connected to a Varian Saturn 2000R MS. Identifications were also determined by comparing the retention time of each compound with that of known compounds (Adams, 2001; McLafferty and Stauffer, 1989).

RESULTS AND DISCUSSION

Flavor compounds of Page mandarin flower

GC-MS analyze of the flavor compounds extracted from Page mandarin flower by using ultrasound water bath allowed identification of 52 volatile components (Table 2, Figure 1): 19 oxygenated terpenes (4 aldehydes, 13 alcohols, 1 esters, 1 ketones), 33 non oxygenated terpenes (14 monoterpens hydrocarbones, 8 sesquiterpenes hydrocarbons), and 11 other components.

Flavor compounds of Page mandarin leaf

GC-MS analyze of the flavor compounds extracted from Page mandarin leaf by using water distillation allowed identification of 69 volatile components (Table 3, Figure 2): 33 oxygenated terpenes (10 aldehydes, 19 alcohols, 4 esters), 36 non oxygenated terpenes (18 monoterpenes hydrocarbons, 16 sesquiterpenes hydrocarbons), and 2 other component.

Aldehydes

Ten aldehyde components that were identified in this analyze octanal, citronellal, decanal, neral, geranial, (E) 2,4-decadienal, dodecanal, tetradecanal, β-sinensal and α -sinensal (Table 5). In addition, they were quantified (from 3.17 to 6.74%) that it was determined and reported as relative amount of those compounds in leaf oil in this study. These findings were similar to the previous study undertaken by Salem (2003) Lota et al. (2001). Tangerine oil is easily distinguished from other citrus oils by its content of various aliphatic aldehydes. Two main aliphatic aldehydes were β -sinensal and citronellal. In addition, tangerine oil also contained α-sinensal (Salem, 2003). β -sinensal has a woody aroma, and is considered one of the major contributors to mandarin flavor (Sawamura et al., 2004).

Since the aldehyde content of citrus oil is considered as one of the more important indicators of high quality, rootstock apparently has a profound influence on Citrus oil quality (Hendrickson et al., 1970). Among the eight rootstocks examined, Yuzu and Swingle citrumello showed the highest content of aldehydes (Tables 4 and 5).

Alcohols

Nineteen alcohol components were identified in this anal*yze* were benzene methanol, linalool, terpinene -1-ol, isopulegol, p-mentha-1, 5-dien-8-ol, terpinene-4-ol, α-terpineol, myrtenol, (z)-piperitol, β-citronellol, cis-carveol, geraniol, elemol, (E) nerolidol, germacrene D-4-ol, spathulenol, α-muurolol, α-cadinol, (z)- β-santalol (Tables 5).

The total amount of alcohols range (from 6.30 to 27.31%) that it was determined and reported as relative amount of those compounds in leaf oil. Linalool was the primary component in this study. Linalool, the most significant alcohol compound of mandarin leaf and flower, is recognized as being very important to good mandarin flavor (Salem, 2003). Linalool has a flowery (rose-like) aroma (Sawamura et al., 2004) and its level is important for flavor characteristics in mandarin leaf oil (Salem, 2003).

Among the eight rootstocks examined, Yuzu and Swingle citrumello showed the highest content of alcohols (Tables 4 and 5).

Esters

Four ester components identified in this analysis were linalyl acetate, citronellyl acetate, neryl acetate and geranyl acetate (Salem, 2003; Lota et al., 2001). The total amount of esters range (from 0.77 to 1.63%) in leaf oil and the geranyl acetate was the most abundant. Among the eight rootstocks examined, Yuzu showed the highest content of esters in leaf oil and Swingle citromelo showed the highest content of esters in flower oil (Tables 4 and 5).

Ketone

The only ketone compound identified in the flower oil analysis was cis-jasmone. Among the eight rootstocks examined sour orange showed the highest content of ketone (Table 4).

Monoterpenes hydrocarbons

The total amount of monoterpene hydrocarbons range (from 54.99 to 76.38%) in leaf oil. Sabinene was the major component among the monoterpene hydrocarbons of Page mandarin flower and leaf oil. Sabinene has a woody aroma (Sawamura et al., 2004) and is considered one of the major contributors to mandarin leaf flavor (Salem, 2003).

Table 2. Chemical composition of essential oils of the flowers of Page mandarin.

	Component	KI		Component	KI
1	α- Thujene	930	27	(Z)-2,6-dimethyl-2,7-octadien-1,6-diol^a	1366
2	α - Pinene	939	28	Tetradecen	1400
3	Sabinene	975	29	Cis-jasmone	1391
4	β -Pinene	979	30	(Z)- β -caryophyllene	1409
5	β -myrcene	991	31	(Z)- β - farnesene	1443
6	α - Phellandrene	1003	32	α - humulene	1455
7	δ - 3 – carene	1031	33	Pentadecane	1500
8	Limonene	1029	34	E,E, α - farnesene	1506
9	(Z) β - Ocimene	1037	35	γ - cadinene	1514
10	(E)- β - Ocimene	1050	36	β -sesquiephellandrene	1523
11	(E)sabinene hydrate	1070	37	(E) – nerolidol	1563
12	(z)linalool oxide	1073	38	Germacrene D – 4 – ol	1576
13	α -terpinolene	1089	39	Spathulenol	1578
14	Linalool	1097	40	Hexadecane	1600
15	Phenyl ethyl alcohol	1107	41	α - cadinol	1654
16	Allo ocimene	1132	42	8-heptadecene*	1676
17	Pentyl benzen	1157	43	Heptadecane	1700
18	Terpinene-4-ol	1177	44	β - sinensal	1700
19	α - terpineol	1189	45	E,E-cis-farnesol	1725
20	Decanal	1202	46	α -sinensal	1757
21	Lilace alcohol B*	1217	47	Octadecane	1800
22	Lilace alcohol D*	1233	48	Unknown	1832
23	Linalyl acetate	1257	49	Caffeine*	1842
24	Perilla aldehyde	1272	50	Nonadecane	1900
25	Indol	1291	51	Eicosane	2000
26	δ- elemene	1338	52	Heneicosane	2100

* The identification was based on the NBS72K mass spectra library and on published MS (Alissandrakis et al., 2003) or MS and NMR data of Alistair et al. (1993).

Among the eight rootstocks examined, Citrange and Trifoliate orange showed the highest content of monoterpenes hydrocarbons in leaf oil (Table 5).

Sesquiterpenes hydrocarbons

The total amount of sesquiterpen hydrocarbons range (from 4.01 to 11.85%) in leaf oil. (Z)- β-farnesene and bicyclogermacrene were the major components among the sesquiterpenes hydrocarbons of Page mandarin leaf oil. Among the eight rootstocks examined, Citrange showed the highest content of sesquiterpenes hydrocarbons in leaf oil (Table 5).

Result of correlation

The results of intercorrellation analysis 13 components are presented in a correlation matrix (Table 6). The highest positive values or r(correlation coefficient) were between [(z)- β-farnesene and bicyclogermacrene (97%); ocimene and β -myrcene (97%); linalool and terpinene-4-ol (96%); α-terpinene and β-myrcen (95%); ocimene and α-terpinene (94%)]. The highest significant negative correlations were between [linalool and ocimene (98%); ocimene and terpinene-4-ol (98%); linalool and β-myrcen (97%); linalool and α-terpinene (96%)].

When 13 components were cluster analyzed, there was clustering of only 6 components into 3 two-compound factors above the 95% level of function. These 3 factors resulted from the clustering of highly positively interrelated compounds shuch as [(z)- β-farnesene and bicyclogermacrene; ocimene and β -myrcene; linalool and terpinene-4-ol] (Table 6).

Statistical analyses

The Duncan’s multiple range test was used to separate the significant rootstocks. Of the 30 individual oil

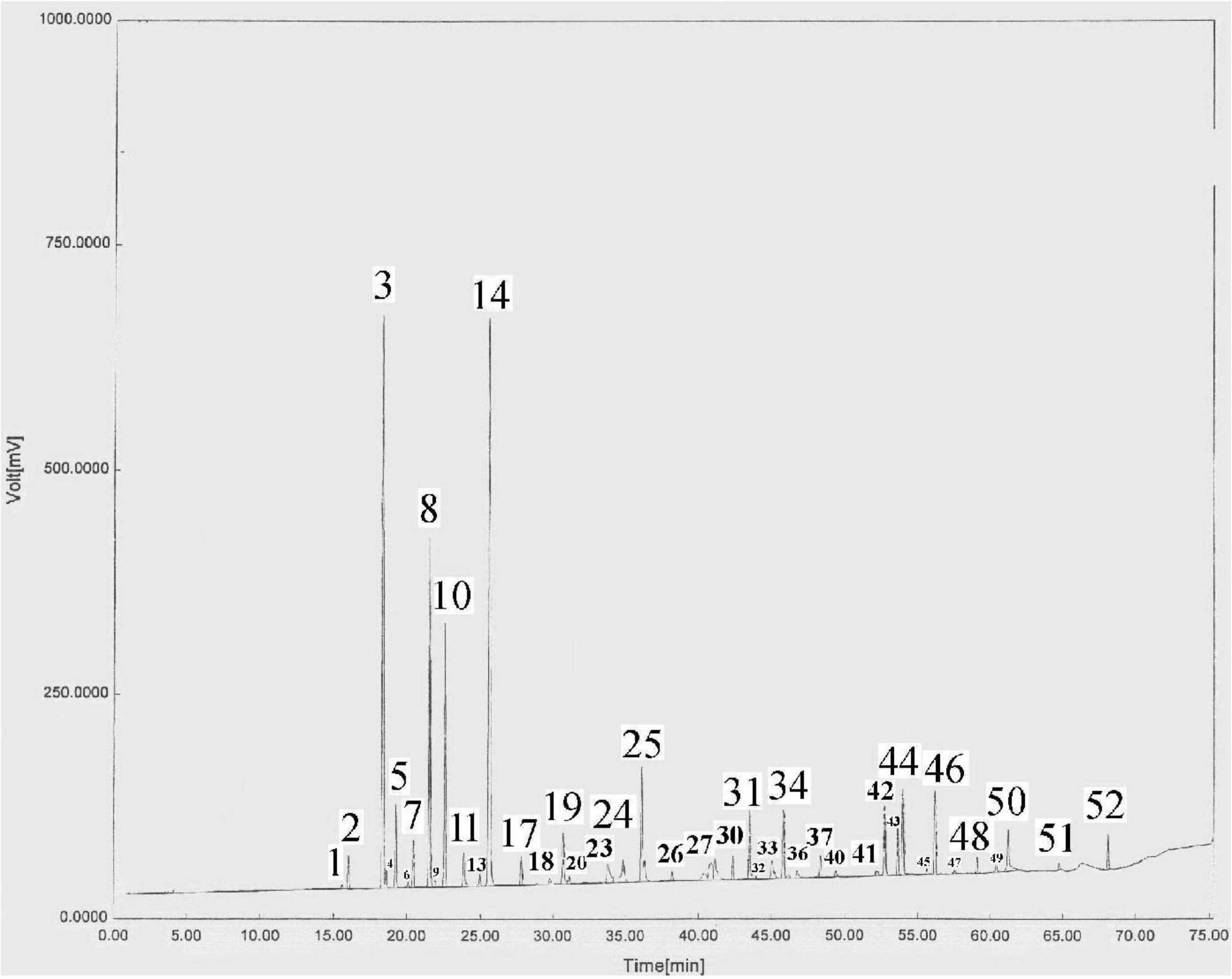

Figure 1. HRGC chromatogram of page mandarin flower oil.

Table 3. Chemical composition of essential oils of the leaves of Page mandarin.

	Component	KI		Component	KI
1	α- thujene	930	36	Geranial	1267
2	α - Pinene	939	37	(E) 2,4-decadieneal	1317
3	Camphene	954	38	δ- elemene	1338
4	Sabinene	975	39	Citronellyl acetate	1353
5	β -myrcene	991	40	α - cubebene	1351
6	Octanal	999	41	Neryl acetate	1362
7	α - phellandrene	1003	42	geranyl acetate	1381
8	δ - 3 – carene	1031	43	β - elemene	1391
9	α - terpinene	1017	44	Dodecanal	1409
10	P-cymene	1025	45	(Z)- β -caryophyllene	1409
11	Limonene	1029	46	γ - elemene	1437
12	(Z)- β - ocimene	1037	47	(Z)- β - farnesene	1443
13	(E)- β - ocimene	1050	48	α - humulene	1455

Table 3. Contd.

14	γ- terpinene	1060	49	α - amorphene	1485
15	(E)sabinene hydrate	1070	50	Germacrene D	1485
16	(z)linalool oxide	1073	51	Bicyclogermacrene	1500
17	α -terpinolene	1089	52	α - muurolene	1500
18	α –methyl-benzene methanol	1063	53	E,E, α - farnesene	1506
19	Linalool	1097	54	γ - cadinene	1514
20	Cis-rose oxide	1108	55	β - sesquiphellandrene	1523
21	Allo ocimene	1132	56	(z)cadina-1,4-diene	1535
22	Terpinene-1-ol	1134	57	Elemol	1550
23	Citronellal	1153	58	(E) – nerolidol	1563
24	Isopulegol	1160	59	Germacrene D – 4 – ol	1576
25	P-mentha-1,5-dien-8-ol	1170	60	Spathulenol	1578
26	Terpinen-4-ol	1177	61	Caryophyllene oxide	1583
27	α - terpineol	1189	62	Tetradecanal	1613
28	Myrtenol	1196	63	α - muurolol	1646
29	Decanal	1202	64	α - cadinol	1654
30	(z)-piperitol	1208	65	β - sinensal	1700
31	β - citronellol	1226	66	(Z)- β -santalol	1716
32	Cis-carveol	1229	67	α -sinensal	1757
33	Neral	1238	68	Caffeine	1842
34	Linalyl acetate	1257	69	Tricosane	2300
35	Geraniol	1253	70		

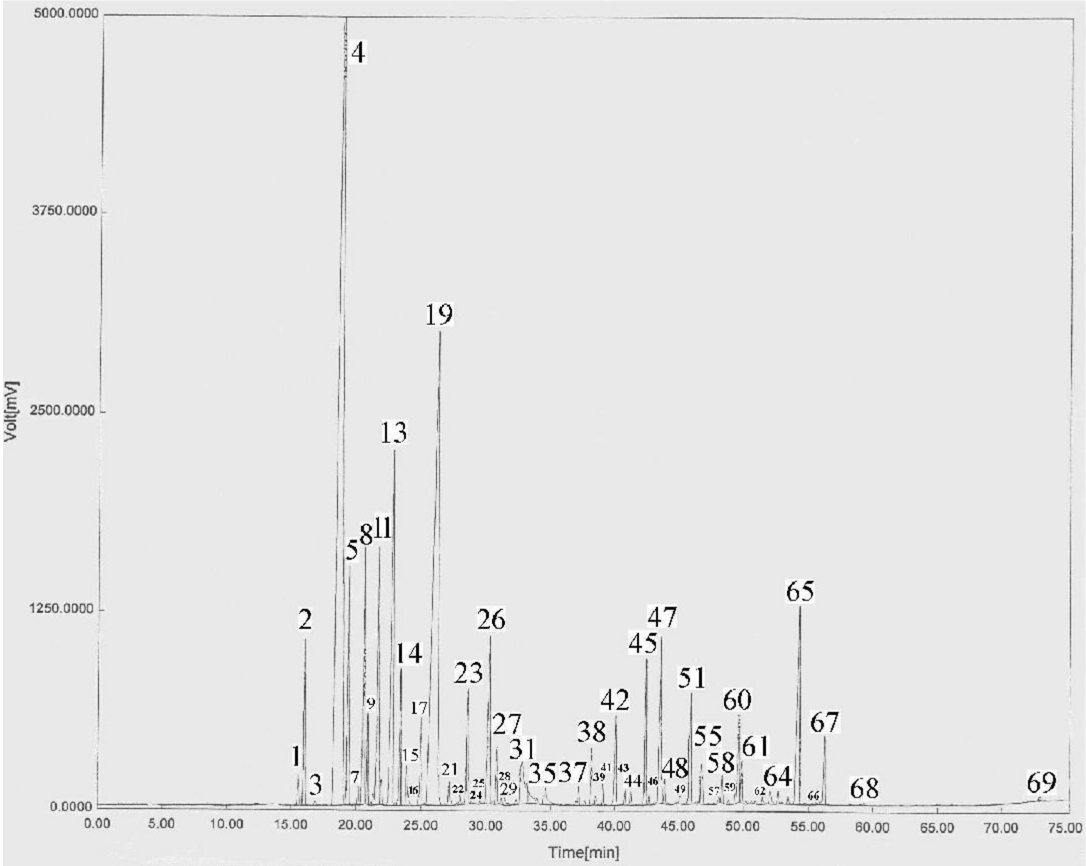

Figure 2. HRGC chromatogram of page mandarin leaf oil.

Table 4. Statistical analysis of variation in volatile flavor components of page mandarin flower budded on eight different rootstocks.

Compound	**Sour orange**		**Yuzu**		**Citrumelo swingle**		**Bakraei**		**Changsha**		**Cleopatra Mandarin**		**Troyer Citrange**		**Trifoliate Orange**		**F value**
	Mean	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	
Oxygenated compound																	
Aldehyds																	
Decanal	0.11	0.02	0.16	0.04	0.21	0.05	0.09	0.01	0.00	0.00	0.16	0.003	0.17	0.005	0.1	0.03	
Perilla aldehyde	0.78	0.29	0.00	0.00	1.20	0.12	0.43	0.01	0.43	0.06	0.62	0.09	0.87	0.04	0.18	0.13	
β-sinensal	2.03	0.20	3.20	0.28	2.29	0.02	2.33	0.03	2.03	0.13	1.98	0.14	1.30	0.04	2.09	0.18	F**
α- sinensal	2.50	0.19	3.80	0.30	2.53	0.03	2.75	0.43	2.47	0.11	2.41	0.12	2.06	0.02	2.50	0.10	F**
Total	5.42	0.70	7.16	0.62	6.23	0.22	5.60	0.48	4.93	0.30	5.17	0.35	4.40	0.10	4.87	0.44	
Alcohols																	
1) Linalool	18	0.72	16.82	0.61	24.39	1.04	18.30	2.06	18.45	1.74	19.60	0.55	15.96	0.19	16.50	0.40	F**
Phenylethyl alcohol	0.39	0.01	8.16	0.96	0.06	0.006	0.07	0.02	2.10	0.69	2.70	0.12	0.05	0.001	3.32	0.25	
Terpinene-4-ol	0.40	0.04	0.60	0.05	0.28	0.05	0.43	0.01	0.55	0.14	0.42	0.03	0.29	0.05	0.40	0.12	
α- terpineol	1.41	0.04	2.19	0.16	1.69	0.02	1.67	0.37	1.29	0.22	1.41	0.15	1.03	0.01	1.55	0.15	F**
Lilace alcohol B	0.14	0.05	0.38	0.03	0.00	0.00	0.11	0.05	0.23	0.02	0.10	0.03	0.00	0.00	0.20	0.01	
Lilace alcohol D	0.11	0.02	0.43	0.12	0.04	0.01	0.07	0.02	0.26	0.04	0.39	0.01	0.00	0.00	0.40	0.12	
Indol	3.91	0.15	1.81	0.16	4.90	0.16	2.31	0.44	3.37	0.20	2.70	0.12	5.48	0.52	2.71	0.08	F**
(Z) 2,6-Dimethyl-2,7-octadiene-1,6-diol	3.91	0.34	3.42	0.55	1.60	0.14	1.50	0.74	3.43	1.67	4.69	0.06	1.88	0.28	4.07	0.12	F**
(E)- Nerolidol	1.09	0.23	2.00	0.30	0.69	0.13	0.43	0.01	1.34	0.24	1.28	0.09	0.85	0.29	0.94	0.10	F**
Germacrene D-4-ol	0.37	0.08	0.49	0.05	0.20	0.06	0.21	0.07	0.25	0.06	0.37	0.07	0.42	0.03	0.38	0.14	
Spathulenol	0.00	0.00	1.39	0.27	0.00	0.00	0.43	0.01	0.29	0.01	0.00	0.00	0.00	0.00	0.40	0.12	
α- Cadinol	0.34	0.01	0.79	0.29	0.28	0.05	0.00	0.00	0.43	0.06	0.41	0.04	0.00	0.00	0.31	0.03	
E,E,Cis-farnesol	0.28	0.04	0.79	0.29	0.00	0.00	0.00	0.00	0.55	0.14	0.42	0.03	0.00	0.00	1.44	0.18	
Total	30.35	1.73	39.27	3.84	34.13	1.66	25.53	3.80	32.54	5.23	34.49	1.30	25.96	1.37	32.62	1.82	
Esters																	
Linalyl acetate	0.62	0.28	0.00	0.00	1.79	0.19	0.65	0.20	0.88	0.47	0.85	0.06	1.37	0.29	0.61	0.25	F**
Ketone																	
Cis-jasmone	2.66	0.59	0.79	0.29	1.20	0.29	0.24	0.01	0.56	0.13	0.42	0.03	0.00	0.00	0.31	0.03	F**
Monoterpenes																	
α -thujene	0.09	0.003	0.00	0.00	0.10	0.01	0.11	0.04	0.00	0.00	0.08	0.007	0.10	0.001	0.10	0.006	
α -Pinene	0.78	0.26	0.60	0.05	0.79	0.10	0.94	0.15	0.56	0.13	0.85	0.06	1.03	0.01	0.72	0.09	

Table 4. Contd.

Sabinene	18.78	1.45	11.81	0.97	18.53	0.67	21.56	1.78	17.54	2.57	18.19	0.40	22.65	0.58	17.92	0.70	F**
β-Pinene	0.94	0.02	0.60	0.05	0.07	0.02	1.44	0.47	0.23	0.02	0.42	0.03	0.86	0.30	0.03	0.003	
β-Myrcene	2.19	0.24	1.58	0.22	2,18	0.14	2.67	0.56	2.36	0.21	2.14	0.16	2.57	0.03	2.19	0.11	F**
α -Phellandrene	0.29	0.01	0.00	0.00	0.20	0.03	0.20	0.02	0.17	0.01	0.18	0.03	0.20	0.002	0.25	0.01	
δ -3-Carene	0.94	0.02	0.79	0.31	1.09	0.07	1.59	0.21	1.01	0.06	1.28	0.09	1.37	0.31	1.35	0.04	
Limonene	7.51	0.29	9.01	0.45	10.08	0.29	10.17	1.31	10.59	0.85	8.67	0.13	11.67	0.43	10.87	0.11	F**
(Z)- β -ocimene	0.18	0.006	0.00	0.00	0.18	0.01	0.21	0.006	0.24	0.01	0.20	0.03	0.22	0.02	0.24	0.01	
(E)- β -ocimene	8.14	0.47	6.38	0.40	6.97	0.21	8.93	1.06	7.83	2.51	7.66	0.27	9.10	0.40	8.14	0.24	NS
(Z)-sabinene hydrate	0.94	0.02	0.41	0.03	1.29	0.13	0.94	0.15	0.56	0.13	0.70	0.21	0.51	0.006	0.72	0.09	
(Z)-Linalool oxide	0.09	0.003	0.00	0.00	0.15	0.01	0.00	0.00	0.00	0.00	0.08	0.007	0.00	0.00	0.03	0.003	
α –Terpinolene	0.40	0.02	0.40	0.003	0.30	0.03	0.43	0.01	0.38	0.03	0.42	0.03	0.46	0.006	0.31	0.03	
Allo ocimene	0.29	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.01	0.00	0.00	0.00	0.00	
Total	41.56	2.84	31.58	2.48	39.75	1.72	49.19	5.76	41.47	6.53	40.99	1.46	50.74	2.09	42.87	1.44	
Sesquiterpenes																	
δ-elemene	0.37	0.04	0.55	0.02	0.29	0.04	0.40	0.05	0.41	0.08	0.42	0.03	0.41	0.05	0.42	0.20	
(Z)- β -caryophyllene	0.94	0.02	1.20	0.11	0.60	0.06	1.15	0.22	0.84	0.29	0.85	0.06	1.03	0.01	0.72	0.09	
(Z)- β - farnesene	2.19	0.24	3.39	0.06	1.81	0.36	3.39	0.79	2.47	0.11	2.41	0.12	2.74	0.31	2.30	0.09	F**
α -humulene	0.04	0.001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.003	0.00	0.00	0.00	0.00	
Pentadecane	0.94	0.02	0.00	0.00	0.21	0.02	0.00	0.00	0.00	0.00	0.85	0.06	0.00	0.00	0.00	0.00	
E,E- α -Farnesene	2.97	0.17	3.58	0.33	2.30	0.32	3.75	0.91	2.63	0.16	2.97	0.20	3.26	0.32	2.82	0.11	F**
γ-cadinene	0.25	0.07	0.30	0.02	0.19	0.04	0.21	0.04	0.00	0.00	0.29	0.02	0.20	0.002	0.19	0.007	
β - sesquiphellandrene	0.37	0.08	0.56	0.04	0.30	0.03	0.43	0.01	0.38	0.02	0.39	0.01	0.41	0.005	0.31	0.03	
Total	8.07	0.64	9.58	0.58	5.70	0.87	9.33	2.02	6.73	0.66	8.22	0.50	8.05	0.69	6.76	0.52	
Other compound																	
Pentyl benzen	0.47	0.01	0.54	0.02	2.01	1.16	1.29	0.39	1.84	0.75	0.85	0.06	0.51	0.006	0.72	0.09	
Tetradecen	0.00	0.00	0.00	0.00	0.00	0.00	1.80	0.58	0.00	0.00	0.0	0.00	0.00	0.00	0.00	0.00	
Hexadecane	0.29	0.01	0.38	0.03	0.08	0.02	0.13	0.04	0.18	0.03	0.12	0.04	0.00	0.00	0.13	0.004	
Heptadecene	3.28	0.38	4.00	0.03	2.79	0.08	1.08	0.20	4.09	1.11	2.84	0.12	2.57	0.48	3.03	0.07	F**
Heptadecane	0.94	0.02	0.98	0.27	0.90	0.09	0.36	0.11	1.13	0.26	0.85	0.06	0.85	0.29	0.94	0.10	
Octadecane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0..00	0.34	0.10	0.00	0.00	0.00	0.00	
Unknown	0.47	0.01	1.20	0.11	0.39	0.14	0.43	0.01	0.68	0.33	0.57	0.24	0.51	0.006	0.72	0.09	
Caffeine	0.00	0.00	0.00	0.00	0.13	0.04	0.00	0.00	0.00	0.00	0.04	0.003	0.00	0.00	0.17	0.07	
Nonadecane	1.24	0.49	1.20	0.11	1.61	0.65	0.36	0.11	0.96	0.45	1.56	0.49	1.36	0.77	1.16	0.70	NS
Eicosane	0.43	0.03	0.44	0.03	0.25	0.04	0.19	0.03	0.33	0.08	0.04	0.003	0.49	0.02	0.42	0.23	
Heneicosane	0.78	0.26	1.20	0.11	0.60	0.06	1.15	0.22	1.62	0.23	0.85	0.06	0.68	0.28	2.63	0.56	

Table 4. Contd.

Total	7.90	1.21	9.94	0.71	8.76	2.28	6.79	1.69	10.83	3.24	8.06	1.17	6.97	1.85	9.92	1.91	
Total oxygenated compounds	39.05	3.30	47.22	4.75	43.35	2.36	32.02	4.49	38.91	6.13	40.93	1.74	31.73	1.76	38.41	2.54	
Total	96.58	7.99	98.32	8.52	97.56	7.23	97.33	13.96	97.94	16.56	98.20	4.87	97.49	6.39	97.96	6.41	

Mean is average composition in % over the different rootstocks used with three replicates. St. err. = standard error. F value is accompanied by its significance, indicated by: NS = not significant, * = significant at P = 0.05, ** = significant at P = 0.01.

Table 5. Statistical analysis of variation in leaf oil components of page mandarin budded on eight different rootstocks.

Compound	**Sour orange**		**Yuzu**		**Citrumelo swingle**		**Bakraei**		**Changsha**		**Cleopatra mandarin**		**Troyer citrange**		**Trifoliate orange**		**F value**
	Mean	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	**Mean**	**St.err**	
Oxygenated compound																	
Aldehydes																	
Octanal	0.00	0.00	0.00	0.00	0.00	0.00	0.009	0.00	0.02	0.001	0.00	0.00	0.00	0.00	0.01	0.001	
Citronellal	1.45	0.04	2.96	0.03	1.41	0.04	1.27	0.02	1.41	0.01	1.16	0.03	0.69	0.02	1.48	0.02	F**
Decanal	0.05	0.001	0.09	0.009	0.11	0.03	0.14	0.02	0.08	0.002	0.08	0.02	0.03	0.01	0.04	0.01	
Neral	0.07	0.001	0.01	0.005	0.11	0.02	0.22	0.08	0.2	0.03	0.00	0.00	0.00	0.00	0.01	0.003	
Geranial	0.009	0.004	0.01	0.002	0.01	0.008	0.007	0.003	0.009	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
(E)2,4-decadienal	0.16	0.02	0.15	0.04	0.19	0.03	0.16	0.03	0.11	0.01	0.12	0.009	0.14	0.01	0.16	0.01	
Dodecanal	0.05	0.001	0.07	0.001	0.13	0.01	0.08	0.01	0.06	0.002	0.10	0.009	0.19	0.006	0.13	0.004	
Tetradecanal	0.00	0.00	0.00	0.00	0.03	0.01	0.05	0.03	0.06	0.002	0.00	0.00	0.00	0.00	0.04	0.01	
β-sinensal	2.58	0.03	2.34	0.08	3.91	0.11	2.33	0.15	2.34	0.04	2.55	0.08	1.79	0.20	1.76	0.07	F**
α-sinensal	0.63	0.01	0.64	0.02	0.84	0.05	0.57	0.04	0.71	0.01	0.62	0.02	0.33	0.05	0.34	0.01	
Total	4.99	0.10	6.27	0.18	6.74	0.30	4.83	0.38	4.99	0.10	4.63	0.16	3.17	0.29	3.97	0.13	
Alcohols																	
Benzene methanol	0.00	0.00	0.00	0.00	0.00	0.00	0.007	0.00	0.00	0.00	0.00	0.00	0.01	0.001	0.01	0.00	
Linalool	15.27	0.16	21.47	0.08	18.62	0.48	16.82	0.05	18.61	0.20	10.52	0.21	4.48	0.31	5.58	0.04	F**
Terpinene-1-ol	0.14	0.01	0.08	0.01	0.15	0.04	0.09	0.02	0.08	0.01	0.09	0.01	0.008	0.00	0.01	0.001	
Isopulegol	0.04	0.01	0.10	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
P-mentha-1,5-dien-8-ol	0.03	0.00	0.00	0.00	0.00	0.00	0.09	0.01	0.06	0.002	0.00	0.00	0.00	0.00	0.00	0.00	
Terpinene-4-ol	2.39	0.007	2.53	0.04	2.71	0.04	2.56	0.04	2.41	0.01	1.64	0.004	1.01	0.05	1.11	0.01	F**
α- Terpineol	0.32	0.01	0.38	0.007	0.61	0.04	0.39	0.04	0.43	0.002	0.16	0.01	0.07	0.01	0.06	0.01	
Myrtenol	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
(Z)-Piperitol	0.08	0.001	0.09	0.009	0.00	0.00	0.11	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
β-citronellol	0.71	0.02	0.57	0.01	1.73	0.45	0.67	0.04	0.49	0.22	0.50	0.007	0.00	0.00	0.00	0.00	

Table 5. Contd.

Cis-carveol	0.38	0.07	0.54	0.02	0.43	0.05	0.74	0.03	0.28	0.01	0.46	0.03	0.18	0.02	0.23	0.007	
Geraniol	0.38	0.01	0.43	0.01	0.53	0.09	0.3	0.07	0.37	0.003	0.00	0.00	0.00	0.00	0.06	0.01	
Elemol	0.005	0.002	0.008	0.001	0.09	0.03	0.01	0.002	0.01	0.002	0.01	0.00	0.01	0.001	0.02	0.001	
(E) Nerolidol	0.32	0.007	0.29	0.008	0.29	0.04	0.3	0.02	0.37	0.02	0.29	0.01	0.18	0.02	0.14	0.02	
Germacrene-D-4-ol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.02	0.08	0.01	0.07	0.002	
Spathulenol	0.56	0.004	0.58	0.01	1.26	0.07	0.71	0.07	0.87	0.02	0.35	0.03	0.2	0.05	0.18	0.005	
α-Muurolol	0.14	0.002	0.10	0.01	0.24	0.02	0.14	0.02	0.17	0.009	0.08	0.009	0.05	0.002	0.06	0.01	
α-Cadinol	0.09	0.009	0.12	0.001	0.14	0.03	0.09	0.01	0.12	0.01	0.09	0.01	0.03	0.01	0.02	0.001	
(z) – β-Santalol	0.02	0.01	0.00	0.00	0.03	0.01	0.02	0.01	0.02	0.001	0.00	0.00	0.00	0.00	0.00	0.00	
Total	20.87	0.33	27.31	0.22	26.83	1.39	23.04	0.48	24.29	0.51	14.32	0.35	6.30	0.48	7.55	0.11	
Esters																	
Linalyl acetate	0.005	0.00	0.00	0.00	0.00	0.00	0.005	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Citronellyl acetate	0.08	0.001	0.09	0.001	0.11	0.01	0.08	0.002	0.09	0.01	0.09	0.002	0.09	0.01	0.12	0.01	
Neryl acetate	0.18	0.008	0.22	0.008	0.2	0.008	0.18	0.005	0.14	0.009	0.13	0.01	0.10	0.005	0.16	0.01	
Granyl acetate	1.08	0.01	1.32	0.02	1.01	0.009	0.72	0.02	0.89	0.003	0.67	0.01	0.58	0.02	1.27	0.01	F**
Total	1.34	0.01	1.63	0.02	1.32	0.02	0.98	0.02	1.12	0.02	0.89	0.02	0.77	0.03	1.55	0.03	
Monoterpenes																	
α -Thujene	0.48	0.008	0.39	0.01	0.40	0.03	0.44	0.002	0.42	0.02	0.54	0.006	0.6	0.01	0.58	0.04	
α -Pinene	2.07	0.01	1.72	0.03	1.85	0.04	2.01	0.03	2.00	0.05	2.28	0.006	2.68	0.05	2.42	0.05	F**
Camphene	0.08	0.001	0.07	0.001	0.07	0.02	0.08	0.002	0.06	0.002	0.07	0.002	0.08	0.01	0.09	0.01	
Sabinene	40.71	0.30	35.91	0.23	32.26	0.82	40.66	0.92	37.38	0.37	42.95	0.08	43.62	0.59	44.12	0.49	F**
β-Myrcene	3.2	0.03	2.48	0.02	2.74	0.02	2.77	0.03	2.92	0.03	3.38	0.01	3.78	0.05	3.79	0.05	F**
α -phellandrene	0.33	0.006	0.27	0.02	0.26	0.02	0.29	0.02	0.29	0.01	0.34	0.005	0.36	0.01	0.34	0.02	
δ -3-Carene	3.74	0.02	3.24	0.01	3.14	0.03	3.03	0.02	4.05	0.02	3.44	0.05	4.2	0.08	4.80	0.06	F**
α-Terpinene	0.73	0.008	0.71	0.01	0.72	0.03	0.71	0.003	0.63	0.02	0.88	0.01	1.21	0.02	1.03	0.04	
p-Cymene	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.03	0.01	0.01	0.00	0.02	0.001	0.02	0.001	
Limonene	4.47	0.02	4.13	0.04	3.86	0.03	4.60	0.05	3.96	0.04	5.24	0.04	5.59	0.09	5.92	0.09	F**
(Z)- β -Ocimene	0.29	0.01	0.24	0.01	0.26	0.03	0.26	0.01	0.29	0.01	0.32	0.005	0.39	0.01	0.34	0.02	
(E)- β -Ocimene	7.35	0.05	6.27	0.01	6.38	0.10	6.45	0.06	6.69	0.01	8.43	0.03	9.56	0.14	9.39	0.28	F**
γ - Terpinene	1.27	0.006	1.25	0.02	1.25	0.02	1.21	0.006	1.06	0.02	1.53	0.01	2.08	0.03	1.94	0.05	
(z) Sabinene hydrate	0.60	0.004	0.55	0.01	0.59	0.02	0.57	0.006	0.58	0.01	0.27	0.007	0.08	0.01	0.10	0.003	
(z) Linalool oxide	0.01	0.00	0.00	0.00	0.009	0.00	0.007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
α-Terpinolene	0.92	0.008	0.82	0.03	0.9	0.04	0.74	0.02	0.72	0.03	0.97	0.01	1.21	0.02	1.37	0.05	
Cis-rose-oxide	0.01	0.00	0.01	0.00	0.02	0.003	0.01	0.00	0.02	0.001	0.00	0.00	0.01	0.005	0.01	0.001	
Allo ocimene	0.24	0.003	0.24	0.01	0.28	0.01	0.27	0.01	0.28	0.006	0.17	0.004	0.14	0.009	0.12	0.01	

Table 5. Contd.

Total	66.51	0.48	58.31	0.46	54.99	1.26	64.11	1.18	61.38	0.65	70.82	0.27	75.61	1.13	76.38	1.26	
Sesquiterpenes																	
δ-Elemene	0.26	0.007	0.26	0.003	0.48	0.01	0.27	0.01	0.31	0.008	0.50	0.01	0.69	0.06	0.54	0.009	
α-Cubebene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.001	0.008	0.00	
β-Elemene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.01	0.00	0.00	
(Z)- β -Caryophyllene	0.88	0.01	0.96	0.008	1.82	0.02	1.15	0.05	1.20	0.008	1.70	0.02	2.91	0.08	2.09	0.05	
γ-Elemene	0.03	0.00	0.05	0.001	0.10	0.01	0.05	0.01	0.06	0.002	0.08	0.009	0.14	0.01	0.09	0.01	
(Z)- β - Farnesene	1.07	0.01	1.16	0.01	2.21	0.01	1.24	0.08	1.11	0.01	1.92	0.02	3.33	0.15	2.33	0.04	F**
α -Humulene	0.10	0.001	0.10	0.001	0.23	0.01	0.14	0.01	0.15	0.004	0.19	0.005	0.34	0.02	0.23	0.007	
α -Amorphen	0.01	0.00	0.01	0.00	0.03	0.01	0.01	0.00	0.01	0.006	0.02	0.00	0.03	0.01	0.02	0.001	
Germacrene D	0.05	0.009	0.05	0.001	0.10	0.03	0.07	0.01	0.11	0.003	0.07	0.002	0.13	0.04	0.06	0.01	
Bicyclogermacrene	1.00	0.01	1.04	0.01	1.83	0.05	1.05	0.05	1.05	0.24	1.90	0.02	2.90	0.20	2.11	0.07	F**
α -Muurolene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.001	0.01	0.00	
E,E, α-farnesene	0.005	0.00	0.009	0.00	0.00	0.00	0.02	0.01	0.01	0.005	0.01	0.00	0.02	0.001	0.01	0.00	
γ-cadinene	0.01	0.001	0.00	0.00	0.02	0.002	0.005	0.00	0.02	0.001	0.01	0.001	0.02	0.001	0.01	0.002	
β-Sesquiphellandrene	0.32	0.007	0.35	0.008	0.61	0.02	0.39	0.02	0.20	0.03	0.50	0.01	0.89	0.02	0.60	0.01	
(Z)- cadina-1,4-diene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.001	0.00	0.00	
Caryophyllene oxide	0.28	0.003	0.25	0.003	0.47	0.03	0.35	0.03	0.43	0.01	0.21	0.02	0.19	0.03	0.13	0.01	
Total	4.01	0.05	4.23	0.04	7.90	0.20	4.74	0.28	4.66	0.32	7.12	0.11	11.85	0.63	8.23	0.21	
Other compound																	
Caffeine	0.01	0.00	0.01	0.00	0.02	0.001	0.01	0.00	0.02	0.001	0.02	0.00	0.02	0.002	0.02	0.001	
Tricosane	0.00	0.00	0.003	0.001	0.00	0.00	0.00	0.00	0.004	0.003	0.004	0.00	0.005	0.00	0.08	0.01	
Total oxygenated compounds	27.20	0.44	35.21	0.42	34.89	1.71	28.85	0.88	30.40	0.63	19.84	0.53	10.24	0.80	13.07	0.27	
Total	97.73	0.97	97.76	0.92	97.80	3.17	97.71	2.34	96.46	1.60	97.80	0.91	97.72	2.56	97.78	1.75	

Mean is average composition in % over the different rootstocks used with three replicates. St. err. = standard error. F value is accompanied by its significance, indicated by: NS = not significant, * = Significant at P = 0.05, ** = Significant at P = 0.01.

Table 6. Correlation matrix (numbers in this Table correspond with main components mentioned in Table 5).

	Linalool	**Sabinene**	**δ -3-Carene**	**Limonene**	**Ocimene**	**Citronellal**	**β -Sinensal**	**Terpinene-4-ol**	**Geranyl acetate**	**α Terpinene**	**β -Myrcene**	**(z)- β Farnesene**
Sabinene	-0.82**											
δ -3-Carene	-0.71**	0.58**										
Limonene	-094**	0.89**	0.65**									
Ocimene	-0.98**	0.82**	0.75**	0.93**								

Table 6. countd.

Citronellal	0.70**	-0.53**	-0.22	-0.55**	-0.60**							
β -Sinensal	0.65**	-0.83**	-0.54**	-0.79**	-0.66**	0.15						
Terpinene-4-ol	0.96**	-0.80**	-0.75**	-0.94**	-0.98**	0.52**	0.71**					
Geranyl acetate	0.33	-0.34	0.14	-0.21	-0.25	0.72**	0.45	0.24				
α terpinene	-0.96**	0.83**	0.69**	0.88**	0.94**	-074**	-064**	-0.92**	-0.48*			
β -Myrcene	-0.97**	0.82**	0.80**	0.89**	0.97**	-0.67**	-060**	-0.93**	-0.27	0.95**		
(z)- β Farnesene	-0.78**	0.35	0.42*	0.64**	0.75**	-0.64**	-0.27	-0.76**	-0.36	0.75**	0.69**	
Bicyclogermacrene	-0.82**	0.42*	0.46*	0.69**	0.80**	-0.64**	-031	-0.81**	-0.37	0.79**	0.74**	0.97**

*Significant at 0.05; ** significant at 0.01.

components analyzed, only 28 showed statistically significant differences due to the influence of individual rootstocks and even here differences were small. These differences on the 1 % level occurred in citronellal, β-sinensal, α-sinensal, citronellal, α -terpineol, linalool, terpinene-4-ol, indol, (E)-nerolidol, (E)-2,6-dimethyl,2,7-octadien-1,6-diol, geranyl acetate, linalyl acetate, cis-jasmone, sabinene, β-myrcene, delta-3-carene, limonenes, (E)- β -ocimene, (Z) - β - Farnesene, E,E- α - farnesene, bicyclogermacrene, 8-heptadecane. The non affected oil components were (E)- β - ocimene, nonadecane in flower oil and they are provided only for convenience of the reader (Tables 4 and 5).

Conclusion

Our observations that changing rootstock has an effect on some of the components of leaf oil is accord with other observations involving roots (Scora et al., 1981). For example, cytokinins synthesized in the roots and transported in the transpiration stream, have been detected in the xylem sap of woody plants. In addition, cytokinin level in the xylem sap also appears to vary with rootstock and appears to direct source to sink relationships by making tissues strong sinks for mineral elements and other metabolites including amino acids (Gordon et al., 1984). Cytokinins have a somewhat positive relationship with essential oil synthesis (Stoeva and Iliev, 1997; Zlatev et al., 1978; El-Keltawi and Croteau, 1987).

Microelements have a positive relationship with essential oil synthesis in plants (El-Sawi and Mohamed, 2002; Zehtab-Salmasi et al., 2008). Microelements increase leaf area and photosynthesis of plant and could therefore improve growth, dry matter production of plants and essential oil (percent and yield). Micronutrient contents in scions are greatly influenced by rootstock and higher Mn, Fe and Cu and N was reported in the scion grafted on Yuzu rootstock (Embleton et al., 1963). Rootstock affects the stomatal conductance, transpiration rate (Klamkowski and Treder, 2002) and photosynthetic efficiency indirectly by providing vigor through the translocation of micro and macro nutrients and water to the upper part the tree. The rootstock influence on root hydraulic conductivity and a higher root hydraulic conductivity results in the increased water uptake per unit root mass varies from rootstock to rootstock. The higher root hydraulic conductivity of Yuzu and Swingl citomelo may be responsible for vigorous growth and higher essential oil percent.

The pronounced enhancement in the amount of oxygenated compounds, when Yuzu and Swingle citromelo were used as the rootstocks, showed that either the synthesis of geranyl pyrophosphate (GPP) is enhanced or activities of both enzymes (isopentenyl pyrophosphate isomerase and geranyl pyrophosphate synthase) increased (Hay and Waterman, 1995).

High positive correlations between two terpenes such as [(z) - β -farnesene and bicyclogermacrene; ocimene and β -myrcene; linalool, and terpinene-4-ol; α -terpinene and β -myrcen; ocimmene and α -terpinene) suggest a genetic control (Scora et al., 1976) Whether such a dependence between two terpenes is due to their derivation of one from another is not known. Similarly, high negative correlations observed between (linalool and ocimene; ocimene and terpinene-4-ol; linalool and β - myrcen; linalool and α -terpinene) suggest that one of the two compounds is being synthesized at the expense of the other or of its precursor. Non-significant negative and positive correlations can imply genetic and /or biosynthetic independence. However, without a thorough knowledge of the biosynthetic pathway leading to each terpenoid compound, the true significance of these observed correlations is not clear. Further research on the relationship between rootstock and essential oil (oxygenated terpenes) is necessary.

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