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Distribution of Aroma Volatiles in a Population of Tangerine Hybrids

PAULINE KERBIRIOU[2†], ANNE PLOTTO[1*], KEVIN GOODNER[1], ELIZABETH BALDWIN[1], AND FRED G. GMITTER, JR.[2]

[1]*USDA/ARS Citrus & Subtropical Products Laboratory, Winter Haven, FL 33881*

[2]*University of Florida–IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850*

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While orange juice volatile composition has been well studied, little is known about volatiles in tangerines. This study was undertaken to determine the most common compounds present in 56 tangerine hybrids, and to find relationships among these hybrids based on volatile content. Fruits were harvested from Nov. 2006 to Mar. 2007. A composite sample of juice from approximately 20 fruits was analyzed by gas chromatography mass-spectrometry. Among the more than 200 identified volatiles in total, ethanol, α-pinene, cymene, and *d*-limonene were found in all the samples. These compounds were previously reported in citrus products (essence, peel oil, juice, etc.). A principal component analysis showed that hybrids 9-1, 8-10, and 'Hongju' were different from the others because a larger than average number of volatiles was detected in those samples. A cluster analysis based on presence/absence of volatiles revealed 10 main clusters influenced by harvest date and/or progenitors, highlighting relationships among certain hybrids based on their volatile composition. Cluster 2 mainly grouped samples having 'Fallglo' and 'Fairchild' in their parentage, while cluster 4 mainly grouped samples having a common parent, 'Murcott'. Cluster 10 grouped samples having sweet oranges in their genetic background and being rich in esters, which are known to give a fruity note to orange juices. This method provided useful information on tangerine hybrid volatile content in relation to their genetic makeup.

Fresh fruit production in Florida primarily consists of oranges (*Citrus sinensis* L. Osb.), grapefruits (*Citrus paradisi* Macf.), and tangerines (*Citrus reticulata* Blanco). However, grapefruit is very sensitive to citrus canker, a recent endemic disease, whereas tangerines are much more tolerant (Gottwald et al., 2002) and could replace grapefruit production in the long term for the fresh fruit market. From 1990 to 2000, seven million 95-lb tangerine boxes were produced in Florida, and in 2000, 60% to 75% of Florida's tangerines were utilized for the fresh fruit market (FDOC, 2000). In 2000, the value of tangerine shipments for 1990–2000 was estimated around $60 million for early tangerines ('Fallglo', 'Dancy', 'Robinson', and 'Sunburst') and around $44 million for 'Honey' tangerines. US standards for grades of Florida tangerines focus only on fruit appearance, such as damage, color, size, etc. (US Department of Agriculture, 1997). There is a growing need for fruit quality criteria to be used in the selection of high quality fruits, and this has been an aim of fresh fruit breeding programs in Florida. Indeed, improvements in fruit quality and characteristics, as well as improvements in pest and disease resistance, tolerance to various environmental stress factors, horticultural performance, and productivity are the primary goals of citrus breeding (Gmitter et al., 2007). These genetic improvements, leading to the release of new superior rootstocks and scions, would benefit the citrus industry because they can result in greater economic returns to growers, processors, packers, and shippers by reducing input unit costs and increasing product marketability.

Understanding plant genetics and genomes is essential to achieving these goals, incorporating the most appropriate methods and technologies for plant breeding. Genomic research could be very helpful in selecting superior individuals (that produce remarkable tasting fruits, for example) via marker-assisted selection (MAS). This method enables the selection of recombinants improved for multiple traits by conventional breeding (Gmitter et al., 2007). Molecular markers constitute useful tools for further selection of individuals presenting the desirable traits at earlier stages. Volatile compounds involved in fruit flavor are among traits of interest for tangerine breeding.

Unfortunately, little information is available regarding volatile compounds of tangerines, compared to oranges or lemons (*Citrus limon* Burm.). Coleman and Shaw (1972) studied the possibility of using water-soluble fractions of tangerine essence oils, containing volatile components with desirable essence-like aroma and flavor, in order to impart fresher flavor to orange juice. More recently, Pérez-López and collaborators (2006) quantified volatile compounds contained in tangerine juices by gas chromatography and mass spectrometry (GC-MS), but the contribution of these compounds to the flavor and aroma of the juice was not investigated. This study focused on the influence of pasteurization on the volatile compounds, and only volatile content of two cultivars ('Clemenule' and 'Fortuna' mandarins) was investigated. In addition, only five compounds were quantified. A screening of volatiles present among tangerine varieties of diverse origins would provide information about fruit quality, and constitute a useful tool to develop early DNA-based MAS of interesting individuals, thus improving the efficiency of the breeding processes.

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†Student presenter.

*Corresponding author; email: Anne.plotto@ars.usda.gov; phone: (863) 293-4133, ext. 123

The objective of this study was to analyze the volatile compounds in a population of tangerine hybrids and investigate commonalties among hybrids based on quality or quantity of volatile compounds. It was hypothesized that if similarities were present, they could be due to common genetic background and/or harvest maturity. In addition, knowledge of tangerine volatile composition may be useful to understand tangerine fruit quality in future studies.

In this study, fruit from 56 tangerine selections were harvested one or more times over the season, according to a preliminary flavor screening and their parentage. Juice samples were analyzed using GC-MS to determine their volatile profile. A statistical analysis of the data was performed by principal component analysis (PCA) and cluster analysis (CA), in order to create sample groups based on their similarities or differences in volatile composition.

Material and Methods

Tangerine trees were grown under the same conditions of soil, irrigation, and illumination at the University of Florida Citrus Research and Education Center (UFCREC) groves. Fruits were collected biweekly, from Nov. 2006 until Apr. 2007. Fruit were collected from individual trees that resulted from crosses made by the UFCREC breeding program, or named cultivars as references. The breeder and assistant pre-selected the test hybrids based on their parentage and a preliminary flavor screening to represent a wide range of flavors. Each sample was a juice composite from approximately 20 fruits harvested once or multiple times from one tree (Table 1). Selections harvested multiple times were: 8-9, 8-10, 'Murcott', 'Ortanique', 'Temple', and two selections from the cross of 8-9 x 'Murcott', and selections from the crosses of 'Fortune' x 'Murcott' and 8-9 x Val4x. A total of 89 samples was analyzed.

Sample preparation. Fruits were first washed with 16 L warm water containing 200 mL detergent (DECCO 241 Fruit and Vegetable Kleen, Monrovia, CA). Fruits were soaked, rubbed for about 1 min, rinsed, and sanitized using a 100 ppm peroxyacetic acid solution at 30 to 35 °C (Biosafe Systems, East Hartford, CT) for 3 min prior to processing in the lab. Individual fruits were cut in half on sterile foil, and juiced by hand with an electric juicer (model 3183; Oster, Rye, NY). The fruits were juiced carefully for 3 s, avoiding any scraping of the albedo or squeezing of the flavedo to prevent potential peel components from contaminating the juice. Most of the seeds were removed, and saturated sodium chloride (2.5 mL) was added to 2.5 mL tangerine juice in 20-mL vials to help drive volatiles into the headspace. The vials were capped with lids containing Teflon-coated septa for solid phase microextraction (SPME) sampling (Sides et al., 2000), and stored at –20 °C until analyzed.

Headspace sampling and GC-MS analysis. An MPS-2 autosampler (Gerstel, Inc., Baltimore, MD) was used for SPME analyses. The vial was incubated at 40 °C for 30 min and then a 2-cm SPME fiber (50/30 μm VB/Carboxen/PDMS; Supelco, Bellefonte, PA) was inserted into the headspace of the sample vial and exposed for 60 min. Subsequently, the fiber was thermally desorbed in the GC injector (splitless mode) port for 3 min at 250 °C. The separation of the volatile compounds was accomplished using an Agilent 6890 GC equipped with a 60 m × 0.25 mm × 0.25 μm DB-5ms column, coupled with a 5973N MS detector (Agilent Technologies, Palo Alto, CA). The column oven was programmed to increase at 6 °C·min^{-1} from the initial 50 to 250 °C, then ramped at 100 °C·min^{-1} to 300 °C and held for 11 min for a total run time of 45 min. Helium was used as carrier gas at a flow rate of 2 mL·min^{-1}. Inlet, ionizing source, and transfer-line temperatures were kept at 250, 230, and 280 °C, respectively. Data were collected using the ChemStation G1701 AA data system (Hewlett-Packard, Palo Alto, CA). Retention indices (Kovats) were determined using a mixture of n-alkanes (C-5 to C-20).

Volatile compound identification. The compounds were identified according to their spectral mass, using the NIST Mass Spectral search program and the NIST/EPA/NIH Mass Spectral Library (version 2.0 d, built 26 Apr. 2005 and distributed by Agilent Technologies). Compound identities were confirmed with their retention indices (Kondjoyan and Berlingué, 1996).

Statistical analyses. A PCA using XL Stats (Addinsoft, Paris) was performed to identify outliers. Furthermore, two cluster analyses, on both peak area and presence/absence of volatiles (Nageswara Rao et al., 2007), were performed to find correlations between volatile composition and sample origin.

Results and Discussion

In total, 225 compounds were identified by GC-MS in the 89 samples. The average number of volatiles per sample detected by GC-MS was 47, ranging from 21 to 86 volatiles per sample. More than 47 volatiles were detected in 34 samples, while 47 or fewer were detected in the remaining 55 samples.

Many compounds listed in Table 2 have been already reported in tangerine oils, essence, and in other citrus fruit (Arena et al., 2006; Buettner et al., 2003; Fanciullino et al., 2005; Moshonas and Shaw, 1972, 1974, 1990, 1994; Pérez-López et al., 2006a, 2006b; Shaw, 1991; Song et al., 2000a, 2000b). For instance, Pérez-López et al. (2006a) identified the following compounds in tangerine juice using the dynamic headspace technique: α-pinene, β-pinene, myrcene, α-terpinene, *d*-limonene, sabinene, γ-terpinene, *p*-cymene, α-terpineol, valencene, terpinen-4-ol, and linalool. On the other hand, some compounds, detected in fewer than 10 samples, might be more cultivar specific. Examples of such compounds are: ethyl octanoate; 2-methyl-3-buten-2-ol; 2-methyl-1-butanol; *o*-methyl-thymol; α-caryophyllene; β-terpineol; 2-pentanone; 2-ethyl-furan; methyl hexanoate; 2,4-heptadienal (E,E); 3-methyl-2-butenal; 2-heptenal; heptanol; 2,4-nonadienal (E,E); 1-decanol; geraniol acetate; limonene oxide (E); nootkatone; acetic acid; 2,3-pentanedione; 2-penten-1-ol (Z); 3-methyl-2-buten-1-ol; 4-methyl-1-pentanol; 2,4-hexadienal, (E,E); phenol; 1-octan-3-ol; 2-ethyl-hexanoate; ethyl 3-oxo-hexanoate; nerol; undecanal; 2,4-decadienal; 2-methyl pentanoate; 3-pentanone; 2-methyl-2-butenal; 2-methyl ethylpropanoate; 3-hexen-1-ol (E); 2-hexen-1-ol (E); 2- methyl-2-hexenal,; benzaldehyde; 2-ethyl-1-hexanol; dihydrocarveol; 1-nonanol; geranial; β-cyclocitral; perillal; 1-propanol; 2-butenal; 3-methyl butanal; 2-methyl butanal; 3-hydroxy-2-butanone; 4-heptanone; 3-hydroxy ethylbutanoate; 5-ethyl-2(5H)-furanone; 2,3-octanedione; hexyl acetate; 2,4-heptadienal (E,E); heptanoic acid; ionone. Table 2 gives the distribution of other compounds among samples.

Principal components analysis (PCA). The main goal of PCA is to reduce a dataset in a multidimensional space into fewer meaningful dimensions in order to explain observed similarities and dissimilarities (Iezzoni and Pritts, 1991; Morales et al., 1995). It is based on the calculation of correlations between variables (each volatile being a variable). PCA was performed on the data using peak area from volatile chromatograms, corresponding

Table 1. List of sample number and corresponding selection names and tree code, with each sample harvest date.

Sample no.	Selection name	Tree code	Harvest date
3	'Sunburst'	A	1 Dec. 2006
4	'Daisy'	A	1 Dec. 2006
7	'Fallglo' x 'Sunburst'	A	1 Dec. 2006
8	'Fallglo' x 'Fairchild'	A	1 Dec. 2006
9	8-9 x 'Orlando'	A	1 Dec. 2006
11	Unknown parentage	A	14 Dec. 2006
12	Unknown parentage	B	14 Dec. 2006
15	8-9 x 'Orlando'	B	14 Dec. 2006
17	'ThongDee' x 'Minneola'	A	14 Dec. 2006
19	'Ponkan'	A	14 Dec. 2006
20	8-9 x 'Murcott'	B	14 Dec. 2006
22	'Fallglo' x 'Sunburst'	B	14 Dec. 2006
25	'Clementine'	A	14 Dec. 2006
26	'Dancy'	A	22 Dec. 2006
28	'Fairchild'	A	22 Dec. 2006
31	'Minneola'	A	22 Dec. 2006
32	8-1	A	22 Dec. 2006
33	9-1	A	22 Dec. 2006
34	9-7	A	22 Dec. 2006
35	9-11	A	22 Dec. 2006
37	8-9 x 'Murcott'	C	5 Jan. 2007
38	8-9 x 'Murcott'	D	5 Jan. 2007
39	'Lee' x 'Fairchild'	A	5 Jan. 2007
44	8-8	A	26 Jan. 2007
48	'Hongju'	A	26 Jan. 2007
50	'Lee' x 'Murcott'	A	26 Jan. 2007
52	'Fortune' x 'Murcott'	B	26 Jan. 2007
54	'Robinson' x 'Fairchild'	A	9 Nov. 2006
55	'Fallglo' x 'Fairchild'	B	9 Nov. 2006
56	'Fallglo' x 'Fairchild'	C	9 Nov. 2006
57	'Fallglo' x 'Fairchild'	D	9 Nov. 2006
58	'Fallglo' x 'Fairchild'	E	9 Nov. 2006
59	'Fallglo' x 'Sunburst'	C	9 Nov. 2006
60	'Fallglo' x 'Sunburst'	D	9 Nov. 2006
61	8-9 x 'Murcott'	F	21 Nov. 2006
62	'Robinson' x 'Fairchild'	B	21 Nov. 2006
63	'Fallglo' x 'Fairchild'	F	21 Nov. 2006
64	'Robinson' x 'Fairchild'	C	21 Nov. 2006
65	'Fallglo' x 'Fairchild'	G	21 Nov. 2006
66	8-8 x 'Fortune'	A	9 Feb. 2007
69	'Fortune' x 'Murcott'	C	9 Feb. 2007
72	'Sanguinelli'	A	9 Feb. 2007
75	'Fortune' x 'Murcott'	D	21 Feb. 2007
76	'Fortune' x 'Murcott'	E	21 Feb. 2007
77	'Fortune' x 'Orlando'	A	21 Feb. 2007
80	ANNA SR Seedling	A	21 Feb. 2007
81	'Kinnow'	A	21 Feb. 2007
1, 16, 47	8-10	A	1 Dec., 14 Dec., 26 Jan.
2, 14, 45	8-9	A	1 Dec., 14 Dec., 26 Jan.
27, 46, 73, 82	'Murcott'	A	22 Dec., 26 Jan., 9 Feb., 21 Feb.
41, 70, 78, 85	'Temple'	A	5 Jan., 9 Feb., 21 Feb., 23 Mar.
42, 71, 79, 88	'Ortanique'	A	5 Jan., 9 Feb., 21 Feb., 23 Mar.
5, 13, 30, 43, 89	'Fortune' x 'Murcott'	A	1 Dec., 14 Dec., 22 Dec., 5 Jan., 23 Mar.
6, 36	8-9 x 'Murcott'	A	1 Dec., 5 Jan.
40, 49, 74	8-9 x 'Murcott'	E	5 Jan., 26 Jan., 9 Feb.
68, 83, 84	8-9 x VAL4x	A	9 Feb., 21 Feb., 23 Mar.

Table 2. Distribution of the volatile compounds among samples.[z] Volatiles are listed according to their frequency of appearance in samples, except for those appearing in fewer than 11 samples, which are reported in the text.

25%[y]	26% to 50%[x]	51% to 75%[w]	76% to 99%[v]	100%
Geranyl acetone	α-Selinene	2-Nonenal (E)	Octanal	Ethanol
β-Humulene	Cadinene	α-Phellandrene	Acetaldehyde	α-Pinene
3-Carene	α-Thujene	Citronellol	Heptanal	Cymene
1-Pentanol	1-Octanol	Decanal	Pentanal	*d*-Limonene
Geranyl acetate	2-Methyl-1,3-butadiene	Sabinene	Nonanal	
γ-Gurjunene	γ-Selinene	β-Terpinene	Terpinolene	
(+/–) 4-acetyl-1-methylcyclohexene (tentative)	2-Pentenal (E)	Nonanoic acid	α-Terpineol	
β-Elemene	Copaene	Dihydrocarvone	β-Phellandrene	
1-Penten-3-ol	Ethyl propanoate	Perillaldehyde	2-Hexenal (E)	
6-Methyl-5-hepten-2-one	Hexanoic acid	β-Pinene	2-Octenal (E)	
Octyl acetate	1-Hexanol	1-Penten-3-one	Terpinen-4-ol	
Neral	2-Methyl-furan	Acetone	Hexanal	
Caryophyllene	3-Methyl-1-butanol	3-Hexenal (Z)	α-Terpinene	
2-Pentyl-furan	1-Octen-3-one	Propanal	γ-Terpinene	
Benzoic acid			Ethyl acetate	
Carveol (Z)			*d*-Carvone	
Thymol			Valencene	
β-Gurjunene			β-Myrcene	
Ethyl butanoate			Linalool	
Ethyl hexanoate				
Nerol				
Ethyl-3-hydroxyhexanoate				
2-Decenal (E)				
Methyl butanoate				
Ethyl-2-methylbutanoate				
Butanal				
Eucalyptol				
Citronellal acetate				
Dodecanal				

[z]The volatiles were detected by GC-MS.
[y]Volatiles detected in 11 to 22 samples.
[x]Volatiles detected in 24 to 45 samples.
[w]Volatiles detected in 46 to 66 samples.
[v]Volatiles detected in 66 to 88 samples.

to an approximate volatile concentration. Less than 30% of the variance was explained by choosing the two first axes, as well as by choosing the first and third axes (Fig. 1). A plot of the scores using first two principal components as axes clearly shows that samples 48 ('Hongju') and 33 (9-1) are different from the others due to their volatile profile (Fig. 1A). An examination of the third principal component additionally indicates that sample 47 (8-10 harvested in January) is also different from the other samples (Fig. 1B). These three samples may be distinguished by their richness in relatively high molecular weight volatiles that appear at the end of the GC-MS analysis (sesquiterpenes). Moreover, these samples contain more volatiles than the average (47 volatiles), since 69, 79, and 53 volatile compounds were detected by GC-MS in these samples, respectively.

This statistical method is largely used for interpreting the aroma of food products. For instance, Jella et al. (1998) determined the key flavor components of processed grapefruit juices. Aishima (1983) used this method to group soy sauces according to their volatile compounds. PCA is also regularly used to discriminate wines according to their volatile content (Noble and Ebeler, 2002). However, datasets are often smaller than the one used in the present study. More than 200 volatiles were identified in the 89 tangerine juice samples, many of them being in only one sample. This explains the diminished variation accounted for by the first two or three factors (axes on Fig. 1), as the variation is spread among the 225 variables (samples volatiles), demonstrating that many variables are uncorrelated. In this case, PCA was useful to emphasize outliers. Another type of analysis was needed to understand similarities between samples based on volatile content.

Cluster analyses (CA). CA, like PCA, is broadly used to discriminate and to create groups of samples (Aishima, 1983; Chastrette et al., 1991; Fundira et al., 2002). In this multivariate analysis, distances between groups of variables (clusters) are measured. Two types of CA were performed, using different kinds of data.

CA based on volatile peak area measurements (CA1). Clusters were formed using the unweighted pair-group average agglomeration method (UPGMA). The Euclidean distance between groups was measured because this method is robust and widely applicable. The Euclidean distance is the distance between two points in a Euclidean space R^n. It comes from the generalization of the Pythagorean theorem to more than two coordinates. Clusters were therefore joined based on the average distance between all

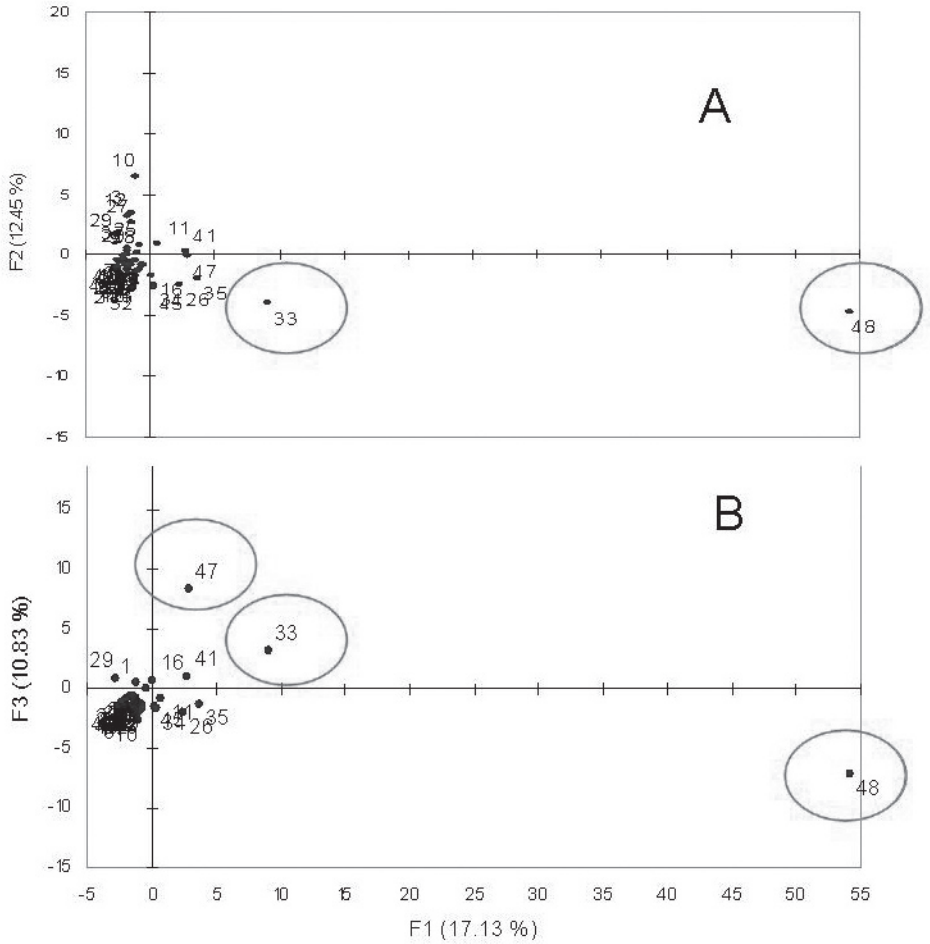

Fig. 1. Principal component analysis, using volatile peak area per sample. The percent contribution to the variance of each factor is given in parenthesis. The 89 samples are represented, and outliers (samples 48, 33, and 47) are circled.

members in the two groups.

Six clusters were determined using this method: four clusters containing one sample each, one cluster containing 14 samples, and an undetermined cluster containing all other samples. Samples 48 ('Hongju'), 55 ('Fallglo' x 'Fairchild'), 85 ('Temple'), and 75 ('Fortune' x 'Murcott') were set apart from the others (Fig. 2). These samples, like those highlighted by the PCA, contained many volatiles when compared with the other samples. Sample 85 ('Temple', harvested on 23 Mar. 2007) contained the highest number of volatiles (86). Seventy-nine and 58 volatiles were identified in both samples 48 and 75, respectively ('Hongju' and 'Fortune' x 'Murcott'), and 66 volatiles were identified in sample 55 ('Fallglo' x 'Fairchild'). It can be concluded that these four samples (48, 55, 75, and 85) are different from the others in their volatile profile because they contributed to the overall variation in volatile peak areas.

Moreover, the automatic truncature gives, in addition to the previously cited samples, indications for the formation of a cluster containing samples 8 ('Fallglo' x 'Fairchild'), 22 ('Fallglo' x 'Sunburst'), 31 ('Minneola'), 14 (8-9), and several selections of the hybrids resulting from crosses of 8-9 x 'Murcott' (samples 20, 38, 40, 49, and 74). This cluster is distinctive because it includes samples originated from the crosses between 8-9 and 'Murcott', grouped together with their parent 8-9 (sample 14). Moreover, the presence of two samples having 'Fallglo' as a parent in that cluster, suggests that samples 8 ('Fallglo' x 'Fairchild') and 22 ('Fallglo' x 'Sunburst') have a similar quantity and quality of volatile compounds. Furthermore, this cluster is interesting because it groups samples having the 'Minneola' tangelo in their genetic background. Indeed, 8-9 is a hybrid between 'Clementine' and 'Minneola'.

CA based on presence/absence of volatiles (CA2). Further relationships and contributions of varieties in the crosses can be explored by another type of CA, based on presence/absence of volatiles. This method is often used in genetics to discriminate individuals that do or do not carry certain genes or alleles (Nageswara Rao et al., 2007). While the cluster analysis by peak area clusters samples by differentiating them with respect to their volatile composition as well as their quantity, a cluster analysis taking into account the presence/absence of volatiles will give a general overview of the distribution of the volatiles among samples.

The agglomeration method used for this cluster analysis was the same as that used in CA1 (UPGMA). The Kulzinski coefficient was employed to measure similarities between samples. From this analysis, the dendrogram in Figure 3 shows 10 clusters of interest, which can be interpreted according to volatile distribution among the samples (Table 3). Generally the clusters were uniform based

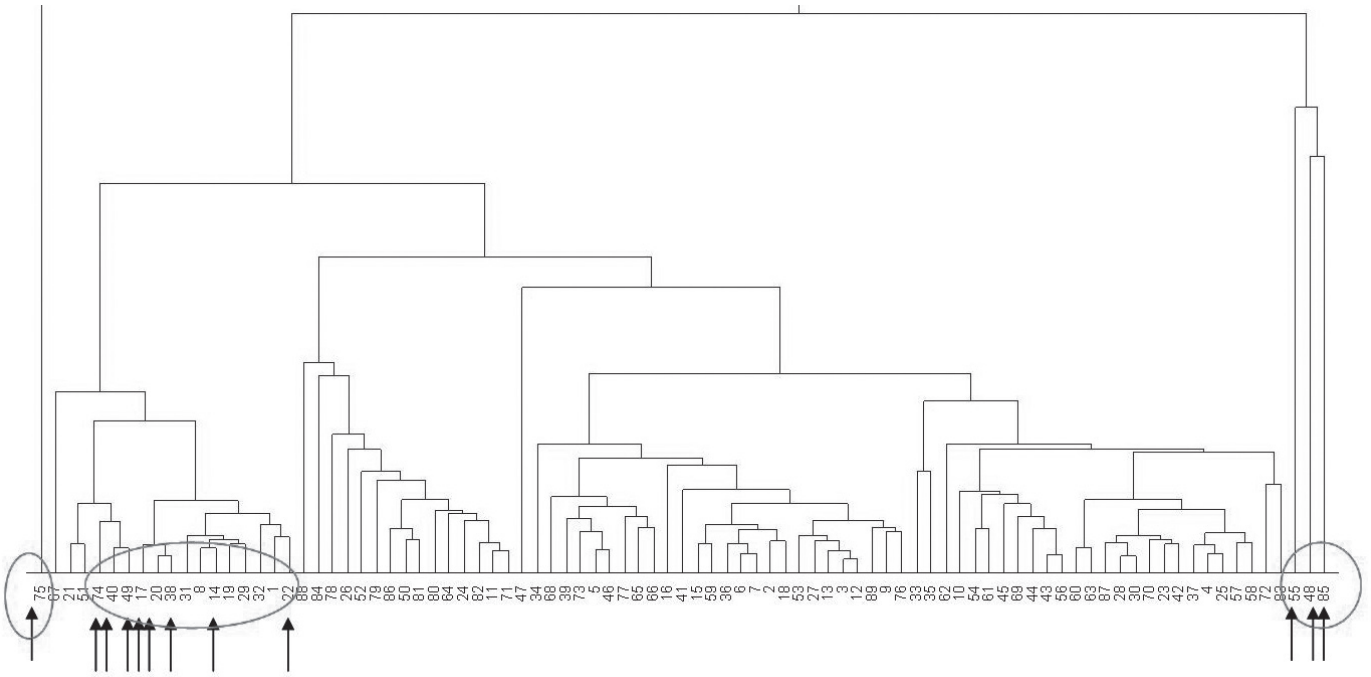

Fig. 2. Cluster analysis using volatile peak area per sample. Outlier samples, different from the other samples (because of their high content in sesquiterpenoids) are highlighted. The interesting cluster, grouping samples having a common parent (with arrows), is represented by a circle.

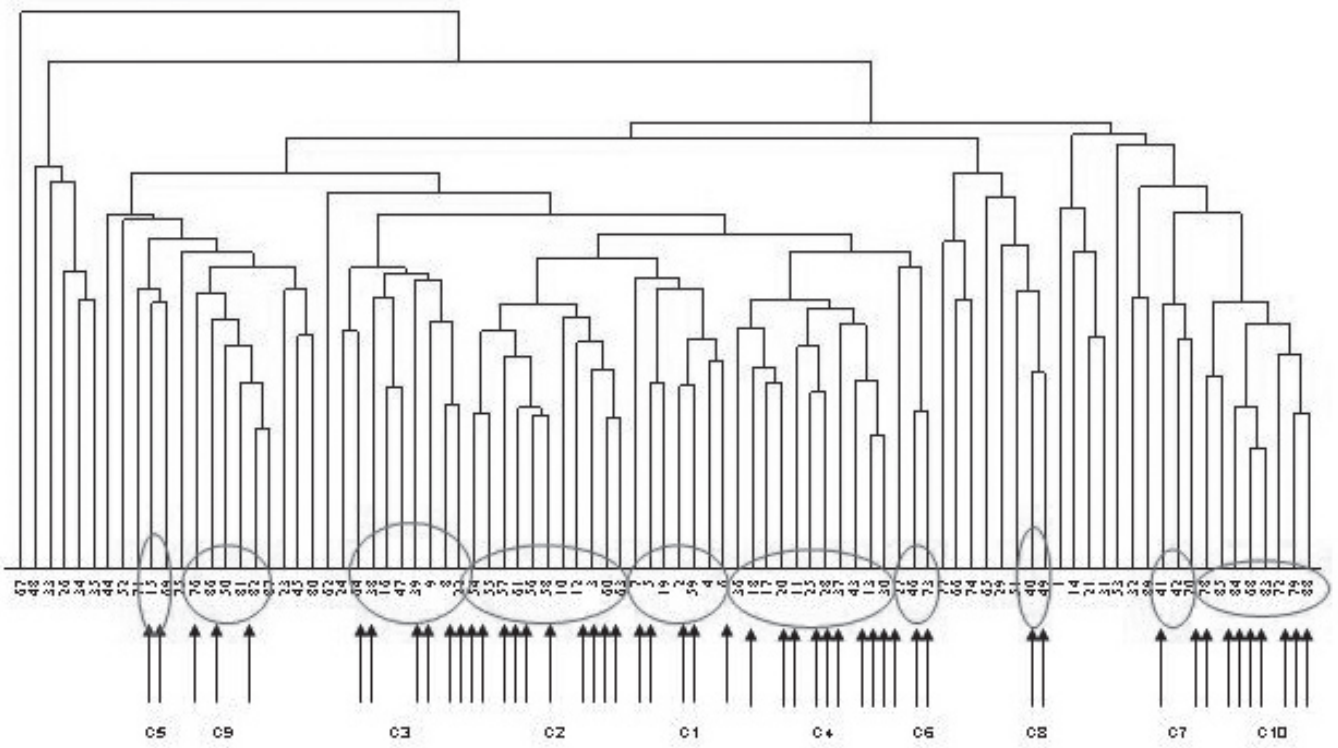

Fig. 3. Cluster analysis, using the presence/absence of volatiles per sample. Clusters of interest are emphasized, and samples among clusters (indicated by arrows) present similar traits, in terms of volatile composition, volatile number and genetic background.

Table 3. Cluster Analysis 2. The clusters are formed according to the presence/absence of volatiles among samples; therefore the samples are listed in the clusters according to their similar composition in volatiles. CLEM = 'Clementine'; F = 'Fortune'; FC = 'Fairchild'; FG = 'Fallglo'; L = 'Lee'; M = 'Murcott'; O = 'Orlando'; ORT = 'Ortanique'; R = 'Robinson'; SANG = 'Sanguinelli'; SB = 'Sunburst'; T= 'Temple'.

C1[z]	C2[z]	C3[z]	C4[z]	C5[z]	C6[z]	C7[z]	C8[z]	C9[z]	C10[z]
2[y] 8-9[x]	3[y] SB[x]	8[y] FG x FC[x]	11[y] R x US119[x]	15[y] 8-9 x O[x]	27[y] M[x]	41[y] T[x]	40[y] 8-9 x M[x]	50[y] L x M[x]	68[y] 8-9 x Val4x
4 DAISY	12 R x US119	9 8-9 x O	13 F x M	69 F x M	46 M	42 ORT	49 8-9 x M	76 F x M	72 SANG
5 F x M	54 R x FC	16 8-10	20 8-9 x M		73 M	70 T		81 KINNOW	78 T
6 8-9 x M	55 FG x FC	22 FG x SB	25 CLEM					82 M	79 ORT
7 FG x SB	56 FG x FC	38 8-9 x M	28 FC						83 8-9 x Val4x
19 PONKAN	57 FG x FC	39 L x FC	30 F x M						84 8-9 x Val4x
59 FG x SB	58 FG x FC	47 8-10	36 8-9 x M						85 T
	60 FG x SB	64 R x FC	37 8-9 x M						88 ORT
	61 8-9 x M		43 F x M						
	63 FG x FC								

[z]Cluster number.
[y]Sample number.
[x]Selection name.

on volatile quality (same volatiles present or absent) detected in the samples of the cluster.

Cluster 1 groups samples harvested at the same time (1 Dec. 2006), except for sample 59, which was harvested earlier (9 Nov. 2006). This cluster is made of samples rich in volatiles, particularly nonanoic acid. This volatile is found only in 31 samples, and in all of the samples of this group. This cluster is also characterized by the absence of C5 alcohols, 1-pentanol, 1-penten-3-ol, (Z)-2-penten-1-ol, but not pentanal and 1-penten-3-one. This could be an indication that the pathway for the C5 alcohol only leads to the latter two compounds in the selections of this cluster. Also, the samples in this cluster contained 39, 28, 44, 41, and 38 volatiles, respectively. Since these samples are grouped in this cluster, it means that they are likely similar to each other in volatile quality. For instance, sample 6 (8-9 x 'Murcott') is similar to sample 2 (selection 8-9), one of its parents, because of its volatile composition. In addition, samples 5 and 6 originated from crosses between 'Fortune' and 'Murcott', and 8-9 and 'Murcott', respectively, and may also be similar in terms of volatile composition, due to their common parent 'Murcott'. Moreover, samples 7 and 59 are from the same cross ('Fallglo' x 'Sunburst') and are in the same cluster, indicating that the volatile composition of these fruits may not be affected by harvest date. Samples 4 and 19, 'Daisy' and 'Ponkan', respectively, are in cluster 1 because they may be similar in volatile quality to those mentioned previously, although unrelated.

Cluster 2 contains samples with a higher than average number of volatiles. Fifty-eight, 53, 54, 66, 46, 48, 47, 57, 51, and 59 volatiles were identified in samples 3, 12, 54, 55, 56, 58, 60, 61, and 63, respectively. The following compounds were present in all the samples of this cluster, in addition to more common volatiles: 1-penten-3-one, β-pinene, α-phellandrene, 3-carene, terpinolene, α-terpineol, and dihydrocarvone. In addition it should be noted that these samples were mainly harvested on 9 and 21 Nov. 2006. Cluster 2 groups five samples from crosses between 'Fallglo' and 'Fairchild' (Table 3). Sample 3 ('Sunburst') is in cluster 2, which also contains sample 60 ('Fallglo' x 'Sunburst'). The parent 'Sunburst' may thus contribute to the volatile composition of fruits originated from crosses between 'Fallglo' and 'Sunburst'. This cluster also contains samples 12 and 54, having the same parent 'Robinson'. They might be similar in flavor because of this genetic background. Moreover, sample 54 ('Robinson' x 'Fairchild') is also close to samples 55, 56, 57, 58, and 63 (all crosses of 'Fallglo' by 'Fairchild'), probably because of their common parent 'Fairchild'.

Cluster 3 groups four samples that contain lower than the average number of different volatiles (samples 8, 9, 16, 22, 38, 39, 47, 64) (Table 3). These samples are listed in that same cluster, probably because they have common parents that are likely to influence their volatile composition. Indeed, samples 39 and 64 are in cluster 3 because of the presence of similar volatiles, probably due to their common parent 'Fairchild', and despite the fact that sample 64 is far richer in volatiles than sample 39 (69 volatiles were identified in sample 64 while only 44 were identified in sample 39). The same relationship exists between samples 9 and 38: 53 volatiles were identified in sample 9 (8-9 x 'Orlando') while only 40 volatiles were identified in sample 38 (8-9 x 'Murcott'), but they both have 8-9 as a parent. Samples 16 and 47 are samples from the same tree (8-10) harvested in mid-December and the end of January (Table 1). 1-Penten-3-one, terpinolene, α-terpineol, dihydrocarvone, *d*-carvone, valencene, and three other sesquiterpenoids are the particular volatiles shared by the samples in this cluster and might contribute to the distinctive taste and aroma of these fruits.

Cluster 4 is made of samples that are distinguished by their nonanal and (E)-2-nonenal content. The number of different volatiles in these samples is close to the average. This cluster contains samples having a common parent 'Murcott' (samples 13, 'Fortune' x 'Murcott'; sample 20, 8-9 x 'Murcott'; sample 30, 'Fortune' x 'Murcott'; sample 36, 8-9 x 'Murcott'; sample 37, 8-9 x 'Murcott'; and sample 43, 'Fortune' x 'Murcott'). It is interesting to note that samples 13, 30, and 43 are multiple harvests of the same tree (14 Dec., 22 Dec., 5 Jan.), but the earlier and later harvests of that tree (sample 5, 1 Dec., and sample 89, 23 Mar.) were not included in that cluster. Sample 25 ('Clementine') is present with 8-9 x 'Murcott' in this cluster probably because 'Clementine' is a parent of 8-9.

'Orlando' and 'Murcott' are similar in terms of volatile composition (both are in cluster 5) and they may have similar flavor because they contain α- and β-phellandrene, *d*-carvone, α-copaene, valencene, and cadinene.

Clusters 6, 7, and 8 confirm the uniformity of the volatile composition for samples coming from the same selections. Clusters 6 and 8 are made of repeated harvests of 'Murcott' and 8-9 x 'Murcott', respectively. They are both characterized by the absence of high MW volatiles, and cluster 6 by the absence of valencene, a

volatile present in more than 76% of the samples. The samples in cluster 7 all contain (E)-2-pentanal, (Z)-3-hexenal, 1-octene-3-one and 1-octanol. 1-Octanol, dihydrocarvone, *d*-carvone, citronellol, perillaldehyde, and geranyl acetate are the common volatiles contributing to the grouping of samples originating from crosses between 8-9 and 'Murcott' (cluster 3). It can be noted that other samples of 8-9 x 'Murcott' are also present in clusters 1, 2, 3, and 4. These samples have the same parents (same crosses) but are harvested from different trees (siblings).

Cluster 9 groups samples in which approximately the same numbers of volatile compounds were identified (44 volatiles were detected in sample 50, 38 in sample 76, 42 in sample 81, and 49 in sample 82). Samples 50, 76, and 82 have a common parent, 'Murcott', and it can be noted that the numbers of volatiles identified in 'Murcott' samples (cluster 6) are close to those found in the samples of cluster 9. 'Kinnow' (sample 81) may be similar to the 'Murcott' hybrids because it also contains 2-methyl-1,3-butadiene, 2-methyl-furan, α- and β-phellandrene, 1-octanol, decanal, citronellol, nonanoic acid, and geranyl acetate.

In cluster 10 are samples with a higher number of volatiles than average. Indeed, samples 68 (8-9 x Val4x'), 72 ('Sanguinelli'), 78 ('Temple'), 79 ('Ortanique'), 83 (8-9 x Val4x'), 84 (8-9 x Val4x'), 85 ('Temple'), and 88 ('Ortanique') contain 54, 65, 79, 62, 56, 60, 88, and 57 volatiles, respectively. These samples are from selections that all have some orange in their background, and therefore produce volatiles that are different from most tangerines. 'Sanguinelli' is a sweet blood orange; 'Val4x' is a tetraploid 'Valencia' sweet orange; and 'Temple' and 'Ortanique' both are presumed to be tangors (hybrids between some unknown tangerine and sweet orange selections). Moreover, except 'Sanguinelli', several samples in this cluster are selections that were harvested multiple times: 68, 83, and 84 are 8-9 x Val4x harvested on 9 and 21 Feb. and 23 Mar.; 78 and 85, and 79 and 88, are samples of 'Temple' and 'Ortanique', respectively, harvested 21 Feb. and 23 Mar. All of these samples have six sesquiterpenes and several esters in common (ethyl butanoate, methyl butanoate, ethyl hexanoate, ethyl 3-hydroxyhexanoate, ethyl propanoate, and ethyl octanoate). Esters usually impart a fruity note to orange juice (Plotto et al., 2004) and these samples might therefore taste fruitier. These unique attributes may be related to the fact that they are not true mandarins.

In addition, to show the relationships between volatile composition and genetic origin of the samples, CA2 shows some grouping between samples due to the effect of volatile composition and harvest date. For instance, cluster 1 contained samples harvested early in the season. Indeed, sample 59 ('Fallglo' x 'Sunburst') was harvested 9 Nov. 2006; samples 2, 4, 5, 6, and 7 were harvested 1 Dec. 2006; and sample 19 was harvested 14 Dec. 2006. In comparison, cluster 10 grouped samples that were harvested late in the season. Samples 68 (8-9 × Val 4x) and 72 ('Sanguinelli') were harvested 9 Feb. 2007; samples 78 ('Temple'), 79 ('Ortanique'), and 83 (8-9 x Val 4x') were harvested 21 Feb. 2007; and samples 84 (8-9 x Val 4x'), 85 ('Temple'), and 88 ('Ortanqiue') were harvested 23 Mar. 2007. On the other hand, it also seems that there is a period in which the volatile composition of the samples remains stable. Before and after that period, the volatile composition seems to change with maturity. For instance, one selection of 'Fortune' x 'Murcott' was harvested multiple times over the season (samples 5, 13, 30, 43). Sample 5 was harvested early (1 Dec. 2006) and is located in cluster 1, while the other samples from the same tree were harvested later (sample 13, 30, and 45 harvested 14 Dec. 2006, 22 Dec. 2006, and 5 Jan. 2007, respectively) and were clustered together in cluster 6. In addition samples 27, 46, and 73 harvested on 22 Dec. 2006, 26 Dec. 2006, and 9 Jan. 2007, respectively, from the same tree of 'Murcott' were grouped together in cluster 6. Sample 82, which came from the same tree of 'Murcott', was harvested later in the season (21 Feb. 2007) and is located in cluster 9.

Conclusion. More than 200 volatiles were identified in the juice of fruit harvested on various dates from a diverse group of 56 tangerine hybrids. PCA modeling based on volatile peak area distinguished four samples containing higher volatile levels from the rest of the samples. This statistical analysis emphasized these outliers but a cluster analysis was more meaningful for finding similarities among samples. The cluster analysis performed on volatile presence/absence data gave the most meaningful results. This analysis revealed the influence of the sample genetic background on volatile composition, providing useful information for future breeding efforts. Further research is needed to find the relationships between volatile composition and sample taste and aroma for fresh fruit and processed juice. Moreover, quantification of other compounds (such as acids and sugars, carotenoids, flavonoids, and phenolics) that are known to contribute to taste, aroma, and nutrition of citrus (Sumida et al., 1999; Widodo et al., 1995) will be performed to understand chemical composition contributing to tangerine juice flavor and nutrition.

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