HORTSCIENCE 52(8):1092–1099. 2017. doi: 10.21273/HORTSCI12002-17

Effect of Vector Control and Foliar Nutrition on the Quality of Orange Juice Affected by Huanglongbing: Sensory Evaluation

Anne Plotto[1], Elizabeth Baldwin, Jinhe Bai, John Manthey, Smita Raithore, Sophie Deterre, and Wei Zhao
USDA/ARS Horticultural Research Laboratory, Ft. Pierce, FL 34945

Cecilia do Nascimento Nunes
Food Quality Laboratory, Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida, Tampa, FL 33620

Philip A. Stansly and James A. Tansey
Southwest Florida Research and Education Center, University of Florida - IFAS, Immokalee, FL 34142

Additional index words. flavor, taste, volatiles, sugars, acids, limonoids, flavonoids

***Abstract*. A 3-year study was undertaken to establish the effect of field nutritional sprays, combined with insecticide treatments or not against Asian Citrus psyllid, on the fruit quality of 'Valencia' orange trees affected by the greening disease Huanglongbing (HLB). Four replicated plots were harvested, juiced, and pasteurized. Nine to twelve trained panelists evaluated the juice using seven flavor, five taste, four mouthfeel and three aftertaste descriptors. There was little difference between treatments in 2013; only orange peel flavor and bitterness were significantly lower for the insecticide treatment. In 2014, positive attributes, such as orange and fruity flavor, sweetness and mouthfeel body, were significantly higher in the insecticide treatment. Sourness was highest in untreated control, and there were no differences between treatments for bitterness. In 2015, negative attributes, such as grapefruit, orange peel and typical HLB flavor, sourness, bitterness, and astringency, were significantly higher in untreated control fruit, suggesting perhaps that the beneficial effect of nutritional and insecticide treatments was cumulative, only manifesting on the 3rd year of the study, and or because of the progression of the disease affecting untreated controls. Data are discussed in relation to juice chemical composition, including volatiles, sugars, acids, limonoids, and flavonoids, adding to the fundamental knowledge concerning chemical drivers of orange flavor.**

Huanglongbing or citrus greening is a devastating disease putatively caused by bacterium *Candidatus* Liberibacter asiaticus (*C*Las) transmitted by an insect vector, the Asian citrus psyllid *Diaphorina citri* (Bové, 2006). This phloem bacterium limits nutrient transport within the plant, leading to leaf yellowing and progressive limb defoliation, die-back, and ultimate tree death within 5–10 years (Bové, 2006). Although HLB has been described in plant pathology journals since the 1960s, only anecdotal information on its effect on fruit eating quality was reported (McClean and Schwarz, 1970). When the disease was first discovered in Florida in 2005, research institutions with industry support investigated many aspects of the disease and its effect on tree decline, including the effect on orange juice quality, as reported in the Proceedings of the First International Research Conference on Huanglongbing, Orlando, 2008 (http://www.plantmanagementnetwork.org/proceedings/irchlb/2008/) and peer reviewed publications (Gottwald et al., 2007; Plotto et al., 2008a). Through the systematic analysis of juice and sensory testing, it was found that fruit showing symptoms of the disease (including lopsided shape and small and green colored fruit) resulted in juice that was less sweet, more sour, more bitter, and had an off flavor described as metallic, umami (savory), salty, fermented, green, and stale (Plotto et al., 2010, 2011). Juice from symptomatic oranges generally had lower sugars, higher acids, higher limonoids and some flavonoids, and lower top-note esters (ethyl acetate and ethyl butanoate) than juice of fruit from healthy trees or asymptomatic fruit from infected trees (Baldwin et al., 2010; Bassanezi et al., 2009; Dagulo et al., 2010). These characteristics were more pronounced in juice from 'Hamlin' than 'Valencia', the two dominant cultivars grown in Florida for orange juice and more significant in early harvests compared with later in the season (Baldwin et al., 2010; Plotto et al., 2010).

HLB has had devastating consequences on the $9 billion juice industry in Florida, and has spread to the other citrus producing states including California, Texas, and Arizona (FDACS, 2016). It also affects other citrus producing areas in the world, such as Brazil and China. Even though a cure for the disease has not yet been found, slowing the progression of the disease has been attempted by 1) limiting the bacterium spread through aggressive insect-vector control (Stansly et al., 2010) and 2) maintaining tree vigor using nutrient sprays that would be absorbed through the leaves (Giles, 2011; Masuoka et al., 2011), or both (Stansly et al., 2014; Tansey et al., 2017). Maintaining citrus trees in production has been a goal for the citrus industry until a cure or tolerant/resistant genotypes are found. One objective of this study was to evaluate the sensory quality of juice made from fruit harvested from trees that received the three following treatments over four growing seasons: foliar nutritionals (*N*), insecticidal sprays (*I*), the combination of both (*I* + *N*), vs. control (*C*) trees that received conventional fertilization and pesticide control.

Orange juice flavor is a combination of taste sensations induced by nonvolatile or soluble compounds (sugars, acids, and flavonoids) and aromas from the retronasal perception of volatile compounds. Much is known about the orange juice flavor, effect of cultivars, processing techniques, and storage (Buettner and Schieberle, 2001; Moshonas et al., 1991; Nisperos-Carriedo and Shaw, 1990; Perez-Cacho and Rouseff, 2008). However, fewer studies show the effect of cultural practices on orange juice quality (Carranca et al., 1993; Jones and Parker, 1949; Koo and Smajstrla, 1984; Quaggio et al., 2006; Roussos, 2011). Those studies mostly analyze the effect of fertilizers on overall fruit quality as measured by soluble solids content (SSC), titratable acidity (TA), and vitamin C content. Even fewer studies show the effect of cultural practices on other components of flavor, such as aroma volatiles, limonoids, and flavonoids in citrus, and their contribution to flavor and taste in relation to sensory panel data. A second objective for this article was to take advantage of a large database of sensory characteristics of orange juice over three seasons, along with the chemical composition of the same juice to gain more fundamental knowledge about chemical drivers of orange flavor of HLB-affected fruit.

Received for publication 6 Apr. 2017. Accepted for publication 9 May 2017.

This article was presented as an oral contribution (HP-24) to the 2016 Florida State Horticultural Society meeting in Stuart, FL, 12–14 June 2016.

[1] Corresponding author. E-mail: anne.plotto@ars.usda.gov.

Materials and Methods

Plant materials. Trees of 'Valencia' oranges were planted in 2001 and subjected to *N* and *I* foliar treatments (Tables 1 and 2) as described by Stansly et al. (2014) and Tansey et al. (2017). Briefly, experiments were carried out on a 5.2-ha grove located in Collier Co., Florida, planted in 2001 with *Citrus sinensis* (L.) Osbeck cv. 'Valencia', on Swingle citrumelo, *C. paradisi* Macf. × *Poncirus trifoliata* L., rootstock. Planting density was 373 trees/ha (151 trees/ac) at 7.3 m between rows and 3.7 m within rows (Stansly et al., 2014; Tansey et al., 2017). Trees were under-tree, microsprinkler-irrigated, and standard weed control and fertilization practices were followed (Davies and Jackson, 2009). The grove was 90% infected with HLB within 18 months of commencing treatments in 2008 as ascertained by sampling every fifth tree using a quantitative polymerase chain reaction (qPCR) detection procedure (Li et al., 2006). The grove was divided into 16 plots in a randomized complete block design with two factors: insecticide and foliar nutrients, each at two levels (with and without) (Stansly et al., 2014). Treatments included two insecticide applications during the winter (dormant season) and during the growing season when a nominal threshold (0.2 ACP adults per tap sample in 2012 and 0.1 in 2013, 2014 and 2015) was exceeded (*I*), two to three applications of foliar nutrition (*N*), a combination of insecticide plus nutrition (*I* + *N*) and an untreated control (*C*). Each treatment was replicated four times (Stansly et al., 2014). Insecticide treatments were grouped by growing season from the end of harvest through the beginning of harvest the next year. The two dormant spray applications of broad-spectrum insecticides were made to the entire study site in the winter of 2012–13 at the grower's request, and to *I* and *I* + *N* treatment trees during the winters of 2013–14 and 2014–15 (Table 1). Foliar nutrition applications were applied during major flush periods (spring, summer, and fall) when leaves were fully expanded but not yet hardened (Stansly et al., 2014; Tansey et al., 2017), with slight differences in the nutrition program in the 2012–Sept. 2013 than for the rest of the experimental period (included *Bacillus subtilis* and boron) (Table 2).

Fruit were harvested on 19 Mar. 2013, 26 Apr. 2014, and 17 Apr. 2015. In 2013, fruit were processed using a JBT commercial extractor (JBT FoodTech, Lakeland, FL). In 2014 and 2015, fruit were processed at the USDA Laboratory using a JBT juicer (Fresh'n Squeeze® Point-of-Sale Juicer; JBT FoodTech) and pasteurized using a pilot pasteurizer (UHT/HTST Laboratory 25EHV Hybrid; Microthermics Inc., Raleigh, NC) at 90 °C for 10 s. The DNA of *C*Las was quantified by qPCR as described in a companion article (Baldwin et al., 2017), to determine the level of HLB infection in each juice sample.

Chemical analysis. Aliquots of juice samples were taken for the following analyses: SSC and TA, individual sugars, citric and malic acid, flavonoids, limonoids. and volatile compounds.

For quality determination, SSC and TA were determined before individual sugar and acid analyses. SSC, determined by refractive

Table 1. Insecticides applied to *I* (insecticide-treated) and *I* + *N* (insecticide + nutrition-treated) trees from 2012 to 2015.[z]

Date	Brand name	Active ingredient	Rate/ha	HMO[y]	Company
1 May 2012	Movento® MPC[x]	Spirotetramat	1.17 L	2%	Bayer CropScience LP
15 June 2012	Imidan® 70-W	Phosmet	1.12 kg	1%	Gowan Company
16 Aug. 2012	Dimethoate 4EC[x]	Dimethoate	1.75 L	2%	Helena Chemical
8 Nov. 2012	Delegate® WG[x]	Spinetoram	0.37 kg	1%	Dow AgroSciences LLC
5 Dec. 2012	Danitol® 2.4 EC[w]	Fenpropathrin	1.17 L	—	Valent
24 Jan. 2013	Movento® MPC[w]	Spirotetramat	1.17 L	2%	Bayer CropScience LP
10 Apr. 2013	VoliamFlexi®[x]	Thiamethoxam + Chlorantraniliprole	0.51 kg	1%	Syngenta
31 Oct. 2013	Closer SC Insecticide[x]	Sulfoxaflor	0.37 L	3%	Dow AgroSciences LLC
19 Dec. 2013	Imidan® 70-W[w]	Phosmet	1.12 kg	1%	Gowan Company
22 Jan. 2014	Danitol® 2.4 EC[w]	Fenpropathrin	1.17 L	—	Valent
22 Mar. 2014	Mustang®[x]	Zeta-Cypermethrin	0.31 L	—	FMC Corporation
7 July 2014	Exirel®[x]	Cyantraniliprole	1.46 L	—	Du Pont
19 Dec. 2014	Lorsban® Advanced[w]	Chlorpyrifos	5.85 L	1%	Dow AgroSciences LLC
14 Jan 2015	Baythroid® XL[w]	Fenpropathrin	0.44 L	—	Bayer CropScience LP
1 May 2015	Agri-Flex®[x]	Thiamethoxam + Abamectin	0.62 L	1%	Bayer CropScience LP
27 July 2015	Apta®[x]	Tolfenpyrad	1.82 L	—	Ninchino America Inc

[z] Tansey et al. (2017).
[y] HMO = horticultural mineral oil.
[x] Growing season sprays.
[w] Dormant spray sprays.

Table 2. Components of foliar nutrition applications 2012–15.[z]

	Product	Function	Rate/ha	Company
2012–Sept. 2013	Serenade Max WP (*Bacillus subtilis* 26.2%)	SAR inducer	2.52 kg	AgraQuest, Inc.
	Saver (Potassium salicylate)	SAR inducer	2.34 L	Plant Food Systems
	3–18–20 w/K-Phite® (KH_2PO_3 + K_2HPO_3)	Macronutrient/fungicide	74.83 L	Plant Food Systems
	13–0–44 fertilizer (KNO_3)	Macronutrient	9.53 kg	Diamond R Fertilizer
	Techmangam ($MnSO_4$)	Micronutrient	9.53 kg	Diamond R Fertilizer
	Zinc sulfate	Micronutrient	3.14 kg	Diamond R Fertilizer
	Sodium molybdate	Micronutrient	0.06 kg	Diamond R Fertilizer
	Epsom salts ($MgSO_4$)	Micronutrient	9.53 kg	Diamond R Fertilizer
	Purespray Green® (435 oil)	Adjuvant	46.77 L	Petro-Canada Lubricants, Inc.
Oct. 2013–Sept. 2015	Saver (Potassium salicylate)	SAR inducer	9.35 L	Plant Food Systems
	K-Phite® (KH_2PO_3 + K_2HPO_3)	Fungicide	4.68 L	Plant Food Systems
	13–0–44 fertilizer (KNO_3)	Macronutrient	9.53 kg	Diamond R Fertilizer
	Techmangan ($MnSO_4$)	Micronutrient	9.53 kg	Diamond R Fertilizer
	Zinc sulfate	Micronutrient	3.14 kg	Diamond R Fertilizer
	Sodium molybdate	Micronutrient	0.06 kg	Diamond R Fertilizer
	Epsom salts ($MgSO_4$)	Micronutrient	9.53 kg	Diamond R Fertilizer
	Purespray Green® (435 oil)	Adjuvant	46.77 L	Petro-Canada Lubricants, Inc.
	Beau-Ron® D (boron)	Micronutrient	1.68 kg	Drexel Chemical Co.

SAR = systemic-acquired resistance.
[z] Tansey et al. (2017).

index, was measured with a digital ATAGO PR-101 refractometer (Atago Co, Tokyo, Japan), and TA and pH were calculated from titration of 10 mL of juice with 0.1 mol·L^{-1} NaOH to a pH 8.1 endpoint using a 808 Titrando (Metrohm, Riverview, FL).

Individual sugars were analyzed with a high-performance liquid chromatography (HPLC) system after an optimized extraction of the juice samples (Baldwin et al., 2012). Twenty grams of juice samples were centrifuged (Avanti J-E centrifuge, Beckman-Coulter, Brea, CA) at 11,952 g_n for 20 min at 10 °C. A total of 10 mL of the supernatant was passed through a C-18 Sep-Pak (Waters/Millipore), and the eluate was filtered with a 0.45-µm Millipore (Siemens-Millipore, Shrewbury, MA) filter before analysis by HPLC. The column used was a Sugar-Pak I (10 µm, 6.5 mm × 300 mm) (Waters, Milford, MA) operated at 90 °C in a CH-30 column heater and a TC-50 controller (FIAtron, Milwaukee, WI). Samples were analyzed by injecting 60 µL of the juice supernatant using a Perkin-Elmer Series 200 autosampler and pump (Perkin-Elmer, Waltham, MA) and running through an isocratic system of 0.001 mol·L^{-1} CaEDTA mobile phase with a flow rate of 0.3 mL·min^{-1}. Detection of peaks was done with an Agilent 1100 series refractive index detector (Agilent Technologies, Santa Clara, CA). Quantification was based on the external standard method (Version 3.3.2. SP2; EZChrom Elite software, Santa Clara, CA) using standards for sucrose, glucose, and fructose. All results are expressed as g·100 mL^{-1} of juice.

Organic acids were also analyzed by HPLC of the same preparation as for the individual sugars. Chromatographic separation was done with an AltechOA1000 Prevail organic acid column (9 µm, 300 mm × 6.5 mm) (Grave Davison Discovery Sciences, Deerfield, IL). Samples were introduced to the HPLC system by injecting 60 µL at a flow rate of 0.2 mL·min^{-1} at 35 °C and a mobile phase of 0.005 mol·L^{-1} H_2SO_4. The analytes of interest (citric and malic acids) were detected with a Spectra System ultraviolet 6000 LP photo diode array detector (Thermo Fisher Scientific, Waltham, MA). Quantification was based on the calibration curves for standards of citric and malic acids, expressed as g·100 mL^{-1} of juice.

Concentrations of limonoids and flavonoids in orange juice were determined by

Table 3. Descriptors and reference standards with suggested intensity for orange juice sensory descriptive panel, using a 16-point intensity scale (1 = low, 7–8 = medium, and 15 = high).

Sensory modality	Descriptor (suggested intensity)	Reference standard
Aroma/flavor	Orange (7)	Orange juice, 100% Florida, Gourmet Pasteurized (Natalie's Orchid Island Juice Company, Fort Pierce, FL)
	Grapefruit (15)	Grapefruit juice, 100% Florida, Gourmet Pasteurized (Natalie's Orchid Island Juice Company)
	Fruity noncitrus (12)	A mixture of passion fruit (Welch's, Westfield, NY), mango (Frito-Lay, Inc., Dallas, TX) and pineapple (Dole Food Company Inc., Westlake Village, CA) juices and guava (Sunshine Bottling Company, Doral, FL) and peach (Santiago Felippelli Conway, Miami, FL) nectars and water
	Orange peel (7)	Zests from Hamlin oranges (washed and sanitized before zesting) cut in ≈50 mm^2 pieces (1.4 ± 0.3 g)
	Green (10)	A mixture of (*Z*)-3-hexenal (2 µL·L^{-1}; Sigma-Aldrich, St. Louis, MO) and (*Z*)-3-hexenol (7 µL·L^{-1}; Sigma-Aldrich) in solution at 0.09% of ethanol
	Stale (10)	0.005% v/v in water of N&A Old Flavor Type, Stale (Givaudan Flavors Corp., Cincinnati, OH)
	Typical HLB flavor	Any off-flavor related to HLB disease
Taste	Sweet (7)	8% sucrose (pure sugar; Publix, Lakeland, FL) in water
	Sour (7)	0.2% citric acid (≥99.5%; Sigma-Aldrich) in water
	Umami (7)	0.08% monosodium glutamate (Ac'cent®, B&G Foods Inc., Parsippany, NJ)
	Bitter (7)	11.5 mg·L^{-1} of quinine monohydrochloride dihydrate (90%; Sigma-Aldrich) in water
	Metallic (10)	Canned orange juice (Ruby Kist®, 100% Orange juice from concentrate (Clement Pappas & Co., Inc., Seabrook, NJ)
Mouthfeel	Body (7)	Orange concentrate at 65 °Brix (pumpout) diluted in water to 11.8 °Brix
	Tingling (15)	Carbonated water, ClubSoda (Publix)
	Astringent (15)	Premium English Breakfast Black tea (Publix)
	Burning (7)	Zests from Hamlin oranges cut in ≈50 mm^2 pieces (1.4 ± 0.3 g), washed and sanitized before zesting
Aftertaste	Bitter (7)	11.5 mg·L^{-1} of quinine monohydrochloride dihydrate (90%; Sigma-Aldrich) in water
	Astringent (15)	Premium English Breakfast black tea (Publix)
	Burning (7)	Zests from Hamlin oranges cut in ≈50 mm^2 pieces (1.4 ± 0.3 g), washed and sanitized before zesting

HLB = Huanglongbing.

Table 4. Attribute sensory ratings (n = 9–12) for 'Valencia' orange juice from trees subjected to nutritional (N), insecticide (I), the combination of N and I ($I + N$), and control (C) evaluated in 2013 (13), 2014 (14), and 2015 (15). Sensory ratings are using a 16-point intensity scale (1 = low, 7–8 = medium, and 15 = high).

	13_C	13_N	13_I	13_I + N	14_C	14_N	14_I	14_I + N	15_C	15_N	15_I	15_I + N
Orange flavor	4.7 a	4.4 a	4.5 a	4.8 a	**3.6 ab**	**3.4 b**	**4.2 a**	**3.4 b**	4.8 a	4.7 a	5.0 a	4.8 a
Grapefruit flavor	1.9 a	2.2 a	2.3 a	2.1 a	**2.2 ab**	**1.9 b**	**1.9 b**	**2.4 a**	**2.1 a**	**1.7 b**	**1.4 b**	**1.6 b**
Fruity noncitrus flavor	0.8 a	0.7 a	0.8 a	0.9 a	**1.3 a**	**0.8 b**	**1.4 a**	**1.1 ab**	1.3 a	1.4 a	1.5 a	1.5 a
Orange peel flavor	**2.8 ab**	**3.0 a**	**2.5 b**	**3.0 a**	2.0 a	1.8 a	2.1 a	1.8 a	**2.2 a**	**1.7 b**	**1.8 b**	**1.7 b**
Green flavor	1.6 a	2.0 a	2.0 a	1.8 a	2.1 a	2.2 a	2.0 a	2.5 a	1.8 a	1.6 a	1.6 a	1.5 a
Stale flavor	1.3 a	1.7 a	1.3 a	1.6 a	1.5 a	1.7 a	1.5 a	2.0 a	1.3 a	1.4 a	1.3 a	1.2 a
Typical HLB flavor	2.6 a	2.9 a	2.5 a	3.3 a	3.5 a	3.5 a	3.2 a	4.0 a	**3.2 a**	**2.9 ab**	**2.5 b**	**2.5 b**
Taste-sweetness	5.3 a	4.8 a	4.9 a	5.1 a	**4.5 ab**	**4.3 b**	**4.8 a**	**4.2 b**	5.2 a	5.2 a	5.3 a	5.3 a
Taste-sourness	5.2 a	5.4 a	5.1 a	4.9 a	**7.6 a**	**6.0 b**	**6.8 ab**	**6.6 b**	**6.2 a**	**5.4 b**	**5.6 b**	**5.4 b**
Taste-umami	1.3 a	1.4 a	1.2 a	1.2 a	**1.5 ab**	**1.7 ab**	**1.4 b**	**1.8 a**	1.6 a	1.2 a	1.4 a	1.4 a
Taste-bitterness	**2.0 ab**	**2.6 a**	**1.8 b**	**2.6 a**	2.2 a	1.9 a	2.3 a	2.1 a	**1.9 a**	**1.6 b**	**1.5 b**	**1.7 ab**
Taste-metallic	1.2 a	1.6 a	1.4 a	1.6 a	1.3 a	1.3 a	1.2 a	1.4 a	1.2 a	1.0 a	1.0 a	1.1 a
Mouthfeel-body	9.1 a	8.9 a	8.8 a	9.1 a	**4.9 a**	**4.5 ab**	**4.9 a**	**4.3 b**	**5.2 a**	**4.7 b**	**5.2 a**	**5.1 a**
Mouthfeel-tingling	1.5 a	1.6 a	1.4 a	1.2 a	1.3 a	1.1 a	1.5 a	1.3 a	1.5 a	1.3 a	1.1 a	1.4 a
Mouthfeel-astringent	1.5 a	1.8 a	1.7 a	1.6 a	1.8 a	1.6 a	2.0 a	1.8 a	**2.0 a**	**1.6 ab**	**1.5 b**	**1.7 ab**
Mouthfeel-burning	1.4 a	1.6 a	1.2 a	1.5 a	**1.8 a**	**1.5 ab**	**1.8 ab**	**1.5 b**	1.8 a	1.6 a	1.5 a	1.6 a
Aftertaste-bitter	1.1 a	1.4 a	0.9 a	1.4 a	**0.8 b**	**1.0 a**	**1.0 ab**	**1.1 a**	1.0 a	1.0 a	0.9 a	1.1 a
Aftertaste-astringent	1.2 a	1.4 a	1.3 a	1.2 a	1.3 a	1.3 a	1.3 a	1.3 a	1.3 a	1.1 a	1.1 a	1.3 a
Aftertaste-burning	1.1 a	1.1 a	0.9 a	0.9 a	1.3 a	1.0 a	1.3 a	1.2 a	1.5 a	1.3 a	1.3 a	1.3 a

HLB = Huanglongbing.

Means followed by a different letter within a row by year are significantly different using the Fisher's LSD test, alpha = 0.05 and are in bold letters.

HPLC–mass spectrometry (HPLC-MS) following a previous method (Baldwin et al., 2010). Each juice sample (10 mL) was added to 30 mL of methanol and 70 µL of 1.8 mg·mL^{-1} mangiferin (internal standard). After manually shaking 60 times, the mixture was incubated at 55 °C for 15 min in a shaking incubator (130 rpm) and then exposed to a –20 °C freezer for 5 min. The cooled mixture was centrifuged at 15,000 g_n for 15 min at 5 °C, and the supernatant was collected. The pellets were extracted again with 10 mL of deionized water and 30 mL of methanol by repeating the previous shaking, incubation, and centrifuging regimen. The supernatants were merged and concentrated using a rotary evaporator to yield 2.5 mL extract. The concentrated sample was then passed through a 0.45-µm PTFE filter for HPLC-MS analysis. A Waters 2695 Alliance HPLC (Waters, Medford, MA) connected in parallel with a Waters 996 PDA detector and a Waters/Micromass ZQ single quadrupole mass spectrometer equipped with an electrospray ionization source was used for the analysis. Compound separations were achieved with a Waters Atlantis dC18 column (2.1 mm × 100 mm), using solvent gradient conditions as reported previously (Baldwin et al., 2010). Elution conditions included a binary solvent gradient composed initially of 0.1 mL formic acid/100 mL water and acetonitrile (90/10 v/v) and increased with linear gradients to 85/15 (v/v) over 10 min, then to 75/25 (v/v), 60/40 (v/v) and 30/70 (v/v) over 15, 23, and 40 min, respectively, and finally equilibrating to the initial condition of 90/10 (v/v) over 60 min, at a flow rate of 0.75 mL·min^{-1}. Postcolumn split to the PDA and mass ZQ detector was 10:1. MS parameters were as follows: ionization mode, ES$^+$; capillary voltage 3.0 kV, extractor voltage 5 V; source temperature 100 °C; desolvation temperature 225 °C; desolvation N_2 flow 465 L·h^{-1}; cone N_2 flow 70 L·h^{-1}. Protonated ions $[M + H]^+$ were monitored in scan mode. Quantification was based on the calibration curves for authentic standards of each flavonoid and limonoid compounds analyzed, expressed as g/100 mL of juice.

Samples for aroma volatiles were also collected. Three milliliters of juice was transferred to a 10 mL crimp-capped vial at the pilot plant, transported on ice to the laboratory, then stored at –80 °C. Frozen samples were thawed under running tap water and injected onto an Agilent 6890 (Agilent Technologies) gas chromatography (GC) using a Gerstel multipurpose autosampler equipped with Stabilwax and HP-5 low bleed columns. The flow rate was split equally to the two columns at 17 mL·min^{-1} at 40 °C with an increase in temperature at 6 °C·min^{-1} up to 180 °C, where the temperature was held constant for an additional 5.8 min. The GC peaks for the aroma volatile compounds were quantified using standard curves as determined by enrichment of deodorized orange juice by known concentrations of authentic volatile compound standards (Baldwin et al., 2010). Volatile compound identification was confirmed by using a headspace and Solid Phase Microextraction (SPME) fibers along with MS following methods described by (Bai et al., 2014). Briefly, juice samples were incubated for 30 min at 40 °C. A 2-cm SPME fiber (50/30 µm DVB/Carboxen/PDMS; Supelco, Bellefonte, PA) was then exposed to the headspace for 30 min at 40 °C. After exposure, the SPME fiber was inserted into the injector of a GC-MS (Model 6890; Agilent) to desorb the extract for 15 min at 250 °C. The GC-MS equipment and settings were DB-5 (60 m length, 0.25 mm i.d., 1.00 µm film thickness; J&W Scientific, Folsom, CA) columns, coupled with an MS detector (5973 N; Agilent). Mass units were monitored from 30 to 250 m/z and ionized at 70 eV. Data were collected using a data system (ChemStation G1701 AA; Hewlett-Packard, Palo Alto, CA). A mixture of C-5 to C-18 n-alkanes was run at the beginning of each day to calculate retention indices.

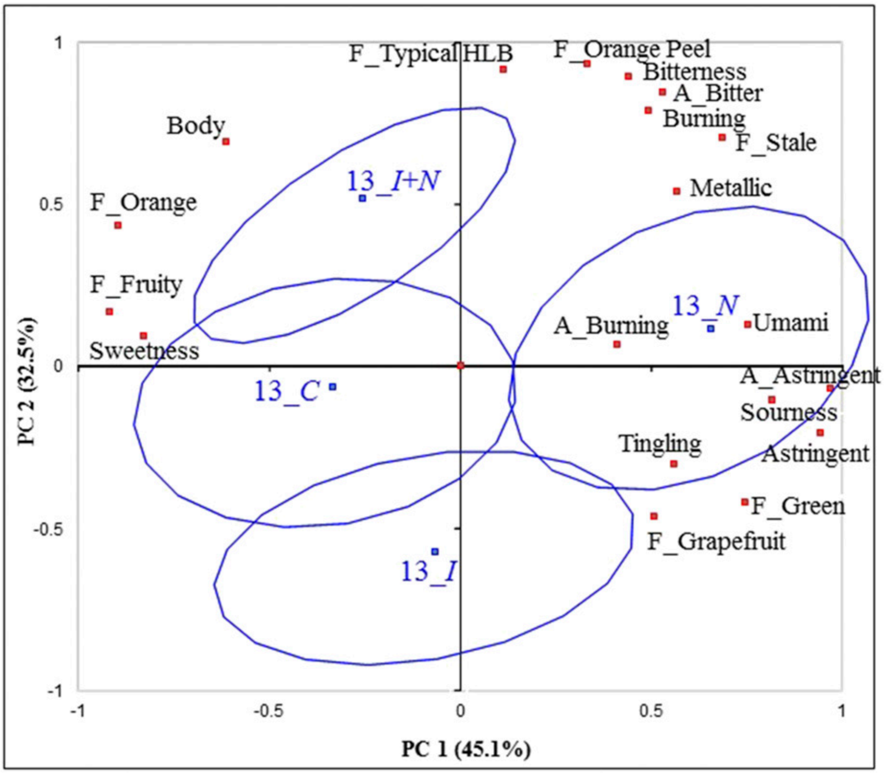

Fig. 1. Principal components analysis (PCA) biplot of sensory ratings for 'Valencia' orange juice from trees subjected to nutritional (*N*), insecticide (*I*), the combination of *N* and *I* (*I* + *N*) field treatments, and control (*C*) evaluated in 2013. Attributes preceded by the letter F and A stand for "Flavor" and "Aftertaste," respectively. Circles around each sample point represent confidence interval for sensory data (average of *n* = 10).

Sensory evaluation. Nine to twelve panelists were specifically trained for orange juice descriptive analysis, with a core of seven panelists having evaluated orange juice samples for over 5 years. Training consisted of twelve 1-h sessions in the 1st and 2nd year, and a "refresher" training using the Compusense® five (Compusense Inc., Ontario, Canada) Feed Back Calibration Method (FMC®) feature in four sessions on the 3rd year. Nineteen descriptors and reference standards were developed including seven descriptors for aroma/flavor, five for taste, four for mouthfeel, and three for aftertaste (Table 3). Only the "typical HLB flavor" descriptor was rated according to each panelist's perception, based on their experience of tasting juice affected with HLB for the last 5 years.

Four samples representing the juice of each treatment (*C*, *N*, *I*, and *I* + *N*) were evaluated at each tasting session. In 2013 and 2014, juice from the field replications was combined and juice was tasted in two sessions to account for panelist variation. In 2015, the four field replications were kept separate, and therefore, panelists evaluated the juice in four sessions, each tasting session representing a field replication. The order of presentation was randomized across the four samples, following a Williams design (Compusense®). The Williams design is a special case of orthogonal Latin square design where the order of sample presentation is balanced across panelists. Samples were served as 50 mL juice in 110 mL cups (Solo® Cups Company, Urbana, IL). Reference standards as well as a "warm-up" sample (orange juice standard) were served at each session. Samples, reference standards and warm up were served at 16 ± 2 °C. First, panelists were asked to taste all the reference standards to review descriptors characteristics and then take a sip of the "warm-up" sample without rating before tasting the juice samples. Panelists rated descriptors using a 16-point intensity scale where 0 = none,

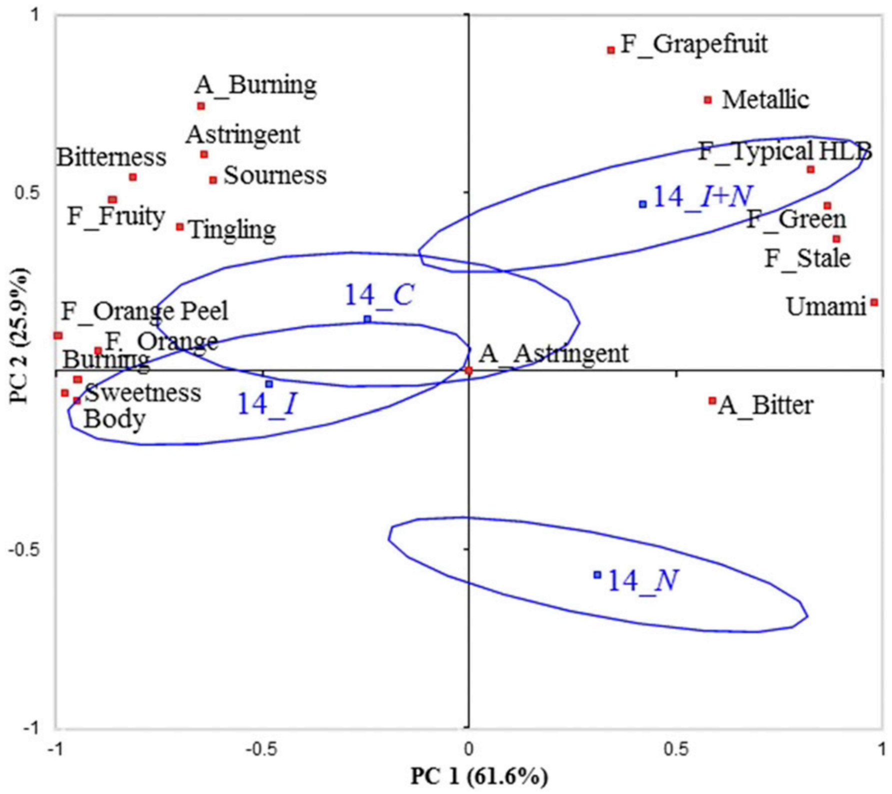

Fig. 2. Principal components analysis (PCA) biplot of sensory ratings for 'Valencia' orange juice from trees subjected to nutritional (*N*), insecticide (*I*), the combination of *N* and *I* (*I* + *N*) field treatments, and control (*C*) evaluated in 2014. Attributes preceded by the letter F and A stand for "Flavor" and "Aftertaste," respectively. Circles around each sample point represent confidence interval for sensory data (average of *n* = 12).

1 = low, 7–8 = medium, and 15 = high, and data were recorded using Compusense® *five*. All taste panels took place in isolated booths equipped with computers, and under positive air pressure and red lighting. Water and unsalted crackers were provided to rinse the mouth between samples as necessary.

Statistical analyses. Sensory data were analyzed for each year by analysis of variance using a mixed model where "panelists" are random, and the main effect is tested against the interaction (Panelist × Sample), and by principal components analysis (PCA) using SenPAQ v. 5.01 (Qi Statistics Ltd., Berkshire, UK). Differences between means were performed using the least significant difference test, with probability error α = 0.05. Relationship between sensory descriptors and chemical components were established using partial least square (PLS) analysis using *X*LSTAT 2014.5.01 (Addinsoft, Paris, France). The sensory ratings were entered in the model as a matrix of dependent variables (*Y*), and chemical components as the independent variables (*X*). PLS calculates a regression model between the components of *Y* and *X*, principal component vectors of the dependent and explanatory variables, respectively (Bastien et al., 2005; Tenenhaus et al., 2005).

Results and Discussion

Sensory characteristics of juice from different nutritional treatments. Differences in sensory characteristics between treatments varied from year to year. In 2013, orange peel flavor and bitterness were the only variables showing significant treatment effect, with *N* and *I* + *N* having higher ratings in both descriptors, and *I* lower ratings (Table 4). The PCA confirmed some overlap between treatments, with PC 1 explaining only 45.1% of the variation, and PC 2 explaining 32.5% (Fig. 1). The nutritional treatment *N* tended to have higher scores on the positive side of PC 1, with attributes indicative of poor quality such as grapefruit flavor, tingling, astringent, sourness, and umami. Orange and fruity noncitrus flavor, and sweetness had high loadings on the negative side of PC 1. Samples from *C*, *I*, and *I* + *N* were on the negative side of PC 1, indicating high scores on orange and fruity flavor, and sweetness. Treatment *I* + *N* also had higher scores for body, typical HLB flavor, orange peel, bitterness (and aftertaste bitterness), burning, stale, and metallic (Fig. 1). Therefore, the nutritional treatments (*N* and *I* + *N*) were associated with both positive and negative flavor characteristics.

In 2014, more descriptors showed significant differences between treatments, with *I* higher in orange and fruity flavor, sweetness and body, and lower in grapefruit flavor and umami, *C* higher in fruity flavor, sourness, body and burning, and *I* + *N* lower in orange flavor, sweetness, sourness, body, and burning, along with *N* except for body and burning, and was highest in grapefruit flavor and umami (Table 4). PCA showed better separation between treatments along PC 1 (61.6% of the variation, Fig. 2) than in 2013, with scores of *N* and *I* + *N* on the positive side of PC 1, describing negative characteristics typical of juice from HLB-affected fruit. However, on the negative side of PC 1, sensory descriptors, such as bitterness, astringent, sourness and tingling, were correlated with a descriptor indicative of juice quality, fruity noncitrus, and burning mouthfeel, and orange peel flavors were correlated with sweetness, body mouthfeel, and orange flavor. The latter set of correlations could be explained by the fact that this juice was made with 'Valencia' oranges, which are usually high in peel oil. Orange peel oil contains flavor components that contribute to orange flavor and sweetness (Perez-Cacho and Rouseff, 2008), but impart a burning sensation, which could be confused with bitterness.

In 2015, *C* had significantly higher ratings for many of the negative quality descriptors indicative of symptomatic fruit: grapefruit, orange peel, and typical HLB flavors, sourness, bitterness, and astringency (Table 4). PCA analysis reflected those results, with a clear separation between *C* and all other treatments on PC 1 (63.9% of the variation), with all the negative attributes of orange juice flavor contributing to the positive loadings on PC 1, except for stale flavor (Fig. 3). Both treatments containing insecticides, *I* and *I* + *N*, were on the negative side of PC 1 with higher scores for quality attributes fruity noncitrus, orange flavor, and sweetness. *N* was also on the negative side of PC 1, with positive orange juice attributes; however, it had a high score for stale flavor on the negative side of PC 2 (18.7% of the variation).

Sensory-chemical relationships. PLS regressions between sensory and chemical data were performed to glean more information on chemical drivers of orange flavor. PLS analysis indicated that 69.3%, 87.3%, and 84.6% of the variation in *Y* (sensory dependent variable) was explained by the two-dimension model in 2013, 2014 and 2015, respectively. Figures 4–6 show the biplots of correlations between sensory and chemical data in the sample score space for each year. In these plots, the variables *X* and *Y* are visualized in such a way that if two variables are close to each other and near the circle, they are positively correlated, whereas if they are also near the circle but opposite from each other, they are negatively correlated. Variables inside the circle have low or no correlations.

In 2013, body, sweetness, orange, and fruity noncitrus flavors were partially explained by octanal, valencene, SSC/TA, and ethyl 3-hydroxyhexanoate (Fig. 4). Octanal (detected at 1.1–1.2 $\mu L \cdot L^{-1}$) has a citrus-like, geranium, and floral aroma (Perez-Cacho and Rouseff, 2008) and was largely above its threshold (*T*) concentration ($T = 0.153\ \mu L \cdot L^{-1}$) (Plotto et al., 2004) in the juice from all treatments. Valencene concentration was at about its detection threshold (T = 3.75 $\mu L \cdot L^{-1}$) (Plotto et al., 2008b), with levels ranging from 5.59 to 6.43 $\mu L \cdot L^{-1}$, and together with a higher SSC/TA ratio in the control treatment, could explain contribution to sweetness and body, mostly in control juice. Fruity flavor was also explained by methyl butanoate and hexanol, but this was not a strong contribution as correlations were less than 1.0. Furthermore, methyl butanoate, an ester imparting spoiled aroma to orange juice when present at its recognition threshold, was at concentration below detection threshold (0.011–0.027 $\mu L \cdot L^{-1}$ in juice; T = 0.146 $\mu L \cdot L^{-1}$) (Plotto et al., 2008b). Sourness, umami and tingling were correlated with the monoterpene hydrocarbons myrcene, limonene, sabinene and α-pinene, adehydes hexanal, and acetaldehyde, and ester ethyl butanoate; TA and citric + malic acid, as well as tangeritin and nobiletin. Although greater sourness can easily be explained by higher TA and citric acid, it can only be speculated that the monoterpene hydrocarbons together with the nonvolatile compounds contribute to umami and tingling taste and mouthfeel and would need to be confirmed in separate experiments. Acetaldehyde (>14.7 $\mu L \cdot L^{-1}$), ethyl butanoate (>0.35 $\mu L \cdot L^{-1}$), and ethyl hexanoate (0.033–0.044 $\mu L \cdot L^{-1}$) were present at concentrations more than 10-fold their taste thresholds (0.152, 0.001, and 0.0023 $\mu L \cdot L^{-1}$, respectively) (Plotto et al., 2008b), which could explain an imbalance in flavor perception contributing to umami, tingling, and burning. A high concentration of ethanol (810–948 $\mu L \cdot L^{-1}$), also above detection threshold in orange juice (313 $\mu L \cdot L^{-1}$, Plotto et al., unpublished data), could explain the burning sensation in the 'Valencia' juice in this study. Astringent, green, and grapefruit flavors were correlated with decanal, 2-methyl propanol, and terpinen-4-ol, the bitter flavonoid sinensetin and the bitter limonoids, limonin and nomilin ($L + N$). Limonin+nomilin concentrations in juice from N (4.06 $mg \cdot L^{-1}$) and I (3.96 $mg \cdot L^{-1}$) treatments were at about recognition level in orange juice (Dea et al., 2013), explaining an association between bitterness and grapefruit flavor. However, nobiletin (2.5–3.2 $mg \cdot L^{-1}$) and tangeritin (0.68–0.91 $mg \cdot L^{-1}$) were at concentrations much below their recognition threshold for bitterness (80–100 $mg \cdot L^{-1}$ in water) (Batenburg et al., 2016), making them unlikely to directly contribute to bitterness in the 'Valencia' juice samples. Chemical compounds that are known to contribute to positive orange juice flavor including linalool, SSC and total sugars (TS), were also correlated with the negative sensory attributes such as "grapefruit," "burning," and "astringent," confirming that high correlations do not necessarily indicate causality, but only that variables change in the same direction (i.e., increase or decrease together).

In 2014, sourness, bitterness, astringent, and tingling were correlated with citric+malic acids, monoterpene hydrocarbons myrcene, limonene, and α-pinene, cis-3-hexenol (although weak correlations with those volatiles),

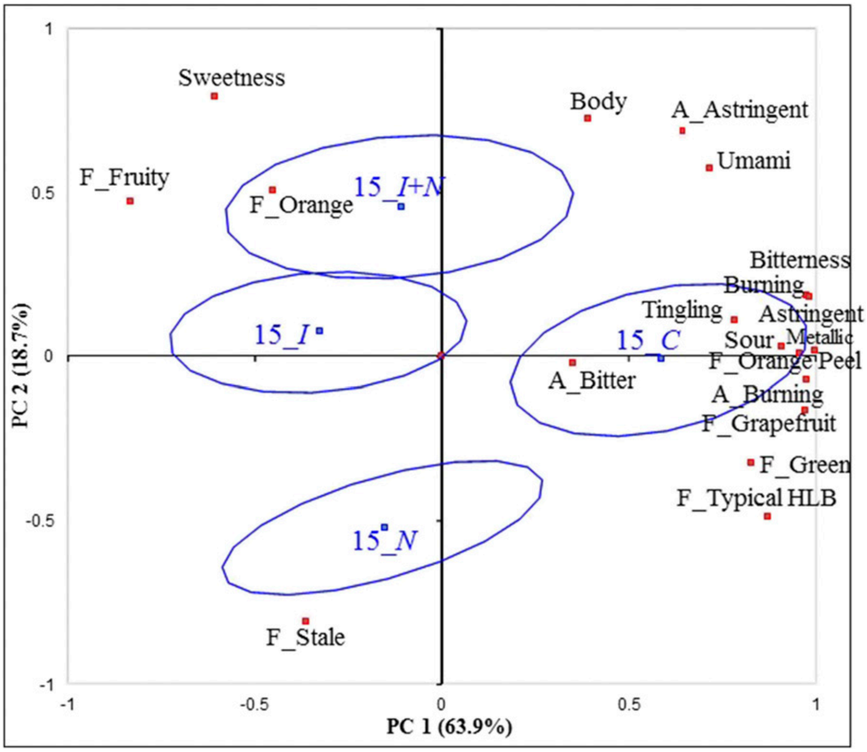

Fig. 3. Principal components analysis (PCA) biplot of sensory ratings for 'Valencia' orange juice from trees subjected to nutritional (N), insecticide (I), the combination of N and I ($I + N$) field treatments, and control (C) evaluated in 2015. Attributes preceded by the letter F and A stand for "Flavor" and "Aftertaste," respectively. Circles around each sample point represent confidence interval for sensory data (average of n = 9).

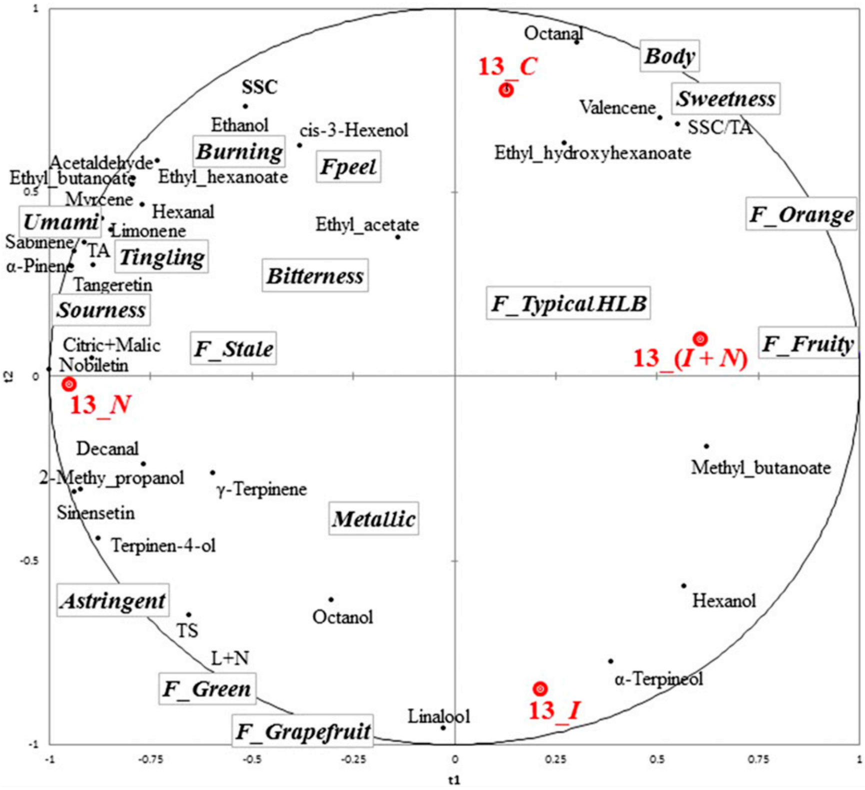

Fig. 4. Partial least square (PLS) regressions biplot of correlations between sensory ratings and chemical measurements in the sample score space (t_1, t_2) for 'Valencia' orange juice from trees subjected to nutritional (N), insecticide (I), the combination of N and I ($I + N$) field treatments, and control (C) evaluated in 2013. Attributes preceded by the letter F and A stand for "Flavor" and "Aftertaste," respectively. SSC = soluble solids content, TA = titratable acidity, TS = total sugars, $L + N$ = limonin + nomilin.

whereas fruity flavor was correlated with TS (Fig. 5). Orange and orange peel flavors, sweetness, burning, and body were correlated with valencene, γ-terpinene, hexanal, and ethyl acetate and weakly with SSC/TA. These correlations do not explain causation because most compounds were below their thresholds in orange juice, except for valencene right at detection threshold (T = 3.75 μL·L^{-1}; 3.30–3.95 μL·L^{-1} in juice) (Plotto et al., 2008b); however, there could be synergistic effects. Grapefruit flavor, metallic, HLB, green, and stale flavors, as well as umami were correlated with α-terpineol, 2-methylpropanol, decanal, octanol, and ethyl hexanoate. The bitter limonoids, $L + N$, TA, the bitter flavonoids (sinensetin, tangeritin, and nobiletin), as well as SSC were negatively correlated with most sensory attributes. The bitter limonoids, $L + N$ (detected at 1.68–2.56 mg·L^{-1}) as well as nobiletin and tangeritin (detected at 2.5–5.6 and 0.4–1.18 mg·L^{-1}, respectively) were at below detection level based on published thresholds in orange juice (Dea et al., 2013) and threshold in water (Batenburg et al., 2016), explaining why it would not be correlated with bitterness.

In 2015, sweetness, orange, and fruity flavors were correlated with octanol, TS, SSC/TA, ethyl-3-hydroxyhexanoate, and valencene (Fig. 6). The latter two volatile compounds were above their thresholds in orange juice (threshold for ethyl-3-hydroxyhexanoate, T = 4.83 μL·L^{-1}, in juice 71.1–86.1 μL·L^{-1}; valencene, T = 3.75 μg·L^{-1}, in juice 4.6–5.4 μL·L^{-1}) (Plotto et al., 2008b). Stale flavor was correlated with the volatiles ethanol (detected at 594–749 mL·L^{-1}), 2-methl propanol (detected at 0.13–0.20 μL·L^{-1}), ethyl butanoate (detected at 0.17–0.22 μL·L^{-1}), as well as with SSC and the two bitter flavonoids sinensetin and nobiletin (detected at 2.5–3.2 mg·L^{-1} and 1.6–2.1 mg·L^{-1}, respectively). Ethanol and ethyl butanoate were at about twice and 40 times their concentration thresholds in orange juice, respectively, and could explain the perception of staleness. On the other hand, 2-methyl propanol was at a concentration below reported threshold in water (T = 1.0 μL·L^{-1}) (Rychlik et al., 1998), as well as nobiletin and sinensetin (Batenburg et al., 2016). Most negative sensory attributes were correlated with the volatile decanal, and "typical HLB" and "green" flavors were correlated with the volatile limonene. Even though detected at lower concentrations than in 2013 and 2014, decanal (0.32–0.39 μL·L^{-1}) was again above its detection threshold (T = 0.07 μL·L^{-1}) (Plotto et al., 2004) in 2015, explaining strong correlation with attributes such as "metallic" and "orange peel." As in 2014, the bitter limonoids $L + N$ were not correlated with any of the negative sensory attributes and were below their detection thresholds.

In summary, SSC/TA and valencene contributed to positive attributes in all 3 years; linalool, ethyl butanoate, and TS only in 2014 and 2015; and ethyl-3-hydroxyhexanoate in 2013 and 2015. Monoterpene hydrocarbons, decanal, and 2-methyl propanol contributed to negative attributes all 3 years, citric + malic

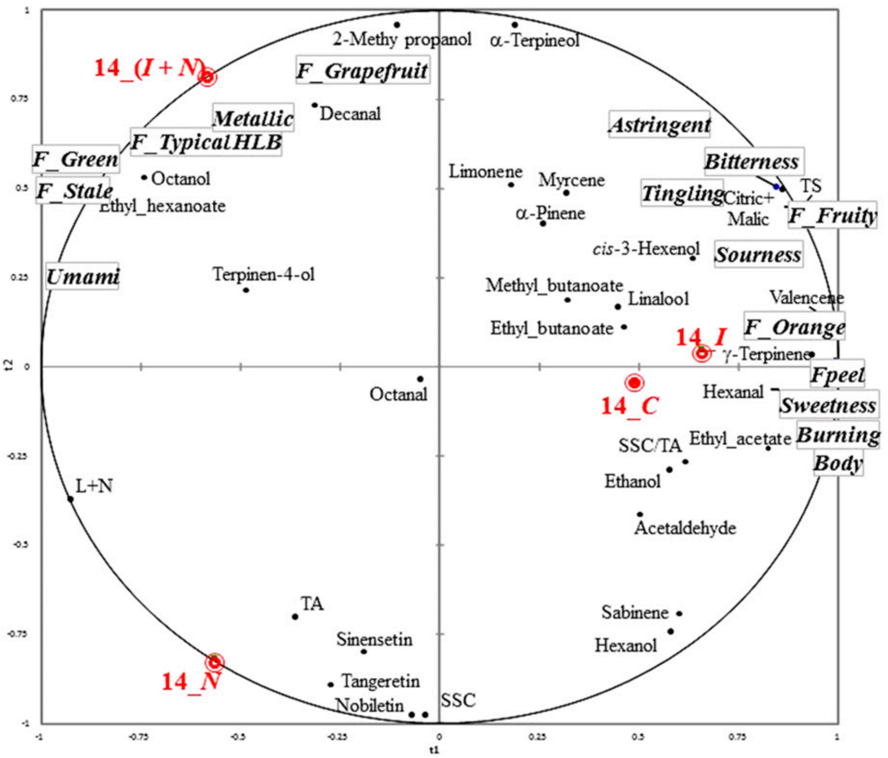

Fig. 5. Partial least square regressions biplot of correlations between sensory ratings and chemical measurements in the sample score space (t_1, t_2) for 'Valencia' orange juice from trees subjected to nutritional (N), insecticide (I), the combination of N and I ($I + N$) field treatments, and control (C) evaluated in 2014. Attributes preceded by the letter F and A stand for "Flavor" and "Aftertaste," respectively. SSC = soluble solids content, TA = titratable acidity, TS = total sugars, $L + N$ = limonin + nomilin.

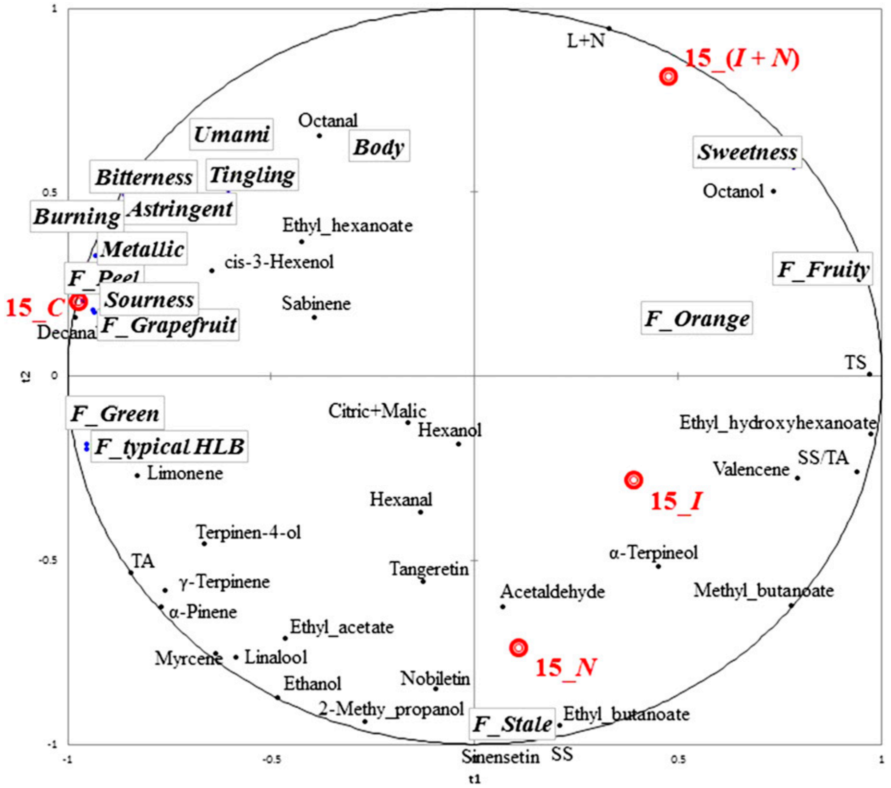

Fig. 6. Partial least square regressions biplot of correlations between sensory ratings and chemical measurements in the sample score space (t_1, t_2) for 'Valencia' orange juice from trees subjected to nutritional (N), insecticide (I), the combination of N and I ($I + N$) field treatments, and control (C) evaluated in 2015. Attributes preceded by the letter F and A stand for "Flavor" and "Aftertaste," respectively. SSC = soluble solids content, TA = titratable acidity, TS = total sugars, $L + N$ = limonin + nomilin.

acids in 2013 and 2014, and bitter limonoids only contributed to bitterness in 2013. The contribution of some of the chemicals, mostly volatiles, to sensory characteristics could be explained by their concentrations above threshold in the juice analyzed, but not always. As with the chemical composition of the juice treatments, sensory quality was not consistent over the 3-years, although either I or $I + N$ was associated with the positive attributes of orange and fruity flavor and sweetness over the 3 years. In the 3rd year of the study (2015), N and I treatments clearly improved quality of orange juice, as measured by sensory characteristics. This could be the result of cumulative effects of field treatments, also shown in chemical composition of higher SSC/TA and lower TA for I and $I + N$ in 2014 and 2015, as well as lower *C*Las levels for those treatments reported in a companion article (Baldwin et al., 2017). *C*Las levels were shown to be negatively correlated with juice quality (Zhao et al., 2015). It is also possible that the control trees, which did not receive as intensive management care with insecticide and nutritional foliar sprays, were declining faster because of *C*Las infection.

Literature Cited

Bai, J., E.A. Baldwin, J. Hearn, R. Driggers, and E. Stover. 2014. Volatile profile comparison of USDA sweet-orange-like hybrids vs. 'Hamlin' and 'Ambersweet'. HortScience 49:1262–1267.

Baldwin, E.A., J. Bai, A. Plotto, R. Cameron, G. Luzio, J. Narciso, J. Manthey, W. Widmer, and B.L. Ford. 2012. Effect of extraction method on quality of orange juice: Hand-squeezed, commercial-fresh squeezed and processed. J. Sci. Food Agr. 92:2029–2042.

Baldwin, E.A., J. Bai, A. Plotto, J. Manthey, S. Raithore, S. Deterre, and W. Zhao. 2017. Effect of vector control and foliar nutrition on quality of orange juice affected by huanglongbing (HLB): Chemical analysis. HortScience 52:1100–1106.

Baldwin, E., A. Plotto, J. Manthey, G. McCollum, J. Bai, M. Irey, R. Cameron, and G. Luzio. 2010. Effect of Liberibacter infection (huanglongbing disease) of citrus on orange fruit physiology and fruit/fruit juice quality: Chemical and physical analyses. J. Agr. Food Chem. 58:1247–1262.

Bassanezi, R., L. Montesino, and E. Stuchi. 2009. Effects of huanglongbing on fruit quality of sweet orange cultivars in Brazil. Eur. J. Plant Pathol. 125(4):565.

Bastien, P., V.E. Vinzi, and M. Tenenhaus. 2005. PLS generalised linear regression. Comput. Stat. Data Anal. 48:17–46.

Batenburg, A.M., T. de Joode, and R.J. Gouka. 2016. Characterization and modulation of the bitterness of polymethoxyflavones using sensory and receptor-based methods. J. Agr. Food Chem. 64:2619–2626.

Bové, J.M. 2006. Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. J. Plant Pathol. 88:7–37.

Buettner, A. and P. Schieberle. 2001. Evaluation of aroma differences between hand-squeezed juices from Valencia late and Navel oranges by quantitation of key odorants and flavor reconstitution experiments. J. Agr. Food Chem. 49:2387–2394.

Carranca, C.F., J. Baeta, and M.A.C. Fragoso. 1993. Effect of NK fertilization on leaf nutrient content and fruit quality of 'Valencia late' orange trees, p. 445–448. In: M.A.C. Fragoso, M.L. Van Beusichem, and A. Houwers (eds.). Optimization of plant nutrition: Refereed papers from the Eighth International Colloquium for the Optimization of Plant Nutrition, 31 Aug.–8 Sept. 1992, Lisbon, Portugal. Springer Netherlands, Dordrecht, The Netherlands.

Dagulo, L., M.D. Danyluk, T.M. Spann, M.F. Valim, R. Goodrich-Schneider, C. Sims, and R. Rouseff. 2010. Chemical characterization of orange juice from trees infected with citrus greening (huanglongbing). J. Food Sci. 75: C199–C207.

Davies, F.S. and L.K. Jackson. 2009. Citrus growing in Florida. 5th ed. University Press of Florida, Gainesville, FL.

Dea, S., A. Plotto, J.A. Manthey, S. Raithore, M. Irey, and E. Baldwin. 2013. Interactions and thresholds of limonin and nomilin in bitterness perception in orange juice and other matrices. J. Sens. Stud. 28:311–323.

FDACS. 2016. Florida citrus statistics. USDA, National Agricultural Statistics Service, Tallahassee, FL.

Giles, F. 2011. An alternative approach. Florida Grower Magazine. 31 Aug. 2011.

Gottwald, T.R., J.V. da Graça, and R.B. Bassanezi. 2007. Citrus huanglongbing: The pathogen and its impact. Plant Health Prog. doi: 10.1094/PHP-2007-0906-01-RV.

Jones, W.W. and E. Parker. 1949. Effects of nitrogen, phosphorus, and potassium fertilizers and of organic materials on the composition of Washington Navel orange juice. Proc. Amer. Soc. Hort. Sci. 53:91–102.

Koo, R.C.J. and A.G. Smajstrla. 1984. Effect of trickle irrigation and fertigation on fruit production and juice quality of 'Valencia' orange. Proc. Florida State Hort. Soc. 97:8–10.

Li, W., J.S. Hartung, and L. Levy. 2006. Quantitative real-time PCR for detection and identification of *Candidatus Liberibacter* species associated with citrus huanglongbing. J. Microbiol. Methods 66:104–115.

Masuoka, Y., A. Pustika, S. Subandiyah, A. Okada, E. Hanundin, B. Purwanto, M. Okuda, Y. Okada, A. Saito, P. Holford, A. Beattie, and T. Iwanami. 2011. Lower concentrations of microelements in leaves of citrus infected with '*Candidatus Liberibacter asiaticus*'. Jpn. Agr. Res. Q. 45:269–275.

McClean, A.P.D. and R.E. Schwarz. 1970. Greening or blotchy-mottle disease of citrus. Phytophylactica 2:177–194.

Moshonas, M.G., P.E. Shaw, and R.D. Carter. 1991. Ambersweet orange hybrid: Compositional evidence for variety classification. J. Agr. Food Chem. 39:1416–1421.

Nisperos-Carriedo, M.O. and P.E. Shaw. 1990. Comparison of volatile flavor components in fresh and processed orange juices. J. Agr. Food Chem. 38:1048–1052.

Perez-Cacho, P.R. and R.L. Rouseff. 2008. Fresh squeezed orange juice odor: A review. Crit. Rev. Food Sci. Nutr. 48:681–695.

Plotto, A., E. Baldwin, G. McCollum, J. Manthey, J. Narciso, and M. Irey. 2010. Effect of *Liberibacter* infection (huanglongbing or "greening" disease) of citrus on orange juice flavor quality by sensory evaluation. J. Food Sci. 75: S220–S230.

Plotto, A., E.A. Baldwin, T.G. McCollum, J.A. Narciso, and M. Irey. 2008a. Effect of early detection huanglongbing on juice flavor and chemistry. Proc. Annu. Mtg. Fla. State Hort. Soc. 121:265–269.

Plotto, A., C.A. Margaría, K.L. Goodner, and E.A. Baldwin. 2008b. Odour and flavour thresholds for key aroma components in an orange juice matrix: Esters and miscellaneous compounds. Flavour Fragr. J. 23:398–406.

Plotto, A., C.A. Margaría, K.L. Goodner, R. Goodrich, and E.A. Baldwin. 2004. Odour and flavour thresholds for key aroma components in an orange juice matrix: Terpenes and aldehydes. Flavour Fragr. J. 19:491–498.

Plotto, A., M.F. Valim, R.L. Rouseff, S. Dea, J. Manthey, J. Narciso, J. Bai, M. Irey, and E. Baldwin. 2011. Sensory evaluation of juice made with fruit from huanglongbing (HLB) affected trees. The Second International Research Conference on Huanglongbing. 10–14 Jan. 2011. American Phytopathology Society (APS), Orlando, FL.

Quaggio, J.A., D. Mattos, and H. Cantarella. 2006. Fruit yield and quality of sweet oranges affected by nitrogen, phosphorus and potassium fertilization in tropical soils. Fruits 61:293–302.

Roussos, P.A. 2011. Phytochemicals and antioxidant capacity of orange (*Citrus sinensis* (L.) Osbeck cv. Salustiana) juice produced under organic and integrated farming system in Greece. Sci. Hort. 129:253–258.

Rychlik, M., P. Schieberle, and W. Grosch. 1998. Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants. Institut für Lebensmittelchemie der Technischen Universität München und Deutsche Forschungsanstalt für Lebensmittelchemie Garching, Germany.

Stansly, P.A., H.A. Arevalo, J.A. Qureshi, M.M. Jones, K. Hendricks, P.D. Roberts, and F.M. Roka. 2014. Vector control and foliar nutrition to maintain economic sustainability of bearing citrus in Florida groves affected by huanglongbing. Pest Mgt. Sci. 70:415–426.

Stansly, P.A., H.A. Arevalo, and M. Zekri. 2010. Area-wide psyllid sprays in southwest Florida: An update on the cooperative program aimed at controlling the HLB vector. Citrus Industry 91:6–8.

Tansey, J.A., P. Vanaclocha, C. Monzo, M. Jones, and P.A. Stansly. 2017. Costs and benefits of insecticide and foliar nutrient applications to huanglongbing-infected citrus trees. Pest Mgt. Sci. 73(5):904–916.

Tenenhaus, M., J. Pagès, L. Ambroisine, and C. Guinot. 2005. PLS methodology to study relationships between hedonic judgements and product characteristics. Food Qual. Prefer. 16:315–325.

Zhao, W., J. Bai, A. Plotto, E.A. Baldwin, and M. Irey. 2015. Method for assessing juice/cider quality and/or safety. U.S. Patent Application Publication US 2015/0093755 A1.