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Effect of Liberibacter Infection (Huanglongbing or "Greening" Disease) of Citrus on Orange Juice Flavor Quality by Sensory Evaluation

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ABSTRACT: Some anecdotal reports suggest that infection of citrus trees with *Candidatus* Liberibacter asiaticus (Las), the suspected causal agent of huanglongbing (HLB) disease, imparts off flavor to orange juice. It is of interest to the industry to know how Las infection affects juice quality with respect to cultivar, maturity, or processing method. Hamlin, Midsweet, and Valencia oranges were harvested over 2 y from trees that tested negative (Las−) or positive (Las+) for Las from different groves and included normal looking (nonsymptomatic) and symptomatic fruit (small, green, and lopsided) from Las+ trees. In the 1st year, fruit were manually juiced, while in the 2nd year, a commercial process was used. Juice from Las+ trees was compared to juice from Las− trees in difference-from-control tests, and by descriptive analysis. Results showed large variability due to tree, harvest date, and cultivar. Juice from Hamlin Las+ trees tended to be more bitter and sour than its Las− counterpart. In contrast, hand processed Valencia juice from Las+ trees was perceived to have some off-flavor and bitterness compared to control, but the following year, commercially processed Valencia juice from Las+ trees was perceived to be only slightly more sour than juice from Las− trees for the April harvest, and to be sweeter for the June harvest. When juice from individual replicates was pooled to be more representative of a commercial situation, there was no difference between Las+ and Las− juice in Valencia. Trained panel differences were noted for juice from Hamlin Las+ fruit, especially for symptomatic fruit.

Practical Application: Assumptions that juice made from oranges harvested from Huanglongbing (from infection with Liberibacter sp.) affected trees is off-flavored appeared to be generally more true for Hamlin juice than for Midsweet or Valencia, especially for Hamlin juice made from symptomatic fruit. For Midsweet and Valencia, flavor differences between juice made from fruit harvested from diseased or healthy trees varied greatly between trees, season, and even processing method. Under a commercial processing situation, where juice is blended from several varieties, seasons, and multiple locations, it is expected that off-flavor will not be a major problem.

Keywords: bitterness, citrus, citrus greening disease, flavor, Huanglongbing

Introduction

Citrus greening (also known as Huanglongbing or HLB) is regarded as a serious threat to worldwide commercial citrus production. It debilitates and eventually kills trees within 5 to 10 y, and to this date, there is no known remedy except preventing trees from becoming infected (Bové 2006). Although a direct causal relationship has not yet been established, HLB disease is associated with the presence of 1 of 3 species of a phloem-limited gram-negative bacterium. In Florida, the species associated with HLB is *Candidatus* Liberibacter asiaticus (Las), which is vectored by the Asian citrus psyllid *Diaphorina citri* (Bové 2006). First described in China, HLB has spread to most citrus producing regions in Asia and Africa, and recently to the Americas, whereas as of January 2006, the Mediterranean basin, Australia, and North- and South-Pacific islands are still free of the disease and its vector (Bové 2006). The presence of trees infected with Las was confirmed in Florida in 2005, and the disease has subsequently been found in all major citrus producing counties of the state (FDOACS 2008). Citrus trees infected with Las are first recognized by having sectors of yellow shoots or branches; trees eventually turn all yellow, defoliate, and die back (Bové 2006). Symptoms such as "yellow-vein" or "blotchy mottled" leaves are commonly described. Some out-of-season flushing and blossoming can be observed (da Graça 1991; McClean and Schwarz 1970). Symptomatic fruit from Las-infected trees do not color properly (hence the name "greening" disease), are small, have an asymmetrical shape (lopsided), and aborted seeds (McClean and Schwarz 1970; da Graça 1991; Bové 2006). Fruit affected by the disease have been described as having a bitter or salty taste, mostly in the early part of the season (McClean and Schwarz 1970). It is also said that the bitter taste is mostly due to "higher acidity and lower sugars" (da Graça 1991). There are other anecdotal reports that trees affected by HLB disease may produce off flavored fruit and subsequent orange juice, but this is not well documented.

There has been no systematic study characterizing the chemical or sensory nature of potential off flavors, or flavor changes during the season and by variety due to Las infection. Although HLB is present in all Florida citrus production areas, there is a 6 to 12 mo latency period between infection and expression of

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disease symptoms (Bové 2006). As a consequence, Las positive trees continue to be productive for some time following infection. In Florida, trees confirmed to be Las positive were initially destroyed as quickly as possible in an effort to reduce inoculum and slow spread of the disease. However, due to the widespread presence of Las and the extensive number of Las positive trees, removal of all Las-infected trees is becoming increasingly difficult; some growers continue to maintain and harvest fruit from Las-infected trees and infected trees remain in abandoned groves. It is therefore of interest to the orange fresh fruit and juice processing industries to determine what affect fruit from trees of various stages of disease development would have on flavor quality.

Results from a preliminary study of early infected trees showed that differences between juice from Las+ compared with Las− Valencia trees were mostly due to lower acid content and higher solids-to-acid ratio (Plotto and others 2008). This resulted in the juice from Las+ trees being perceived as sweeter by a taste panel, compared to juice from fruit of healthy (Las−) trees. The sample size for that study, however, was minimal due to the low incidence of HLB disease in Florida at the time, and fruit were harvested late in the season, masking potential off-flavor reported for Las+ trees earlier in the season (McClean and Schwarz 1970). Subsequently, it has been possible to collect larger volumes of fruit and multiple samples from trees with more advanced disease symptoms. Thus, more trees were sampled in 2007 and 2008 from 3 cultivars and several harvest dates to further understand the effect of the disease on different cultivars over the harvest season and the effect of tree-to-tree variation. Fruit samples were collected from Las+ or Las− trees, juice was extracted by hand in 2007 and using a commercial juice extractor in 2008. Analyses included sensory evaluation, total and individual sugars and acids, color, secondary metabolites, and aroma volatiles. Details of chemical data are presented in a companion paper (Baldwin and others 2010).

Materials and Methods

Fruit and juice material

In 2007, fruit were sampled from the 3 main processing orange cultivars, Hamlin, Midsweet and Valencia. Fruit from 5 replicate trees symptomatic for HLB disease (Las+) and 5 replicate healthy trees (Las−) were washed, sanitized with 200 ppm NaOCl for 30 s, gently hand juiced, lightly pasteurized at 71 °C for 15 s, and frozen at −20 °C until analyzed. The trees, symptomatic for HLB disease, were later confirmed to be diseased by polymerase chain reaction (PCR) used to amplify the DNA of the associated Las bacteria (Jagoueix and others 1996; Bové 2006; Li and others 2006). Control trees were confirmed to be PCR negative for the Las bacterium. Fruit from Las+ trees were generally nonsymptomatic, that is, normal looking, although some were slightly greener and/or smaller than the majority of fruit on the trees and those from Las− trees. Total of 30 fruits were harvested per tree. There was a single harvest of Hamlin and Midsweet varieties (February) and 4 harvests (March, April, May, and June) of Valencia with 5 replicate trees per cultivar, per harvest date, and per disease state.

The "2008 season" included Hamlin harvests in December 2007 and February 2008 and Valencia harvests in April and June, 2008. For each harvest, fruit were sampled from each of 3 Las+ or Las− trees, replicated 3 times (total of 9 infected and 9 healthy trees). For each set of 3 trees, 200 to 400 fruits were harvested and juiced as a separate replicate. Unfortunately, due to removal of diseased trees, it was not possible to use the same trees in 2008, and samples were harvested from different groves for both Hamlin and Valencia. In addition, for Hamlin in February 2008 and Valencia in April 2008, there was an additional harvest of symptomatic fruit (small, green, and asymmetrical) along with fruit from Las− trees in the same vicinity. All the "2008 season" fruit were extracted using a commercial JBT 391 single head extractor (JBT Food Tech, Lakeland, Fla., U.S.A.) with premium juice extractor settings, and pasteurized under simulated commercial conditions (1.2 L/m, 8 to 10 s hold time, 83 to 90 °C) using a pilot pasteurizer, UHT/HTST Lab 25EHV Hybrid (Microthermics, Inc.; Raleigh, N.C., U.S.A.), then immediately frozen at -20 °C until analyzed.

Sensory evaluation

Laboratory staff, 20 to 24 panelists, experienced at orange juice evaluation performed triangle tests (ASTM 2003) and difference-from-control (DFC) tests (Meilgaard and others 1999) comparing juice from Las− and Las+ trees. Preliminary triangle tests were performed before the DFC tests, and when differences were found by the triangle tests, DFC tests were then conducted and differences were subsequently found. For this reason, triangle tests will not be further discussed. In all the difference tests, 15 mL juice was served in 30 mL cups (Solo Cups Co., Urbana, Ill., U.S.A.) with serving temperature at 11 to 13 °C. In the DFC tests, each panelist was served 2 sets of juice. Each set comprised a control (from Las− trees) labeled as "control," and a test sample labeled with a 3-digit code number. The coded sample was either juice from Las+ (diseased) or Las− (healthy/control) trees. The control coded samples were presented to account for the placebo effect (Meilgaard and others 1999). Panelists were asked to compare the coded sample with the control juice, by smell, and by taste, and rate the degree of difference on an 11-point category scale, where "0 = no difference," and "10 = extremely different." They were also asked to describe the quality of the difference using their own vocabulary, although a suggested list of descriptors was provided in 2008. They were also informed that some test samples were identical to the control. The 2 sets were presented to account for panelist variability.

In 2007, for each cultivar, juice from fruit harvested from individual Las+ trees was compared to pooled juice of the 5 Las− trees. In 2008, juice prepared from Las+ trees was compared to juice from Las− trees in replicate pairs (3 trees per replicate), and at the end of the study, all 3 replicate juices were combined (pooled) and all juice from Las+ trees with nonsymptomatic fruit was compared to juice from Las− trees.

Trained panel. In 2008, Hamlin juice of the February harvest was presented to a 16-member panel specifically trained (>50 h) for descriptive analysis of citrus, different from the panel performing the difference tests. Specific descriptors were chosen for the Hamlin Las− and Las+ juice (Table 1). Samples were served as 50 mL juice in 110 mL cups (Solo Cups Co.). A Las− juice sample was served as a warm-up sample and rated in the same way as the test samples, and 3 juices, Las−, Las+ from nonsymptomatic and Las+ from symptomatic fruit, were presented in a randomized order. Panelists rated 9 aroma and 14 flavor/taste/mouth feel descriptors using a 16-point intensity scale where 1 = low, 7 to 8 = medium, and 15 = high. Reference standards were provided and served in 30 mL cups (Solo Cups Co.) (Table 1). Panel evaluation was performed in duplicate, on 2 different days.

All taste panels took place in isolated booths equipped with positive air pressure and under red lighting.

Chemical analysis of juice samples

Juice samples were set aside for chemical analysis before each taste panel session in 2007 and at the time of processing in 2008. Chemical analysis included volatiles by direct headspace, sugars, acids, total soluble solids (SSC), titratable acidity (TA), and specific secondary metabolites (Baldwin and others 2010). Peel oil was measured as the processing plant routine quality control protocol.

Microbiology

Juice samples were tested for the presence of microorganisms before and after pasteurization. Unpasteurized juice from all samples was compared to assess any differences in the microflora in these samples. Juice samples were also evaluated postpasteurization as a standard procedure when juice is served to panelists. For both assessments, juice samples were placed on potato dextrose agar (PDA) and plate count agar (PCA) plates (agars BD/Difco Brand, Sparks, Md., U.S.A.), 250 μL per plate and spread with a sterile glass rod. The plates were incubated for 24 to 36 h at 35 °C and resulting bacteria and yeasts noted. Plates were then left at ambient temperature (approximately 23 °C) for 5 to 7 d to allow for mold growth. Plates were made for qualitative purposes only. Juice samples were also tested for the presence of *Alicyclobacillus terrestris*, which can also cause off-flavor in juices (Jensen and Whitfield 2003). These assessments were done according to the method of (Wisse and Parish 1998).

Table 1 – Descriptors, reference standards, and anchor value when applicable, used in the descriptive sensory evaluation of Hamlin juice harvested February 2008.

Descriptor	Reference standard (suggested intensity)
Aroma and flavor	
Orange	Hand squeezed non pasteurized orange juice
Fruity-noncitrus	A mix of passion-fruit, mango, peach, guava, and pineapple juice
Fresh	A mix of lime oil (10 μg mL^{-1}), *cis*-3-hexenal (1.75 μg mL^{-1}) and *cis*-3-hexenol (6 μg mL^{-1}) in water
Fatty	*Cis*-3-hexenal (3.5 μg mL^{-1}) in water
Peel oil	Orange pumpout (11.8 °Brix) spiked with orange oil (0.03% – Mastertaste, Lakeland, Fla., U.S.A.)
Sour/fermented	The aroma of fermented juice or sour milk
Musty/earthy	Methyl isoborneol (5 μL of 50 μg L^{-1}) on a filter paper
Pungent/peppery	$\frac{1}{2}$ tea spoon of black pepper boiled in 500 mL water for 3 min
Paint	Mineral oil
Taste and mouth feel	
Sweet	8% sucrose solution (7)
Sour	0.25% citric acid in solution (7)
Umami/salty	0.3% MSG (7)
Bitter	0.1% caffeine in water (7)
Metallic	A clean and sterilized penny
Tingling	Perrier mineral sparkling water
Astringent	1% alum in water (7)

Statistical analysis

For the triangle tests, correct answers were tallied and compared to the values in the ASTM standards table (ASTM 2003). Data for the difference-from-control and descriptive panels were analyzed by analysis of variance (ANOVA) using mixed models, with panelists as a random variable. In the difference-from-control tests, the degree of difference from control for the test sample (Las+ juice) was compared against the degree of difference from the placebo control using Senpaq version 4.1 (Qi Statistics, Reading, U.K.). Data from the descriptive panel were analyzed by ANOVA using Senpaq. For all sensory tests, mixed model with random panelists was used in ANOVAs, with the main effect being tested against the interaction (Senpaq, Qi Statistics). Difference between means was performed using the LSD test, $\alpha = 0.05$.

Chemical and sensory data from the difference-from-control ratings and trained panel were compared using Pearson's correlations, linear, nonlinear, and multiple regressions, and Partial Least Square Regressions using XLSTAT 2008 (Addinsoft, Paris, France). Selected chemical data (SSC, TA, SSC/TA, limonin, nomilin) from both years were analyzed by principal components analysis (PCA) using XLSTAT 2008.

Results and Discussion

Hand-squeezed juice (2007 season)

Hamlin. For Hamlin, there was 1 harvest of 5 replicate trees. Panelists found aroma (orthonasal) differences between Las− and Las+ juice from Las+ trees # 1, 2, and 3 (Table 2) in DFC tests. Differences were described as "slightly fermented," "older juice" but also "fresher" and "fruitier." Panelists found flavor (retronasal and taste) differences in juice from all trees except tree # 3 (Table 2). The degree of difference should be noted: Tree # 1 and # 5 had a larger perceived flavor difference (4.83 and 3.66, respectively, on a 0 to 10 scale) than tree # 2 and 4 (2.14 and 2.38, respectively). The differences were described as "sweeter" or "richer flavor," but also "overripe," "fermented," "off flavor," "sour," "bitter taste or aftertaste." There were no differences in SSC, TA, or volatile content that could explain flavor comments such as "fermented" or "overripe" (Baldwin and others 2010). However, the 2 known bitter compounds, limonin and nomilin (Maier and others 1973, 1977, 1980; Rouseff and Matthews 1984; Hasegawa and others 2000; Abbasi and

Table 2 – Sensory evaluation mean difference from control (scale 0 to 10) and probability (*P*) value of the ANOVA test with hypothesis (Ho) that there is no difference between "control," that is, juice from Las− trees, and juice from diseased Las+ trees, and between "control" and blind "control" juice for Hamlin harvested on 9 Feb 2007 and Midsweet harvested on 13 Feb 2007.

		Hamlin			Midsweet		
Tree nb.	Sensory modality	Las+[z]	Las−[y]	*P*-value	Las+	Las−	*P*-value
1	Aroma	3.50	1.90	<.05	1.32	0.98	0.36
	Flavor	4.83	1.29	<.0001	1.23	1.16	0.74
2	Aroma	1.73	0.70	<.01	1.21	1.05	0.57
	Flavor	2.14	1.11	<.01	2.14	1.21	<.01
3	Aroma	2.20	0.92	<.001	1.43	0.74	0.19
	Flavor	1.80	1.55	0.52	2.10	1.17	<.01
4	Aroma	0.90	0.64	0.51	0.90	0.62	0.28
	Flavor	2.38	1.05	<.01	1.83	1.36	0.16
5	Aroma	0.89	1.02	0.69	0.90	0.81	0.76
	Flavor	3.66	0.84	<.0001	2.17	0.79	<.05

[y] Mean rating of the difference between control and blind control, both Las− juices (scale: 0 = no difference; 10 = extremely different).

[z] Mean rating of the difference between Las+ and control (Las−) juice (scale: 0 = no difference; 10 = extremely different).

others 2005) occurred at moderately higher concentrations in the Las+ juice than in the Las− juice: 3.6 and 1.9 μg mL^{-1} in Las+ compared with 2.6 and 1.2 μg mL^{-1} in Las− juice for limonin and nomilin, respectively (Baldwin and others 2010). A positive correlation ($R = 0.860$) was observed between the sensory ratings and limonin contents for the Hamlin juices. Taste threshold for limonin in orange juice was reported to be about 6 μg mL^{-1} (Guadagni and others 1973), and there are reasons to believe that nomilin threshold in orange juice might be similar since its threshold in water was similar to that of limonin, about 1 μg mL^{-1} (Rouseff and Matthews 1984). In the same study, Guadagni and others (1973) found a large variation in limonin thresholds, with values of 0.5 to 32 μg mL^{-1} for the most sensitive and the least sensitive panelist, respectively. We proposed that in our current study, the 1 μg mL^{-1} and 0.7 μg mL^{-1} differences in limonin and nomilin, respectively in the Las− and the Las+ samples, might be perceived as increased bitterness by the most sensitive panelists. This may explain the discrepancies in comments for the Las+ juice in comparison to the Las− juice recorded in the 2007 season Hamlin DFC tests. Also, by the nature of the DFC test, the most sensitive panelists could compare 2 samples and find minute differences in composition.

Midsweet. As for Hamlin, there was only 1 harvest of 5 replicate trees for Midsweet. Panelists did not find aroma differences between Las+ juice and Las− juice in the DFC tests, and they found flavor differences for only trees # 2, 3 and 5 (Table 2). These differences were low (2.10 to 2.17 when significant), and were described as "fermented" or "overripe," "fruitier," "sweeter," and "slight off flavor." The fermented/sweeter/fruitier descriptors might be an indication of fruit maturity. Chemical analysis shows that juice from these specific trees had slightly higher ethanol content (7039, 5271, and 5097 μg mL^{-1}, respectively, compared with 4241 μg mL^{-1} for the Las− trees), but most other volatiles were either not different, or lower in Las− than Las+ juice. In addition, there were no differences in SSC, TA, SSC/TA, individual sugars and acids that could explain sweeter perception for the Las+ juice (data not shown).

Valencia. For Valencia, there were 4 harvests of 5 replicate trees (same trees each harvest) in the 2007 production season. In DFC tests, there were generally no aroma differences due to Las status of the tree, except for tree # 2 harvested in May and June, tree # 3 harvested in April, and tree # 4 harvested in March, (Table 3). For these samples, the aroma difference was described as "off," "sour," and "fermented" by several panelists for juice from tree # 3, but differences were low and descriptors not consistent among panelists for tree # 2. Taste differences were perceived in juice from all trees, but not consistently for all harvests, except for trees # 3 and 4 (Table 3). Juice from Las+ trees was described as "bitter," "more sour" than Las−, and in the May harvest, having a strong unidentified off-flavor. However, by the June harvest, descriptors of the differences between Las+ and Las− juices for trees # 3 and 4 had diminished to such terms as "less sweet" and "more bland." Significant negative correlations were found between sensory difference ratings and chemical data: SSC (−0.650), SSC/TA (−0.673), sucrose (−0.686), glucose (−0.541), fructose (−0.540), and ethyl butanoate (−0.588), while limonin had a positive correlation (0.655). Chemical data showed that indeed, SSC, total sugars, sucrose, and glucose were lower in Las+ juice than in Las− juice, and limonin was higher (Baldwin and others 2010). However, volatiles were either higher or lower, at either harvest date, with no clear pattern, except in the May harvest where many volatiles were lower in the Las+ juice (Baldwin and others 2010). This may explain that an imbalance in volatile compounds in the May harvest generated off-flavor comments from panelists for the Las+ juice. Limonin and nomilin were consistently higher in Las+ juice; however, at levels below thresholds (less than 1.5 μg mL^{-1} and 0.70 μg mL^{-1} for limonin and nomilin, respectively), but combined with lower sugars, the perception of bitterness could be noticeable for the most sensitive panelists in the DFC test.

Commercially processed juice (2008 season)

Hamlin. There were 2 harvests (December 2007 and February 2008) of 3 composite replicates of 3 trees each. Results from the analyses of the Hamlin fruit harvested in December 2007 (included as the early season component of the 2008 study) showed flavor differences between juice from healthy and disease-affected trees in all 3 replications. Aroma differences were only perceived in the 2nd replication (pair 2), and were described as "sharp," and "more citrus oil" in the juice from Las+ trees (Table 4). Indeed, fruit from Las+ trees tended to be smaller (188 and 214 g, for Las+ and Las−, respectively) (Baldwin and others 2010), and thus, the juice-to-peel ratio was lower for the Las+ fruit than with the Las− fruit. In this case, peel oil reported by the processor in juice from Las+ trees was 0.02%, in comparison with juice from Las− tree (0.01%), and that difference was likely to have been enough to be perceived as an aroma difference. Flavor differences were high (average greater than 4.00 on a 0 to 10 point scale) and were described as "bitter," "sour," and "grapefruit-like" in all replications (Table 4). It is to be noted that juice in this experiment had low total soluble solids content (7.7 to 8.2 °Brix), and would probably not enter a commercial juice stream, or would be blended to reach the accepted commercial level of about 11.8 °Brix. The lack of sugar in the juice may have

Table 3– Sensory evaluation mean difference from control (scale 0 to 10) and probability (*P*) value of the ANOVA test with hypothesis (Ho) that there is no difference between "control," that is, juice from Las– trees, and juice from diseased Las+ trees, and between "control" and blind "control" juice for Valencia harvested in March, April, May, and June 2007.

Tree nb.	Sensory modality	March			April			May			June		
		Las+[z]	Las−[y]	*P* value	Las+	Las−	*P* value	Las+	Las−	*P* value	Las+	Las−	*P* value
1	Aroma	0.68	0.85	0.40	1.25	0.89	0.31	1.84	1.28	0.11	2.10	1.78	0.22
	Flavor	4.74	0.89	<.0001	4.71	1.35	<.01	4.04	1.62	<.0001	1.68	1.60	0.82
2	Aroma	0.87	0.73	0.48	1.25	1.22	0.93	1.71	0.96	<.05	1.65	0.70	<.01
	Flavor	1.16	0.95	0.57	1.59	1.53	0.89	2.58	1.67	<.05	2.45	1.00	<.01
3	Aroma	0.83	0.73	0.72	2.13	0.63	<.01	1.25	1.04	0.43	1.29	0.74	0.14
	Flavor	1.82	0.61	<.01	5.43	1.11	<.0001	3.44	1.56	<.01	2.03	0.87	<.05
4	Aroma	1.50	0.56	<.01	1.24	0.79	0.15	1.05	1.24	0.52	1.05	0.75	0.24
	Flavor	2.42	0.50	<.001	3.06	1.00	<.01	4.42	1.63	<.001	3.53	1.08	<.001
5	Aroma	0.58	0.47	0.36	1.13	0.59	0.26	1.17	1.00	0.50	1.47	1.08	0.21
	Flavor	1.08	1.06	0.93	1.41	0.81	0.18	2.02	1.50	0.20	1.72	0.81	<.05

[y] Mean rating of the difference between control and blind control, both Las− juices (scale: 0 = no difference; 10 = extremely different).

[z] Mean rating of the difference between Las+ and control (Las−) juice (scale: 0 = no difference; 10 = extremely different).

Table 4— Sensory evaluation mean difference from control (scale 0 to 10) and probability (*P*) value of the ANOVA test with hypothesis (Ho) that there is no difference between "control," that is, juice from Las− trees, and juice from diseased Las+ trees, and between "control" and blind "control" juice for Hamlin harvested in Dec 2007 and Feb 2008.

Sample	Modality	Las+[z]	Las−[y]	*P*-value	Comments describing differences between (+) and Las− juice
					Hamlin Dec. 2007
Pair 1	Aroma	0.56	0.78	0.21	
	Flavor	4.19	1.31	< 0.001	Bitter, grapefruit-like
Pair 2	Aroma	1.39	0.72	< 0.05	Sharp, citrus oil
	Flavor	4.58	0.56	< 0.0001	Bitter and sour
Pair 3	Aroma	1.00	0.76	0.48	
	Flavor	4.76	0.76	< 0.0001	Bitter, sour, sour milk
					Hamlin Feb. 2008
Pair 1	Aroma	2.00	0.76	< 0.01	Pungent, sour, peppery
	Flavor	3.24	1.32	< 0.01	Pungent, sour, peppery, metallic, bitter, earthy, musty
Pair 2	Aroma	1.22	0.94	0.42	
	Flavor	4.03	0.81	< 0.001	Bitter, astringent, sour, musty, less sweet than control
Pair 3	Aroma	1.78	1.13	0.08	Slightly musty, pungent
	Flavor	3.43	1.63	< 0.01	Bitter, more peel oil, metallic, astringent, tongue tingles
Pair 4[x]	Aroma	1.95	0.51	< 0.05	Fermented, musty earthy
	Flavor	6.00	1.17	< 0.0001	Fermented, sour, bitter, astringent, green, musty, less sweet than control

[x] Pair 4 was from Las+ symptomatic fruit.
[y] Mean rating of the difference between control and blind control, both Las− juices (scale: 0 = no difference; 10 = extremely different).
[z] Mean rating of the difference between Las+ and control (Las−) juice (scale: 0 = no difference; 10 = extremely different).

accentuated the perception of sourness and bitterness, confirmed by juice analysis that showed lower total sugars (5.97 and 6.96 mg mL^{-1} in Las+/- juice, respectively) and higher limonin and nomilin content (3.27 and 0.83 in Las+ compared with 1.45 and 0.43 μg mL^{-1} in Las− juice, respectively) all explaining high sour and bitter characteristics of the juice from disease-affected trees.

Hamlin fruit harvested in February 2008 had a composition more characteristic of commercial juice than did the Hamlin fruit harvested in December 2007 with SSC ranging from 10.3 to 12.4 °Brix and TA from 0.45% to 0.66%. Aroma differences were found for pairs 1 and 4 (Table 4). These differences were small (equal to or less than 2 on a 10-point scale) but significant. Flavor differences between juice from healthy and Las+ trees were highly significant with averages of 3.24 to 4.03 (on a 10-point scale) when juice was made from nonsymptomatic fruit from Las+ trees (pairs 1 to 3), and higher (6.00) when juice was made from symptomatic fruit from Las+ trees (pair 4—Table 4). Juice from Las+ trees was described as "pungent," "sour," "peppery," "metallic," "bitter," "earthy/musty," "astringent," and some panelists described a "tingling" effect on the tongue. Similar descriptors were reported by a consumer panel (Goodrich-Schneider and others 2008). The bitter compounds limonin and nomilin were found at 1.5 and 0.51 μg mL^{-1} in juice from Las+ trees, in comparison with 0.82 and 0.18 μg mL^{-1} in juice from Las− trees, respectively, in addition to a lower total sugar content in Las+ juice (7.62 mg mL^{-1} compared with 9.82 mg mL^{-1}) (Baldwin and others 2010).

Table 5— Sensory descriptive analysis of orange juice from Las− healthy and Las+ Hamlin trees, harvested 13 Feb 2008.[z]

Descriptor	Las−	Las+ nonsymptomatic fruit	Las+ symptomatic fruit
Aroma			
Orange	**4.8 ab**	**5.0 a**	**4.3 b**
Fruity-noncitrus	1.0 a	0.9 a	1.3 a
Fresh	1.4 a	1.9 a	1.3 a
Fatty	**1.9 a**	**1.0 b**	**1.0 b**
Peel oil	2.0 a	1.8 a	1.8 a
Sour/fermented	**1.5 ab**	**1.0 b**	**2.6 a**
Musty/earthy	1.2 a	0.7 a	0.9 a
Pungent/peppery	1.1 a	0.6 a	0.5 a
Paint	1.2 a	1.3 a	0.8 a
Flavor/Taste/Mouth feel			
Orange	**5.0 a**	**3.2 b**	**2.7 b**
Fruity-noncitrus	1.6 a	1.0 a	1.4 a
Fresh	**1.5 a**	**0.8 b**	**0.5 b**
Fatty	1.6 a	1.7 a	0.9 a
Peel oil	1.6 a	2.2 a	1.9 a
Sour/fermented	**1.1 b**	**2.1 b**	**4.5 a**
Musty/earthy	**1.0 b**	**1.7 b**	**2.6 a**
Sweet	**5.3 a**	**3.6 b**	**3.2 b**
Sour	2.7 a	3.2 a	3.3 a
Salty/umami	**0.8 b**	**1.4 ab**	**2.0 a**
Bitter	**0.7 b**	**3.6 a**	**2.7 a**
Metallic	**0.6 b**	**2.1 a**	**2.0 a**
Pungent/peppery	**0.5 b**	**1.3 a**	**1.6 a**
Tingling	0.8 b	1.7 a	1.4 ab
Astringent	**0.6 b**	**1.6 a**	**1.3 a**

[z] Means followed by the same letter are not significantly different by the LSD test within a row ($\alpha = 0.05$). Bold characters indicate descriptors with significant differences.

When commercially processed Hamlin juice from the February 2008 harvest was presented to a trained panel, differences were found between juice from healthy (Las−) and Las+ trees for "fatty" aroma and between Las+ symptomatic and nonsymptomatic fruit for "orange" and "sour/fermented" aroma (Table 5). "Orange" aroma was higher in juice made with nonsymptomatic fruit from Las+ infected trees; "fatty" aroma was higher in juice from Las− trees; and "sour/fermented" aroma was higher in juice made with symptomatic fruit from Las+ affected trees (Table 5). Differences between juices from healthy and diseased trees were more definite for flavor than for aroma (Table 5). Juice from Las+ trees was lower in "orange" and "fresh" flavor and "sweet" taste, and higher in "bitter," "metallic," "pungent/peppery," and "astringent" flavor and taste. Differences between juice from symptomatic and nonsymptomatic fruit were found for "sour/fermented" and "musty/earthy" flavor, and juice made with symptomatic fruit was perceived to have more "salty/umami" taste than Las− juice (Table 5).

To further evaluate correlations between sensory findings from the trained panel and levels of potential chemical markers, a partial least square regression analysis (PLS) was performed with the sensory ratings from the trained panel as the dependent variables (*Y*) and the instrumental data as the explanatory variables (*X*). PLS is a "soft" multivariate modeling method that describes relationships between 2 sets of variables such as sensory and chemical or physical data (Martens and Martens 1986; Martens 2001). Y and X are principal component vectors of the dependent and explanatory variables, respectively, and the components of Ys are connected with the Xs by means of a regression model (Bastien and others 2005; Tenenhaus and others 2005). When all the sensory data were entered in the model, 87% of Y variation was explained by the 2-dimension model, and when only flavor data were considered, the 2-dimension model explained 93% of the variation. Both models were of good quality as per the Q^2 statistic rule (Tenenhaus and others 2005). With all sensory data (including aroma and flavor), $Q^2(\text{cum}) = 0.377$ and $R^2Y(\text{cum}) = 0.866$, and with flavor data, $Q^2(\text{cum}) = 0.584$ and $R^2Y(\text{cum}) = 0.931$. For simplicity of interpretation, only the biplot of the correlations between flavor and chemical data is shown, with the sample scores in the (t_1, t_2) space (Figure 1). In that plot, the variables X and Y are visualized in such a way that if 2 variables are close to each other and near the circle, they are highly positively correlated, while if they are on the opposite side, they are negatively correlated. Variables inside the circle have low or no correlations. "Orange" and "fresh" flavor and "sweetness" were explained by levels of SSC, fructose, glucose, SSC/TA, sucrose, and γ-terpinene (Figure 1). "Fruity-noncitrus" was explained by decanal and ethyl butanoate, and "fatty" flavor was highly correlated with hexanol. Sample scores on the t vectors show that the Las− juice (juice from healthy trees) tended to have these characteristics ("orange, fresh sweet, fruity" descriptors, and high in sugars and the mentioned volatiles). On the other hand, descriptors that were high for juice from Las+ trees ("peel oil, astringent, bitter, tingling, metallic, pungent, salty, musty, sour/fermented") were on the opposite side of the plot. Nomilin was highly correlated with the descriptors "astringent" and "bitter," confirming

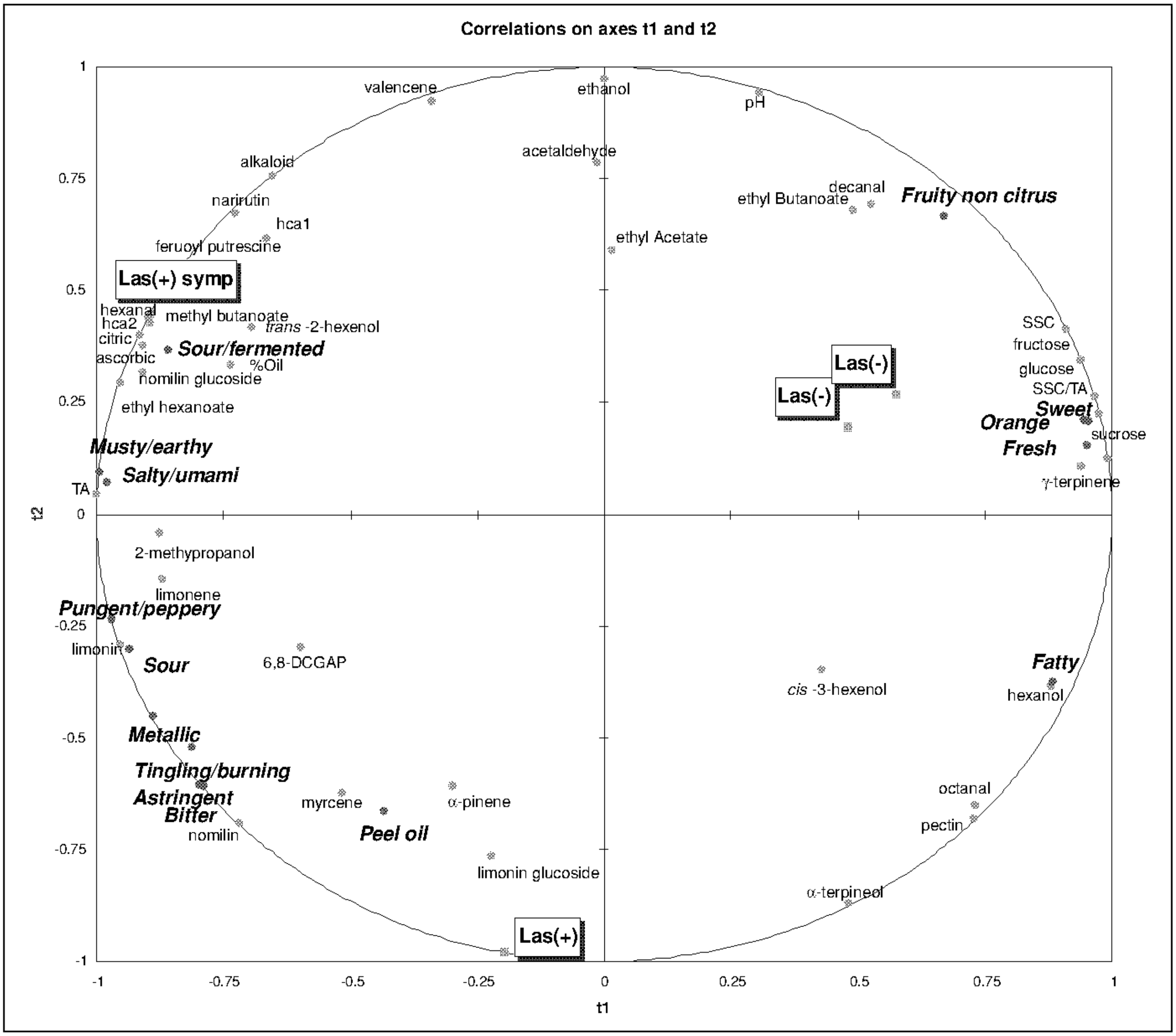

Figure 1—Correlation circle of the samples' sensory and instrumental characteristics with (t_1, t_2) for Hamlin juice harvested in February 2008 and tasted by a trained panel. Las− = juice from fruit harvested from Las− trees, Las+ = juice from nonsymptomatic fruit harvested from Las+ affected trees, Las+ symp = juice from fruit showing the symptoms of Las+ disease (small, lopsided, and green). TA = titratable acidity, SSC = soluble solids content, hca1/2 = hydroxycinnamic acid at 6.3/7.2 min, 6,8-DCGAP = 6,8-di-C-glucosyl apigenin. Chemical data are reported in Baldwin and others (2010).

Table 6— Sensory evaluation mean difference from control (scale 0 to 10) and probability (*P*) value of the ANOVA test with hypothesis (Ho) that there is no difference between "control," that is, juice from Las− trees, and juice from diseased Las+ trees, and between "control" and blind "control" juice for Valencia harvested in April and June 2008.

Sample	Modality	(+)Las^z	(−)Las^y	*P*-value	Comments describing differences between (+) and Las− juice
				Valencia April 2008	
Pair 1	Aroma	0.79	0.63	0.40	
	Flavor	2.53	0.84	< 0.001	More sour and less sweet than control, slight off flavor
Pair 2	Aroma	1.40	0.90	< 0.05	More peel oil, grapefruit-like smell
	Flavor	1.81	0.67	< 0.01	More sour than control, but also sweeter.
Pair 3	Aroma	0.81	0.88	0.75	
	Flavor	1.31	0.59	0.15	
Pair 4^x	Aroma	0.88	0.55	0.31	
	Flavor	2.98	1.18	< 0.01	Sour, sharp, acidic, tangy
				Valencia June 2008	
Pair 1	Aroma	1.89	0.70	< 0.01	Sharper, greener, peel oil
	Flavor	1.85	1.00	< 0.01	Aftertaste, slightly bitter, bland, unidentifiable different flavor
Pair 2	Aroma	0.81	0.76	0.85	
	Flavor	1.55	1.12	0.22	
Pair 3	Aroma	1.76	0.87	0.07	No consistent comments
	Flavor	2.85	1.43	< 0.01	Sweeter than control, slight bitter

[x] Pair 4 was from Las+ symptomatic fruit.
[y] Mean rating of the difference between control and blind control, both Las− juices (scale: 0 = no difference; 10 = extremely different).
[z] Mean rating of the difference between Las+ and control (Las−) juice (scale: 0 = no difference; 10 = extremely different).

Table 7— Sensory evaluation mean difference from control (scale 0 to 10) and probability (*P*) value of the ANOVA test with hypothesis (Ho) that there is no difference between "control," that is, juice from Las− trees, and juice from diseased Las+ trees, and between "control" and blind "control" juice for juice from pooled nonsymptomatic samples of Hamlin harvested in Feb 13 and Valencia harvested on 29 April and 23 June 2008.

Sensory modality	Las+^{z}	Las−^{y}	*P*-value	Comments describing differences between (+) and Las− juice
		Hamlin February 2008		
Aroma	1.48	0.86	0.08	Sweeter smell, slight earthy
Flavor	3.98	1.19	<.001	Bitter, astringent, grapefruit-like, sour
		Valencia April 2008		
Aroma	0.33	0.66	0.12	Similar to control, somehow flat
Flavor	1.40	1.05	0.19	Slightly more sour, astringent, bitter, less fresh
		Valencia June 2008		
Aroma	1.30	0.89	0.32	Slightly more peel oil aroma
Flavor	1.50	1.30	0.77	Sweeter, more peel oil, slightly metallic/astringent

[y] Mean rating of the difference between control and blind control, both Las− juices (scale: 0 = no difference; 10 = extremely different).
[z] Mean rating of the difference between Las+ and control (Las−) juice (scale: 0 = no difference; 10 = extremely different).

panelists' description for this compound in water (Rouseff and Matthews 1984), and limonin was correlated with "sour" and "pungent/peppery." Titratable acidity (TA) was correlated with the descriptors "musty/earthy" and "salty/umami" in these samples. Many compounds were correlated with the "sour/fermented" descriptor, high for the juice made with Las+ symptomatic fruit: hexanal, hydroxycinnamic acid, feruloyl putrescine, citric and ascorbic acid, narirutin, nomilin glucoside, hexanal, methyl butanoate, ethyl hexanoate, trans-2-hexenol, and percent oil. This does not necessarily mean that these compounds are directly responsible for the sour/fermented descriptor, but when these compounds were high in the Las+ symptomatic juice, there was also a high rating for "sour/fermented."

Valencia. Valencia oranges were harvested in April and June 2008. In both harvests, differences between juices from Las− and Las+ trees were either not significant, or less than 3 on a 10-point scale when significant (Table 6). Even though there were no statistical differences in the chemical compositions (sugars, acids, SSC, and TA) of the complete sets of juices from Las+ and Las− trees (Baldwin and others 2010), panelists could perceive sugar and acid differences in individual juice replications presented as pairs 1 and 4 from the April harvest (Table 6). SSC/TA ratios were 16 and 16.7 in juice from healthy trees of pairs 1 and 4, respectively, higher than 12.8 and 15 in juice from Las+ trees from the same pairs. In both pairs, juice from Las+ trees was described as "less sweet" and "more sour" than Las−. Pair 4 involved a comparison between juice prepared from Las+ symptomatic fruit and Las− fruit. Panelists also found aroma and flavor differences between juice from Las− and Las+ trees in pair 2, but these difference were small (less than 2 on a 10-point scale), and inconsistently identified (Table 6).

For fruit harvested in June, differences were found in pair 1 (aroma and flavor) and pair 3 (only flavor). The slight difference in aroma between juices from Las− and Las+ trees in the first pair was most likely due to higher oil content in juice from Las+ trees (0.015% compared with 0.023% in Las− and Las+ juices, respectively). In pair 3, SSC was 13 and 15 in juice from Las− and Las+ trees, respectively, explaining the sweeter perception for juice from Las+ trees. However, that juice was also perceived as "slightly bitter" in both pairs 1 and 3 by some panelists (Table 6). The level of limonin was below its detection threshold in Valencia juice (less than 0.66 μg mL^{-1}), and higher in juice from Las− than in Las+ trees (Baldwin and others 2010), thus unlikely to explain the perception of bitterness, except for very sensitive panelists (Guadagni and others 1973).

Pooled juice. When juice from all 3 replications in each harvest was pooled, differences between juice from healthy and Las+ trees were only perceived in Hamlin harvested in February (Table 7). Only juice from Las+, nonsymptomatic fruit was used in that test, since symptomatic fruit are unlikely to be harvested due to their tendency to abscise from the tree, and the likelihood of small misshapen fruit being culled prior to juicing. Juice from Hamlin Las+ trees was again perceived as more "bitter," "sour," and "astringent" than juice from Las-trees, and as having a "grapefruit-like" flavor. There were no differences due to the disease status of the trees for juices from Valencia harvested in April or June (Table 7).

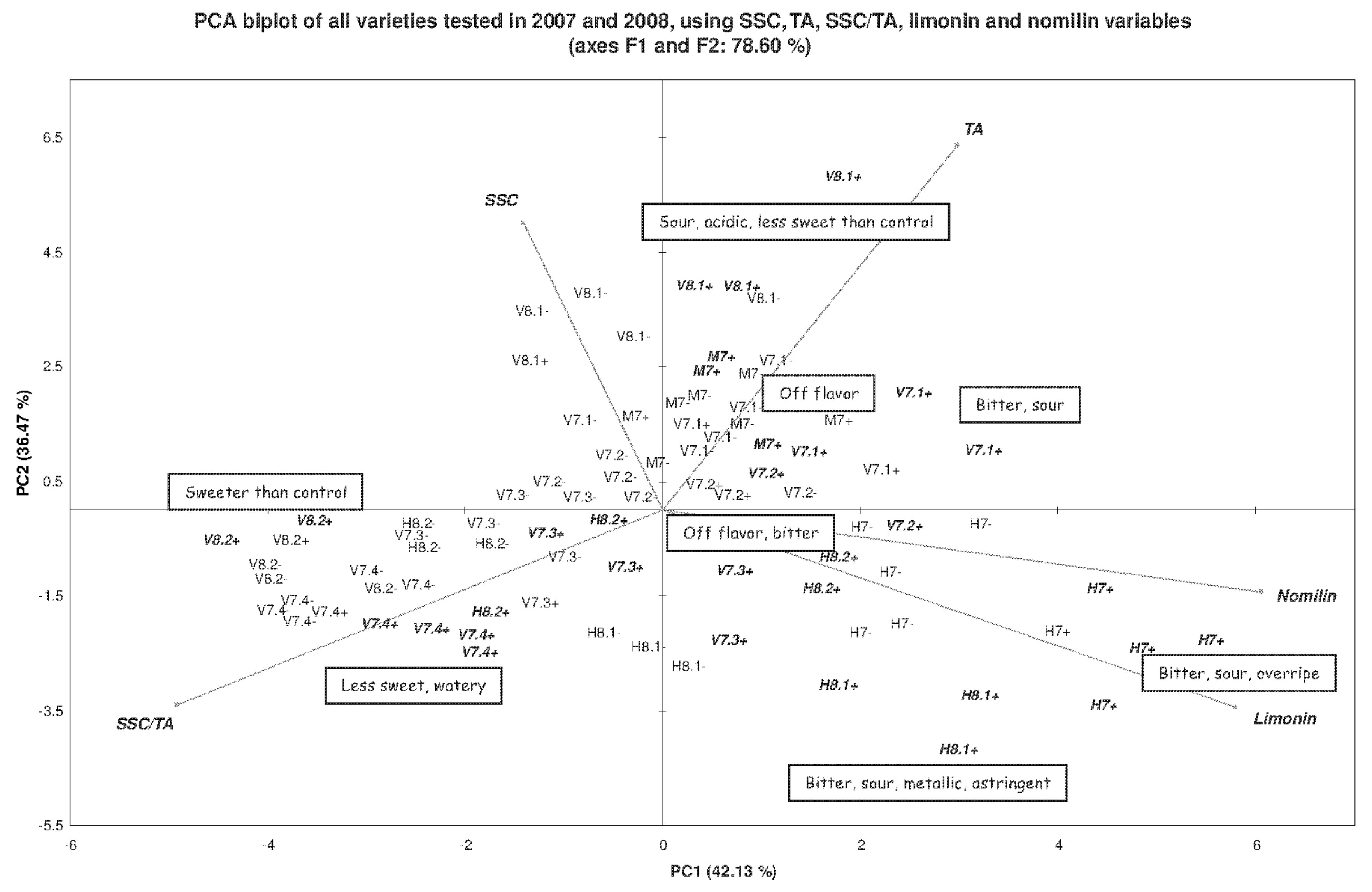

Figure 2–Principal components analysis of all chemical data with indication of sensory ratings for each sample score in the PC1/PC2 biplot. Point labels in italics indicate a significant difference sensory rating between the Las+ sample and its Las– counterpart. Chemical data are reported in Baldwin and others (2010). H = Hamlin, M = Midsweet, V = Valencia, 7 = 2007, 8 = 2008, 1 to 4 = 1st to 4th harvest, –/+ = Las –/+.

Understanding variations across cultivars and seasons

Even though limonin and nomilin, the 2 known bitter compounds in citrus, did not always correlate with sensory data from the DFC tests, there was a trend for Las+ juice to be perceived as bitter by the most sensitive panelists when limonin content was higher in Las+ than in Las− juice. Guadagni and co-authors (1973) showed limonin threshold varied with citric acid, pH, and SSC/TA in juice. Since SSC, TA SSC/TA are standard measures of quality by the citrus juice industry, these variables as well as limonin and nomilin were included in the analysis by PCA for all cultivars and harvests to find patterns among samples that would explain sensory results. Scores of each data point (cultivar/harvest date) are represented in the PCA biplot (Figure 2). When Las+ samples had sensory ratings significantly different from their Las− counterpart in the DFC test, their points in the PCA biplot were highlighted in bold italic characters, and the general comments provided by panelists were added to the graph. The first 2 components of that PCA explained 78.6% of the total variance, with the 1st and 2nd PC accounting for 42.1% and 36.5%, respectively. Juice from Las+ Hamlin harvested in 2007 and 2008 had the highest scores on PC1 explained by limonin/nomilin dimension and partially by TA on the positive side, and SSC/TA on the negative side (Figure 2). Indeed, these samples had recurring descriptors of high bitterness and sourness compared to juice from Las− fruit. Other descriptors such as "overripe" for Hamlin 2007 and "metallic, astringent" for Hamlin 2008 may be explained by volatile or other non volatile compounds for Hamlin 2007 and 2008, respectively, but are not represented in this analysis. Juices from Las+ Valencia harvested in April 2008 were scored high on the TA component, and their differences from the Las− juice were described as "sour/tart," "acidic," and "less sweet" than control. For this cultivar and harvest, SSC and TA appeared to be the main reasons for differences between +/- Las juices. Juices from Valencia harvested in June of 2007 and 2008 were all on the negative sides of PC 1 and PC 2, with high scores on SSC/TA. For the 2 Las+ samples of Valencia 2008 that were different from their Las− counterpart, SSC was slightly higher than the Las− juice, and they were described as "sweeter than control." On the other hand, Las+ juice from the 2007 Valencia harvest were described as "less sweet" than Las− juice, and indeed, had a slightly lower SSC and SSC/TA. Finally, samples of which scores were in the middle were not particularly high in limonin/nomilin, SSC or TA. For these, Midsweet 2007, Valencia 2007 April and May harvests, Las+ samples were described with a slight off flavor with respect to Las− juice, which would be due to some volatiles and not reflected in this biplot. Juice from Las+ Valencia fruit harvested in March 2007 were described as slightly more bitter and sour than their Las− counterpart, and had slightly higher scores on both TA and limonin/nomilin dimensions (Figure 2). In summary, Valencia data points were separated mostly due to harvest date, and Hamlin due to disease status based on SSC, TA, limonin, and nomilin. Midsweet was less affected by Las status.

Modeling sensory differences with sugars, acids, and limonin/nomilin content in the juice

To build a model to predict sensory data with SSC, TA, SSC/TA, limonin, and nomilin, multiple linear regressions with stepwise model selection were performed with the sensory ratings as the dependent variable, and data from the Las+ samples as the independent variables. When using data from both years or only 2007 and all varieties combined, the model did not satisfactorily explain the observed sensory ratings (Adj. R^2 less than 0.30, Table 8). Because fruit was hand juiced in 2007, batches (replicate juices) were not as uniform as commercially processed juice, especially with pulp and volatiles content that could be confounding factors in taste perception (Plotto and others 2008). However, in 2008, with commercially processed juice, limonin could predict 68% of the sensory ratings (Table 8). A nonlinear model was therefore constructed that could predict the sensory rating based on limonin content, the sensory rating still being the difference between Las+ and Las− juices (Figure 3). That model explained 85% of the variation (Adj. $R^2 = 0.85$), and the curve followed an exponential growth with a plateau. This curve appears like the compressive part of the sigmoidal curve of a psychophysical function, with data in the higher range of stimuli (Maes 1985; Chastrette and others 1998; Meilgaard and others 1999; Keast and Breslin 2003). The model was validated using data from the pooled samples (Table 7 and 9). All juice samples with limonin content greater than 1 μg mL^{-1} and sensory difference ratings greater than 3 were from Hamlin oranges (Figure 3), with at least 1 panelist indicating the juice was bitter (Table 4). All data points with limonin less than 1 μg mL^{-1} were from Valencia juice, for which sensory difference ratings were lower then 3 and with various descriptors (Table 6). There was 1 outlier in that model: sensory difference rating for the juice made with symptomatic Hamlin fruit was high, but the limonin content (2.4 μg mL^{-1}) was similar to those of the nonsymptomatic fruits (Figure 3). As discussed earlier, that sample had strong off-flavor described as "sour/fermented, metallic, astringent" (Table 5), probably from components other than limonin alone.

If the major difference between juice prepared with fruit from Las+ and Las− trees is mostly due to limonoids, it would be, therefore, possible to predict bitterness from the limonin content in juice. The most sensitive panelists could perceive bitterness in juice at limonin levels as low as 1 μg mL^{-1} or even lower, which confirms earlier studies by Guadagni and others (1973). However, one needs to keep in mind that a large variation exists in the perception of bitterness in the population (Guadagni and others 1973; Bartoshuk and others 2004). It is very likely that those panelists who could perceive a difference between Las+ and Las− juices due to limonin content could also perceive differences due to other compounds in the juice. Genetic variation in bitterness perception has been shown for phenylthiocarbamide (PTC) and propylthiouracil (PROP) and extends to many other compounds (Hall and others 1975; Bartoshuk and others 1980; Gent and Bartoshuk 1983; Bartoshuk and others 1988; Marino and others 1991; Guo and Reed 2001; Kim and others 2004). The problem of bitterness in orange juice has been studied since the 1970s (Maier and others 1977; Puri 1990; Hasegawa and others 2000), and has led to the detailed sensory study by the group of Guadagni and collaborators (1973). In their limonin (and naringin) threshold study, these researchers demonstrated the effect of pH in the perception of limonin, with the highest limonin threshold (lowest perceived bitterness) at pH

Table 8— Statistical summary of stepwise multiple regressions of soluble solids content (SSC), titratable acidity (TA), SSC/TA, limonin (L), and nomilin (N) to predict sensory difference rating between juices from (+) and Las− affected Hamlin, Midsweet, and Valencia trees in 2007 and 2008. Chemical data are reported in Baldwin and others (2010).

Timeframe	Variables	Adj. R^2	*F*-value
All years combined	L	0.204	4.8*
	SSC	0.271	5.4*
2007, all varieties	SSC	0.151	7.8**
	L	0.243	4.4*
2008, all varieties	L	0.682	28.8***

*, **, *** = Significant at 5%, 1%, and 0.1% levels, respectively.

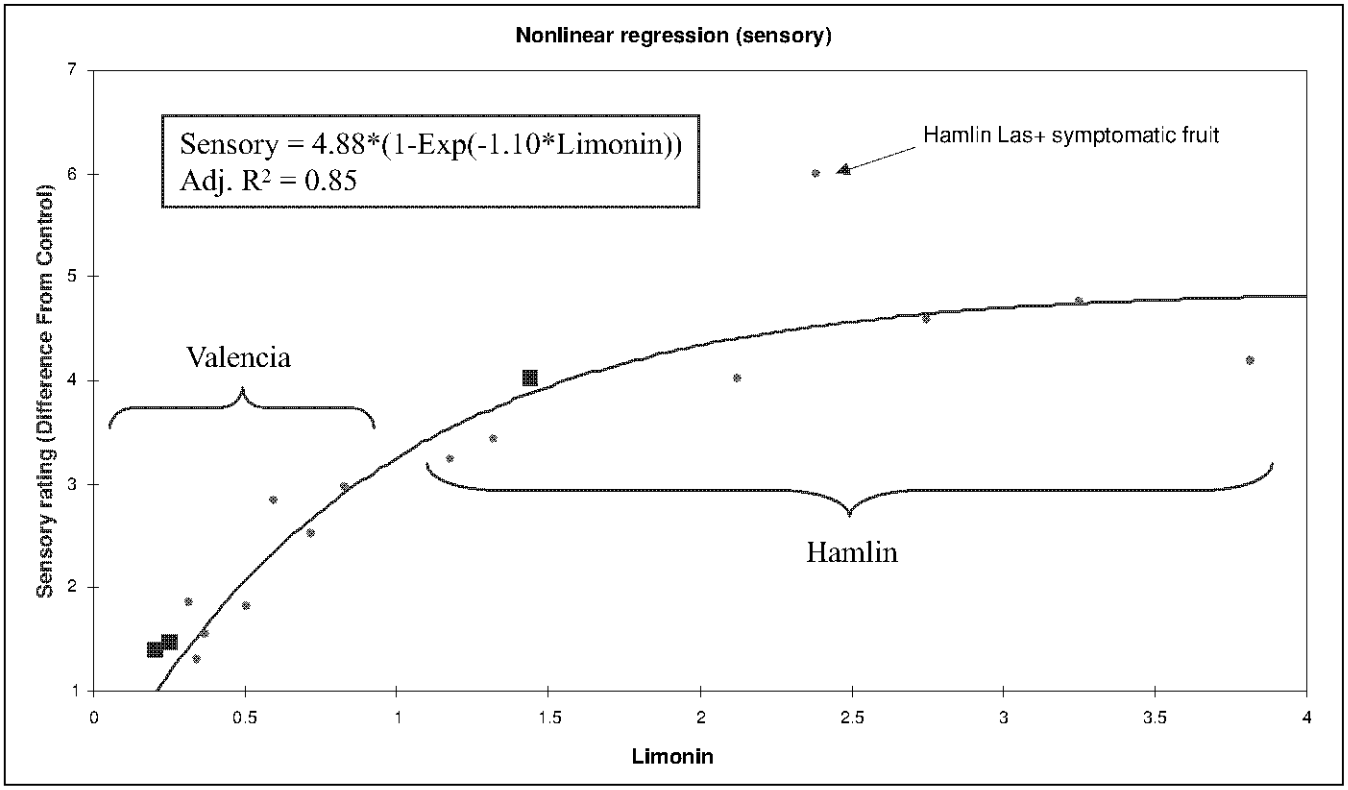

Figure 3–Nonlinear regression between limonin content in juice from Las+ fruit (x variable) and sensory rating by the difference-from-control (DFC) test. This regression was built with data from 2008 commercially processed juice (Hamlin and Valencia), reported in Baldwin and others (2010). (■) are the points from pooled juice that were used to validate the model.

Table 9 – Chemical analysis of pooled juice from 3 replications made with nonsymptomatic fruit from Las+ and Las– trees. SSC = soluble solids content; TA = titratable acidity. Chemical methods are reported in Baldwin and others (2010).

Disease status	SSC (° Brix)	TA (% citric acid)	pH	SSC/ TA	Limonin (μg mL^{-1})	Nomilin (μg mL^{-1})
		Hamlin February 2008				
Las–	11.9	0.50	4.19	23.8	1.01	0.20
Las+	11.4	0.52	4.17	22.1	1.45	0.70
		Valencia April 2008				
Las–	14.5	0.89	3.78	16.2	0.25	0.23
Las+	14.7	1.05	3.68	14.1	0.21	0.21
		Valencia June 2008				
Las–	12.0	0.42	4.37	28.7	0.34	0.04
Las+	13.2	0.46	4.27	28.4	0.25	0.04

between 3.7 and 3.9, regardless of sucrose or citric acid content. In our 2-y study, very few samples had this pH value; they were mostly at or above pH 3.9. In general, bitter and sour compounds at subthreshold levels tend to enhance each other in a hypoadditive way, that is, the threshold of compounds in the mixture is lower than that of compounds alone (Breslin 1996). In orange juice, sugars and acids play an important role in bitterness/sourness perception, as illustrated in this study. In addition, the case of Hamlin juice from symptomatic fruit needs to be further studied as these fruit had additional off-flavors not associated with limonin or nomilin. Understanding taste interactions in binary mixtures (sweet/sour, sweet/bitter, and so on) is complicated, and when compounds are in complex matrices such as orange juice, many other factors enter the picture, including peripheral (taste receptors) interactions and cognitive effects (Keast and Breslin 2003).

Other factors possibly contributing to the difference between Las+ and Las– juice

Some of the off flavors found by panelists could not be attributed to the common compounds found in orange juice. All samples were tested for microbial quality prior to serving to the panelists, but also before pasteurization of the juice to see whether the disease would contribute to some specific microbial population on the fruit surface or in the juice (the Las bacteria, associated with HLB disease, has resisted all attempts to culture to date). In the unpasteurized juice, populations of bacteria, yeasts, and filamentous fungi in relation to each other changed over season, fruit cultivar, maturity, and grove placement. However, there was no difference in the type of microbial population between juice made with fruit from Las+ or Las– trees. In all cases, pasteurized juice samples of both types had no or minimal growth (below 25 cfu's per plate). For the 2007 juice samples, some panelists' comments indicated a "sour," "fermented," or "meaty" aroma and flavor. As this is often indicative of the presence of *Alicyclobacillus* (Jensen and Whitfield 2003; Gocmen and others 2005), all of these juice samples were tested for the presence of *Alicyclobacillus*. There were no positive results from these assays for either Las– or Las+ juice samples (data not shown). Therefore, in spite of the suspicion that some of the off flavors might be due to the presence of microflora in the pre-pasteurized juice, the lack of differences between juices from Las+ and Las– trees counters that hypothesis.

Conclusions

A 2-y study on the effect of infection with *Candidatus* Liberibacter *asiaticus* (Las) on the quality of orange juice showed a large variation due to cultivar, harvest date, maturity, and tree. Nevertheless, it appears that Hamlin juice prepared from fruit harvested from Las+ trees tended to show the most off-flavors, most often described as "bitter" or "sour," and "sour/fermented" for juice made with symptomatic fruit. Hamlin fruit harvested early in the season (December) from young Las+ trees showed most of the "bitter" and "sour" characteristics, enhanced by low sugar content. Valencia fruit harvested early (March, April, or May) from Las+ trees also produced juice with "bitter" or "off-flavors" in 2007 for some trees, but in 2008 and with commercially processed juice, there were no differences between juices from fruit harvested from Las+ and Las− trees. In fact, by the June harvest, Valencia Las+ juice was sweeter than Las− juice, similar to the earlier study (Plotto and others 2008). For commercially processed juice, a model could explain some of the flavor differences between Las+ and Las− juice based on juice limonin content. Limonin at $1\ \mu g\ mL^{-1}$ could be perceived as "bitter" by the most sensitive panelists. This seems to be a low value; however, the perception of bitterness can be reduced by blending juices, increasing SSC and adjusting pH to 3.7 to 3.9, as suggested by Guadagni and others (1973). Since juice is blended under commercial conditions, studies need to be conducted to determine how much Hamlin Las+ juice can be blended to Valencia until bitterness is no longer perceived, and GC-MS and GC-olfactometry analyses will provide improved identification of off flavors undetectable by GC-FID. In conclusion, in spite of taste differences perceived between juices made from individual trees negative or positive for Las, overall differences were low and likely to be not detectable when juice is made on commercial scales, processed, and blended to reach acceptable sugar and acid levels. Similar results were found using consumer panels (Goodrich-Schneider and others 2008). When differences were high with Hamlin juice from symptomatic fruit, the amount of symptomatic fruit that can be added to normal juice before off-flavor or bitterness can be perceived should be investigated.

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