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Effect of Early Detection Huanglongbing on Juice Flavor and Chemistry

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When huanglongbing (HLB) was first discovered in Florida, trees with early symptoms of disease were harvested to determine whether there was any flavor difference between juice made from infected trees but with asymptomatic fruit, and fruit harvested from healthy trees. It is of interest to the processing industry to determine what affect fruit from trees of various stages of infection would have on processed orange juice quality. 'Valencia' oranges were harvested in 2006 from trees that tested positive for Liberibacter asiaticus, the presumed causal agent of HLB, in the early stages of disease development (HLB fruit), and compared to fruit from healthy trees (control). A consumer panel did not perceive differences for taste or smell between control and HLB juice in a triangle test. An experienced panel, however, did perceive that juice from HLB fruit was sweeter than juice from non-HLB fruit. Further tests were performed by using the "difference-from-control" test with filtered or unfiltered juice, to determine the effect of pulp on difference perception. When juice was filtered, panelists could perceive a difference by smell and by taste; when juice was served unfiltered they could only perceive a difference by taste. One of the descriptors that came up frequently for the HLB filtered juice for taste difference was again "sweeter." Chemical analyses showed that the juice from HLB fruit was lower in acids and higher in acetaldehyde content and soluble solids-to-acid ratio than from juice from non-HLB fruit, which is in agreement with the "sweeter" perception. Methanol, 2-methyl propanol, and α-pinene were also higher in juice from HLB fruit. Differences in the remaining volatiles were due to the presence of pulp but not to the health status of the tree.

Citrus greening (also known as huanglongbing or HLB) is regarded as the most devastating of all citrus diseases (Bove, 2006) and poses a serious threat to the viability of the Florida citrus industry. Extensive reviews on the biology, epidemiology, detection, geographical distribution, and control of citrus greening have been published (Bove, 2006; da Graca 1991; Gottwald et al., 2007; Halbert and Manjunath, 2004). In Florida, this disease is associated with the presence of a phloem-limited bacterium, *Candidatus* Liberibacter asiaticus (Las) which is vectored by the Asian citrus psyllid *Diaphorina citri*. At present Las has not been isolated in pure culture so it has not been possible to fulfill Koch's postulates; however, based on a substantial amount of indirect evidence it is generally accepted that greening is caused by Liberibacter species (Bove, 2006). Control measures for citrus greening are currently limited to exclusion of the pathogen, removal of infected trees, and control of the insect vector. Citrus greening disease was confirmed to be present in Florida in Aug. 2005 and the disease has subsequently been found in all major citrus producing regions of the state (FDOACS, 2008). A latency period of months to years between the time of infection and appearance of disease symptoms along with epidemiological data, which indicate that numerous infected but asymptomatic trees are present when an infection is confirmed, suggest that the number of Las-positive trees in Florida is much greater than the number of confirmed positives (Gottwald et al., 2007).

In addition to debilitating and eventually killing trees, greening has a negative impact on fruit quality. Symptomatic fruit from trees expressing greening symptoms are small, lopsided, and do not develop normal color (Bove, 2006; Gottwald et al., 2007). Reports of greening-affected trees producing fruit with off-flavor are fairly common (da Graca 1991; Gottwald et al., 2007). However, there is little published information documenting the effects of greening on citrus juice quality parameters. McClean et al. (1970) reported that fruit from greening affected trees have a bitter taste. Kinnow mandarins harvested from greening affected trees had higher acidity and lower soluble solids than did fruit from non-affected trees (Kapur et al 1978). In Brazil, greening affected fruit were smaller, lighter, "very acid," as well as being lower in Brix, sugar-to-acid ratio, percent juice and pounds solids (Bassanezi et al., 2006).

In an effort to develop strategies for dealing with citrus greening in Florida, it is essential to determine effects of the disease on fruit and juice quality. Currently, fruit are being harvested from groves that possibly contain both fruit from healthy trees and non-symptomatic fruit from Las-infected trees. Theoretically, symptomatic fruit would not enter the juice stream as they would abscise prior to harvest or be graded out. In order to gain a better understanding of how greening may impact juice quality, our objective was to conduct compositional and sensory analysis of citrus juice prepared from healthy and non-symptomatic fruit from Las-positive trees.

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Materials and Methods

Fruit sampling. 'Valencia' oranges, cultivar V-1-18-31 on Swingle rootstocks, were harvested from a 17-year-old commercial grove in Hendry County (southern growing region, Florida) on 7 July 2006. This was when huanglongbing was first discovered in Florida, and the degree of infection in that specific grove was less than 0.5%. Samples were collected from individual trees that had been tested for presence of *Candidatus* Liberibacter asiaticus by polymerase chain reaction (PCR) following standard protocol (Li et al., 2006). Six individual trees were sampled, three of which had tested negative for Las and three of which had tested positive for Las. Las-positive trees showed symptoms of the disease, but fruit were asymptomatic. About a bushel bag was harvested from each tree. Juice was manually extracted from fruit using a Sunkist model 412 juicer and immediately frozen at –20 °C.

Sensory evaluation. Frozen juice was thawed overnight prior to sensory evaluation. In triangle tests, juice was served with the pulp, while two tests were performed using the difference-from-control technique, serving juice with or without pulp. To remove pulp, juice was filtered with a kitchen stainless-steel sieve, then flash pasteurized at 71 °C for 10 s and immediately cooled in an ice bath, then served. An earlier taste panel by triangle test showed that this pasteurization method did not affect orange juice flavor but yet was effective at removing microorganisms from the juice (data not shown). Juice with pulp was also pasteurized using the same procedure as filtered juice.

Triangle tests (ASTM, 2003) were performed at the USDA-ARS laboratories in Fort Pierce (6 Sept. 2006) and Winter Haven (7 Sept. 2006), with 55 and 26 panelists in each test, respectively. Juice (15 mL) was served in 30-mL cups (Solo cup company, Urbana, IL), capped, and marked with a 3-digit code number. Juice was served at room temperature (Fort Pierce) or at 12 to 14 °C (Winter Haven). Panelists were served with three cups, and were told that two were the same, and one was different, and were asked to determine which one was different, by either smell or taste. Panelists were served two sets of samples with different codes: one set was for smell, and the other for taste. This was done to avoid bias between aroma and flavor. Samples were served in a completed randomized order and balanced, so that they were AAB, ABA, BAA, BBA, BAB, or ABB.

Since the triangle tests showed sample differences between juices from Las-positive and Las-negative trees, difference-from-control tests (Meilgaard et al., 1999) were performed in the Winter Haven laboratory. Each panelist was served two sets of juice presented in the same cups as in the triangle test. Each set comprised a control labeled as "control," and a test sample labeled with a 3-digit code number. The test sample was either juice from Las-positive (HLB) or Las-negative (control) trees. The control coded samples were presented to account for the placebo effect (Meilgaard et al., 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 informed that some test samples were identical to the control. The two sets were presented in duplicate to each panelist to account for panelist variability.

Chemical analysis. For sugar and acid analyses, orange juice samples (same juice as used for taste panels, filtered and unfiltered) were thawed, centrifuged at 18,000 × *g* at 5 °C and the supernatant filtered through cheesecloth. Titratable acidity was determined by titrating to pH 8.2 with 0.1 N NaOH using an autotitrator and soluble solids using a refractometer (Baldwin et al., 1998). Individual sugar analysis was performed using high performance liquid chromatography (HPLC) described by Baldwin et al. (1991, 1998). Approximately 40 g juice was extracted using 70 mL 80% ethanol/deionized water solution. The mixture was then boiled for 15 min, cooled and filtered (Whatman #4 filter paper). The filtered solution was brought to 100 mL with 80% ethanol. The 10 mL filtered solutions were then passed through a C-18 Sep-pak (Waters/Millipore, Milford, MA), followed by a 0.45-μM Millipore filter. Sugars (were analyzed using HPLC (Perkin Elmer, Series 400) with a refractive index detector (Agilent 1100 series) equipped with a Waters Sugar Pak column using 10^{-4} M ethylenediaminetetraacetic acid disodium calcium salt (CaEDTA) as mobile phase (0.5 mL·min^{-1} flow rate at 90 °C) and a Perkin Elmer Series 200 autosampler at 4 °C . Organic acids and total ascorbic acid were separated on an Alltech OA-1000 organic acid column with a mobile phase of 0.01 N H_2SO_4 at a flow rate of 0.2 mL·min^{-1} at 35 °C, using a Perkin Elmer Series 200 autosampler at 4 °C and a Spectra System P4000 pump and Spectra System UV 6000 LP detector.

Orange juice volatile compounds were identified and quantified by gas chromatography (GC) using the headspace analysis technique described by Baldwin et al. (1995) and Nisperos-Carriedo et al. (1990) with modification. Before sensory evaluation, thawed juice samples (3 mL) were placed in 10-mL GC vials with crimp top and Teflon-silicone septums, and frozen at –20 °C until analysis. Samples were thawed and placed into an autosampler (Gerstel Multipurpose Sampler) and held at a temperature of 10 °C. Headspace (2 mL) was injected into an Agilent 6890 gas chromatograph (Agilent Technologies) equipped with a 0.53 mm × 30 m polar stabilwax capillary column (1.0-μm film thickness, Restek Corp.), a HP-5 low bleed column (J&W Scientific, Agilent Technologies) and a flame ionization detector. The column flow rate was 8.5 mL·min^{-1}. Initial column temperature was held at 40 °C for 6 min, and then raised to 180 °C at a rate of 6 °C·min^{-1} where it was held constant for an additional 5.8 min. The GC peaks for the aroma volatile compounds were quantified in μL·L^{-1} using standard curves as determined by enrichment of deodorized orange juice (reconstituted concentrate, termed "pump out" by the industry) mixed with known concentrations of authentic volatile compounds standards for GC analysis.

Juice color was measured with a Minolta CR-300 Chroma Meter (Minolta, Tokyo, Japan) calibrated to a white plate using the CIE L*, a*, and b* system.

Statistical analysis. For the triangle tests, correct answers were tallied and compared to the values in the ASTM standards table (ASTM, 2003). For the difference-from-control tests, the degree of difference from control of the test sample (Las-positive juice) was compared against the degree of difference from the placebo control using a *t*-test. All chemical data were analyzed by analysis of variance (ANOVA) using the XLStat software (Addinsoft, Paris, France). Difference between means were performed using the LSD test, $\alpha = 0.05$.

Results and Discussion

Sensory evaluation. For the triangle test performed in Fort Pierce, 12 panelists out of 55 correctly identified the different sample by smell, which is nonsignificant, and 21 correctly identified the different sample by taste, which is significant at $\alpha = 0.30$ (Table 1). In Winter Haven, where panelists are more experienced in tasting orange juice, 12 out of 26 panelists correctly identified

Table 1. Significance levels of the differences between juice from Liberibacter asiaticus (Las) -positive and Las-negative trees in two triangle tests performed at two locations.

	Smell	Taste
Fort Pierce (55 panelists)	NS	0.30
Winter Haven (26 panelists)	0.30	0.01

[NS]Nonsignificant.

Table 2. Mean degree of difference (on a 0 to 10 scale) from control in a "difference-from-control" test.[z]

	Las-positive[y]	Las-negative[y]	LSD[x]	Probability
Filtered juice				
Smell	2.83	1.73	1.02	0.048
Taste	4.06	1.33	1.03	<0.001
Juice with pulp				
Smell	1.83	1.15	0.69	0.054
Taste	2.54	1.48	0.73	0.006

[z]In this test, the degree of difference in the "control" column represents the difference between the labeled control and the coded control (placebo).
[y]Las-positive/negative = Liberibacter asiaticus (Las) – positive/negative trees
[x]LSD = Least significant difference.

Table 3. pH, titratable acidity (TA), soluble solids content (SSC) and ratio SSC/TA of orange juice, filtered or not, from Liberibacter asiaticus (Las) -positive or Las-negative trees.[z]

	pH	TA (% citric)	SSC (°Brix)	SSC/TA
Filtered juice				
Las-positive	4.78 a	0.538 b	11.27 a	20.95 a
Las-negative	4.62 b	0.639 a	12.03 a	18.83 b
Juice with pulp				
Las-positive	4.74 ab	0.465 c	10.05 b	21.58 a
Las-negative	4.60 b	0.632 a	11.57 a	18.32 b

[z]Means followed by the same letter in a column are not significantly different by the Fisher's least significant difference test at $\alpha = 0.05$.

the different sample by smell (significant at $\alpha = 0.30$), and 15 out of 26 correctly identified the different sample by taste (significant at $\alpha = 0.01$). Panelists were free to comment on the juice. Some comments applied for either Las-positive or Las-negative juices included "sweeter," "more sour," and "fruitier."

In the difference-from-control test, when the juice was filtered, panelists found significant differences between control (Las-negative) and the test samples (Las-positive) (Table 2). Differences were larger by taste (4.06, $P<0.001$) than by smell (2.83, $P<0.05$). Descriptors that came the most frequently for the juice from Las-positive trees, by taste, were "sweeter," but also "fermented" or "overripe." When the juice remained unfiltered (with pulp), panelists could not find any difference between samples by smell, and the difference was only 2.54 ($P<0.01$) by taste (Table 2). There were no consistent comments for either Las-positive or Las-negative juice. Juice from Las-positive trees was found to be "stronger," "slightly more acidic," but also "weaker," or "sweeter" than control. A difference was clearly and repeatedly perceived, but it was difficult for panelists to pinpoint the characteristic to describe difference.

Chemical analysis. Juice from Las-positive trees always had higher pH, lower TA, and higher SSC/TA ratio, thus explaining the sweeter or fruitier comments from panelists (Table 3). SSC in juice with pulp from Las-positive trees was lower, so were sucrose, fructose and glucose, but these were nonsignificant (Table 3 and 4). Citric and malic acid were lower in Las-positive trees, especially in juice with pulp (Table 4), explaining the lower TA found in these juices and also the perception of tasting sweeter. Total ascorbic acid ranged from 62.30 to 81.14 mg·100 mL^{-1}, without differences between samples.

Among volatiles sampled from the headspace, acetaldehyde, methanol, 2-methylpropanol and α-pinene were higher in juice from Las-positive trees (Table 5). Acetaldehyde is an indicator of physiological stress in the plant and its higher level in fruit from Las-infected trees could be an early indicator of stress. The fact that ethanol and ethyl acetate, two other indicators of storage

Table 4. Individual sugars and acids of orange juice, filtered or not, from Liberibacter asiaticus (Las) -positive or Las-negative trees[z].

	Sucrose (%)	Fructose (%)	Glucose (%)	Citric (mg·100 mL^{-1})	Malic (mg·100 mL^{-1})
Filtered juice					
Las-positive	4.09 a	1.92 a	2.84 a	453.61 b	103.61 b
Las-negative	4.33 a	1.90 a	2.76 a	517.49 a	132.82 a
Juice with pulp					
Las-positive	3.68 b	1.69 b	2.46 b	398.71 c	86.39 c
Las-negative	4.05 ab	1.79 ab	2.61 ab	480.51 ab	111.76 b

[z]Means followed by the same letter in a column are not significantly different by the Fisher's least significant difference test at $\alpha = 0.05$.

Table 5. Volatiles (μL·L^{-1}) showing differences between orange juice, filtered or not, from Liberibacter asiaticus (Las) -positive or Las-negative trees[z].

	Acetaldehyde	Methanol	2-Methylpropanol	α-Pinene
Filtered juice				
Las-positive	13.27 a	1426 ab	0.032 b	0.013 b
Las-negative	8.41 b	1053 c	0.019 c	0.010 c
Juice with pulp				
Las-positive	14.38 a	1533 a	0.059 a	0.017 a
Las-negative	9.06 b	1145 bc	0.033 b	0.012 bc

[z]Means followed by the same letter in a column are not significantly different by the Fisher's least significant difference test at $\alpha = 0.05$.

Table 6. Color (L*, a*, b*, chroma and hue) of orange juice, filtered or not, from Liberibacter asiaticus (Las) -positive or Las-negative trees[z].

	L*	a*	b*	Chroma	Hue angle
Filtered juice					
Las-positive	26.68 b	–1.94 a	3.45 b	3.95 b	119.2 a
Las-negative	27.56 b	–2.52 b	5.10 b	5.68 b	116.3 b
Juice with pulp					
Las-positive	38.67 a	–6.01 c	18.51 a	19.46 a	108.1 c
Las-negative	39.22 a	–5.70 c	20.55 a	21.32 a	105.5 c

[z]Means followed by the same letter in a column are not significantly different by the Fisher's least significant difference test at $\alpha = 0.05$.

stress in fruit, were not different in juice from Las-positive trees precludes a possible postharvest stress effect. The acetaldehyde level present in the juice was much higher than its threshold in orange juice (0.187 and 0.152 ppm for orthonasal and retronasal thresholds, respectively), which could explain that juice from infected trees was perceived as fruitier, fermented and overripe (Plotto et al., 2008). Higher methanol in juice from Las-positive trees would indicate higher pectin methyl esterase activity that releases methyl groups from pectin (Daas et al., 1998), thus possibly explaining also higher 2-methyl propanol in this juice. Finally, it is unclear why α-pinene was higher in juice from Las-positive trees. The level of α-pinene was below its threshold in orange juice, making it unlikely to have any relevant effect on the sensory perception of differences between juices from Las-positive and Las-negative trees (Plotto et al., 2004).

There were no differences between juices from Las-positive and Las-negative trees for all other volatiles although filtering did have a significant impact on volatile amount released to the headspace (Figs. 1 and 2). Berlinet et al. (2007) demonstrated that juice filtering results in lower volatile amount, especially for hydrophobic compounds that bind more to the pulp. Differences in color between juices were also mostly due to filtering for L*, b*, and chroma* (Table 6). Hue angle and a* values were higher in filtered juice from Las-positive trees. This difference, even slight, could be visually perceived under natural lighting when samples are presented side-by-side. To avoid visual bias in taste panels, samples were served under red lighting (materials and methods).

To the authors' knowledge, this study presents the first report of a formal sensory evaluation of juice from Las-positive trees. Because the disease had just been discovered in Florida, samples were taken late in the season from trees confirmed Las-positive by PCR. Experienced panelists were able to detect a difference by taste between juices from Las-positive (asymptomatic fruit) and Las-negative trees. That difference was significant but small in all the tests, and was not described with negative terms (such as off flavor) in any of the tests. Fruit used in this study were at the early stage of Las detection. These fruit had lower acid and higher acetaldehyde contents, which explains flavor differences. Higher acetaldehyde in juice might be used as an early indicator of Las contamination when molecular techniques such as PCR are not available.

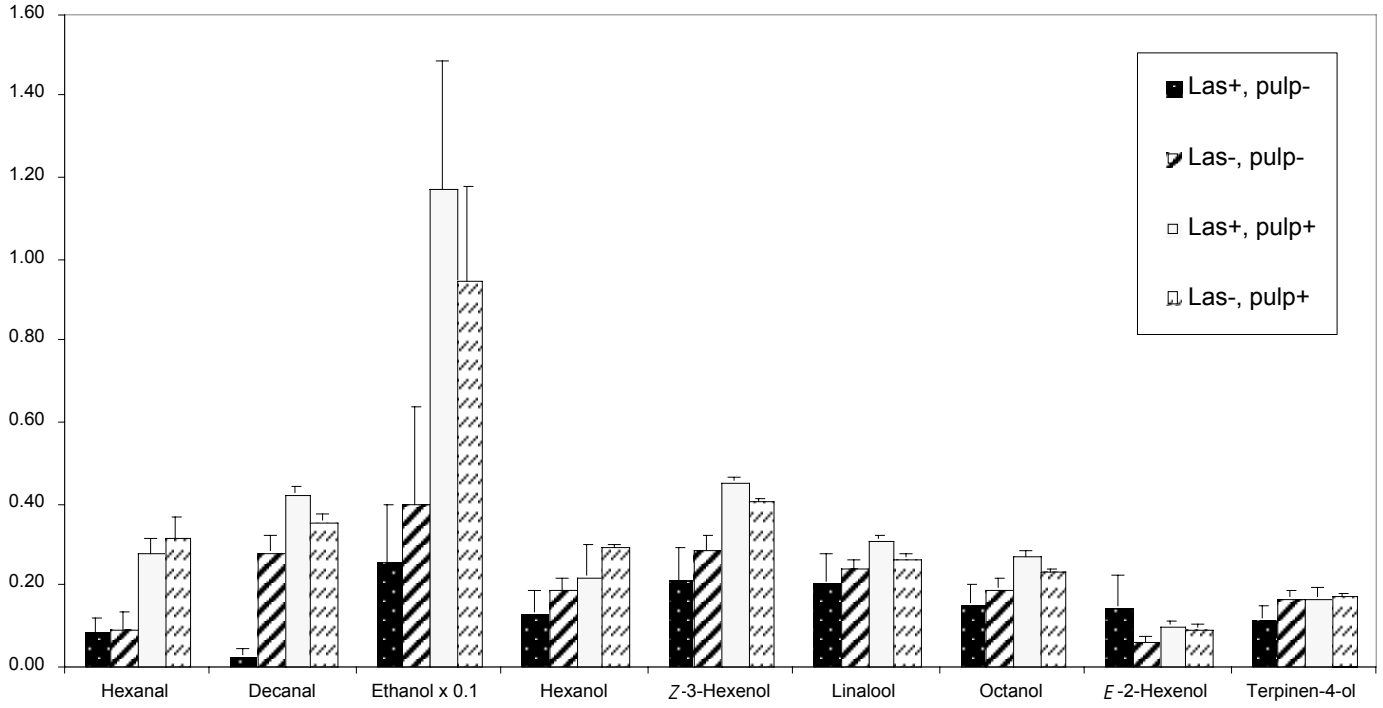

Fig. 1. Volatile aldehydes and alcohols (μL·L^{-1}) in orange juice, filtered (pulp-) or not (pulp+), from Liberibacter asiaticus (Las) -positive (Las+) or Las-negative (Las-) trees. Vertical bars are standard errors.

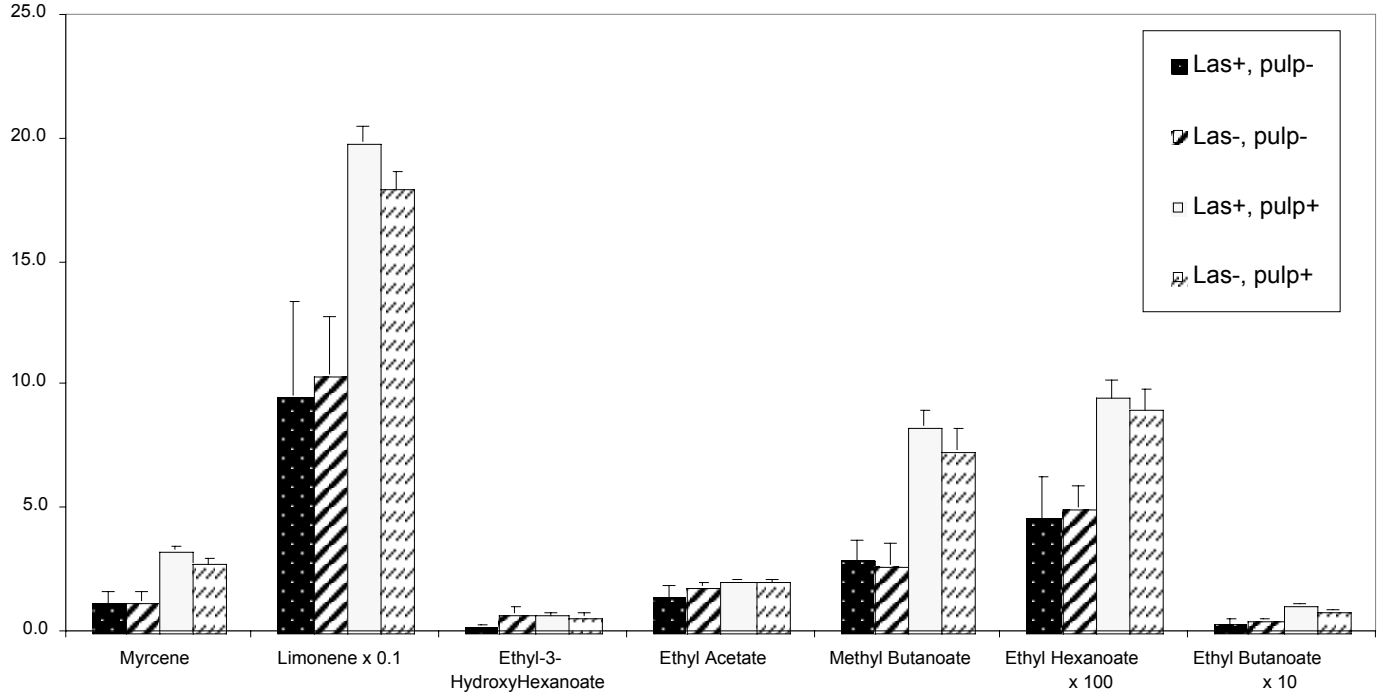

Fig. 2. Volatile terpenes and esters ($\mu L \cdot L^{-1}$) in orange juice, filtered (pulp-) or not (pulp+), from Liberibacter asiaticus (Las) -positive (Las+) or Las-negative (Las-) trees. Vertical bars are standard errors.

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