Effect of Nutritional Sprays on Orange Juice Flavor

-Preliminary Information-

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Asymptomatic vs symptomatic fruit

Asymptomatic or Non-symptomatic

Healthy  Mild  Severe

Symptomatic
Introduction

• Rigorous control, scouting and removal of infected trees is optimal and proven the best practice to limit the spread of HLB.

• When annual infection rates climb over 5%, it may make more sense economically to stop removing trees and attempt to keep the remaining trees productive using a **foliar nutrition program**. International research conference on HLB in Orlando Last January 2011, reported by Allen Morris (UF-IFAS CREC), and Mike Irey (US sugar Corp.)

• Today most growers have reached their actual or perceived economic threshold and can no longer afford to or are no longer willing to remove infected trees. (Spann, T.M., Schuman, A.W., Rouse, B., Ebel, B., 2011, Citrus Industry)

• Therefore, many growers have chosen to maintain the health and productivity of their trees through an enhanced foliar nutrition program.

• This policy could conceivably lead to 100% infection
Asymptomatic vs symptomatic fruit

Asymptomatic or Non-symptomatic

Healthy    Mild    Severe

Symptomatic
Brix to Acid Ratio-Harvest of 2009

- Hamlin 01/2009
- Valencia 04/2009
- Valencia 06/2009

Brix / TA

- Healthy
- Non-symptomatic HBL
- Symptomatic HLB

Tingling
Fruity-non-citrus
Fresh
Metallic
Fatty
Peel oil
Sour / Fermented
Peppery / Musty
Paint
Salty / Umami

Orange
Sour
Sweet

Hamlin 01/2009
Valencia 04/2009
Valencia 06/2009

SSC / TA
Non-symptomatic HBL
Symptomatic HLB
2007 Non volatile compounds

Significant for:
Hamlin L & N
Midsweet N & A
Valencia L & N except June
Valencia A except March
2007 Non volatile compounds

Significant for:
Hamlin L & N
Midsweet N & A
Valencia L & N except June
Valencia A except March
Results

In the Complex Matrix VS Orange Juice:

• Thresholds of limonin and nomilin were lower in ‘Valencia’ and commercial orange juice compared to the thresholds measured in the complex matrix.

<table>
<thead>
<tr>
<th>Matrix Solution</th>
<th>Components</th>
<th>Geometric mean ± geo. stdev. (ppm)</th>
<th>Number of panelists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex matrix</td>
<td>Limonin and Nomilin (2:1)</td>
<td>Detection threshold: 4.18 ± 0.20 (limonin) 2.26 ± 0.26 (nomilin) Recognition Threshold: 8.07 ± 0.17 (limonin) 4.51 ± 0.20 (nomilin)</td>
<td>16</td>
</tr>
<tr>
<td>Orange juice</td>
<td>Limonin and Nomilin (2:1)</td>
<td>Detection threshold: 2.32 ± 0.24 (limonin) 1.73 ± 0.32 (nomilin) Recognition Threshold: 4.07 ± 0.21 (limonin) 2.86 ± 0.27 (nomilin)</td>
<td>24</td>
</tr>
</tbody>
</table>

‘Valencia’ and commercial orange juice:
- 5.2% sucrose
- 2.1% glucose
- 2.5% fructose
- 0.75% citric acid
- 0.25% malic acid
- pH = 3.8
Results

In the simple matrix:

- The **synergetic effect** of limonin and nomilin was significant in decreasing their individual thresholds

<table>
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<th>Geometric mean ± geo. stdev. (ppm)</th>
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<tr>
<td></td>
<td>Detection threshold</td>
<td>Recognition Threshold</td>
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<tr>
<td>Limonin</td>
<td>4.00 ± 0.24</td>
<td>6.15 ± 0.24</td>
</tr>
<tr>
<td>Nomilin</td>
<td>5.86 ± 0.23</td>
<td>8.09 ± 0.19</td>
</tr>
<tr>
<td><strong>Limonin and nomilin</strong></td>
<td><strong>1.89 ± 0.30</strong></td>
<td><strong>3.99 ± 0.20</strong></td>
</tr>
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</table>

Nomilin – metallic, lingering and tingly
Blending: Hamlin 01/09

Detection

Recognition

100% HLB
50% HLB
25% HLB
12.5% HLB
6.25% HLB

Limonin

Nomilin

Healthy
Sooo….If we end up with high infection rates with trees maintained through nutritional programs…we will theoretically have less healthy fruit juice for blends…also will we eventually be forced to harvest fruit that are more symptomatic for the disease?

Could the nutritional program that mitigates tree symptoms also mitigate fruit symptoms including the potential for off-flavor
Maury Boyd initiated the original foliar nutrition program.

HLB symptomatic trees receiving a comprehensive foliar nutrition treatment have improved visually in some cases and appear to be maintaining some level of productivity.

Can fruit can be grown on HLB-infected trees with a good foliar nutrition program and their internal quality not be dramatically compromised by the infection.

The impact of these programs on HLB infected fruit flavor and aroma is unknown.
Symptomatic Huanglongbing (HLB) infected fruit and resulting juice are perceived as being more sour, bitter and off-flavored. (Plotto et al., 2010; Dagulo et al., 2010)

In the symptomatic juice, the off-flavor was correlated with lower sugars, and sometimes with higher acids as well as higher limonin/nomilin. (Baldwin et al., 2010)
This study looked at the effects on fruit flavor of 3 different foliar nutritional treatments:

- Maury Boyd
- Keyplex
- Wettable Powder (WP)

and compared them with a conventional spray program.

Serenade
Oxidate
Renew 14-7-8
Magnesium sulfate
Techmargum
Zinc sulfate
Sodium molybdate
Potassium nitrate 13-0-44 spray grade
435 spray oil
Materials and Methods

3 types of fruit samples:
- **Healthy**: Healthy orange fruit from non-infected trees
- **HLBa**: Asymptomatic fruit from HLB infected trees
- **HLBs**: Symptomatic fruit from HLB infected trees

4 harvest times and 2 cultivars:
- **‘Hamlin’**
  - December 2009
  - December 2010
  - January 2011
- **‘Valencia’**
  - April 2011
Fruits were commercially processed
Materials and methods

Sensory Analysis: Difference from control test

Same or different?

Rate the difference
Smell
0 1 2 3 4 5 6 7 8 9 10
Taste
0 1 2 3 4 5 6 7 8 9 10
Describe the difference

Two replications for statistical analysis (ANOVA)

18-23 panelists
Difference from control test: 2008

Results
Comparing non-symptomatic HLB with healthy juice

- **Valencia June**: No difference
- **Valencia April**: No difference
- **Hamlin February**: Extreme difference

Rating scale:
- No difference
- Extreme difference

Difference described as Bitter

Small sampling unit:
- 3 trees, 200-400 fruits
Materials and methods

Composition analysis:

- Sugars (fructose, glucose, sucrose, total)
- Acids (citric and malic acid)
- Ascorbic acid
- Limonin, limonin glucoside, nomilin content

Samples were also analyzed by electronic tongue and electronic nose (Alpha MOS)
Materials and methods

Electronic tongue

7 electrochemical sensors + reference electrode
Materials and methods

E-nose system

- Sample vials with homogenate
- Heating chamber
- Injection port
- Syringe
- Autosampler control
- Refrigerated holding tray
Results
No significant difference between the treatments:

- Limonin glucoside,
- Fructose
- Glucose
- Sucrose
- Total sugars

Significantly higher citric acid and lower malic acid in the **Maury Boyd symptomatic** treatment.
## Hamlin December 2009

### Difference from control test

<table>
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<tr>
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<th>HLB</th>
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<tr>
<td><strong>Conv HLB asyp vs.</strong></td>
<td></td>
<td></td>
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<tr>
<td>Smell</td>
<td>1.4a</td>
<td>2.1a</td>
</tr>
<tr>
<td>Taste</td>
<td>2.3b</td>
<td>4.1a</td>
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</table>

**Taste:** Less sweet, bitter, watery, cooked, aftertaste, tart, sour, less flavor, grapefruit, less OJ

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<tr>
<td>Smell</td>
<td>1.0b</td>
<td>2.6a</td>
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<tr>
<td>Taste</td>
<td>1.1b</td>
<td>6.3a</td>
</tr>
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**Smell:** Lime, perfume, grapefruit, odd, green, minty, mandarin, sulfur

**Taste:** Bitter, mint, medicinal, acid, astringent, less sweet, sour, sulfur, chemical, green, bland

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<th>MB</th>
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<tr>
<td><strong>Maury Boyd HLB asynp vs. Healthy</strong></td>
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<td>Smell</td>
<td>1.0b</td>
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**Smell:** Grapefruit, green, lemony, bitter, flat, sour, sulfur, chemical, medicinal

**Taste:** Grapefruit, sour, astringent/tart, bitter, less flavor/no citrus/less OJ, off-flavor, medicinal, sulfuric, burnt, metallic, greasy/oily
Hamlin December 2009

Electronic tongue

Discrimination index = 32
## Difference from control test

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<tr>
<td>Smell</td>
<td>0.7b</td>
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<td>1.0b</td>
<td>5.7a</td>
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- **Smell:** Rotten/catty/stinky, burnt, lemon, fruity, chemical
- **Taste:** Bitter, astringent/tart, grapefruit, sour, no citrus/less flavor, off-flavor, metallic, oily/greasy, sulfur, acid

<table>
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<th>Keyplex Healthy vs. Conv. Healthy</th>
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<th>K-H</th>
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<tbody>
<tr>
<td>Smell</td>
<td>0.7a</td>
<td>0.9a</td>
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<th>K-HLB</th>
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<tr>
<td>Smell</td>
<td>0.5b</td>
<td>1.3a</td>
</tr>
<tr>
<td>Taste</td>
<td>0.8b</td>
<td>5.3a</td>
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- **Smell:** Sour milk, less orange, flat
- **Taste:** Bitter, sour, grapefruit, tart, off-flavor, metallic, citrus oil, lingering

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- **Taste:** Grapefruit, bitter, sour, astringent/tart, less sweet, bland/flat, metallic, lingering
### Hamlin December 2010

**Difference from control test**

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<th>WP-H</th>
<th>WP HLB asymp vs. Conv. Healthy</th>
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<tr>
<td><strong>Smell</strong></td>
<td>0.7b</td>
<td>1.1a</td>
<td>Nutty, less orange, flat</td>
<td>0.5b</td>
<td>1.4a</td>
<td>Less orange</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Taste</strong></td>
<td>0.9b</td>
<td>1.5a</td>
<td>Bitter, watery, flat/bland, sour</td>
<td>0.9b</td>
<td>6.6a</td>
<td>Sour, grapefruit, bitter, sulfur, metallic, flat, astringent/tart</td>
<td></td>
<td></td>
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</table>
Hamlin December 2010

Electronic tongue

- Keyplex healthy
- Conv HLBs
- WP Healthy
- Conv HLBa
- WP HLBa

Discrimination index = -17
Electronic nose

Discrimination index = 76
No significant difference between the treatments:
- Nomilin content

Lower amount of citric and malic acid in HLB symptomatic, similar level among other treatments
### Total sugars (%)

- **Healthy**: a
- **HLB asymptomatic**: c
- **HLB symptomatic**: bc
- **Maury Boyd asymptomatic**: c
- **Maury Boyd symptomatic**: b

### Ascorbic Acid (mg/100ml)

- **Healthy**: ab
- **HLB asymptomatic**: ab
- **HLB symptomatic**: a
- **Maury Boyd asymptomatic**: b
- **Maury Boyd symptomatic**: ab
### Conv HLB asyp vs. Healthy

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**Smell:** Less citrus, off, weak  
**Taste:** Off-flavor, bitter, sour, less citrus/OJ, metallic, watery, less sweet, tart/astringent, less flavor, bland/flat, grapefruit

### Conv HLB symp vs. Healthy

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**Smell:** Piney, old, less fruity, rubber/gas, flat, chemical, musty, Less OJ, moldy, fermented, overripe  
**Taste:** overripe, lacking flavor, bitter, rubber, sour, metallic, grapefruit, fermented, moldy, rotten, oily, less sweet
<table>
<thead>
<tr>
<th>Maury Boyd HLB asyp vs. Healthy</th>
<th>MB Healthy</th>
<th>HLBa</th>
<th>Taste: flat/bland, bitter, metallic, less sweet, limey, less fresh, weak/muted/lacked/less flavor, sour, watery</th>
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<th>Maury Boyd HLB symptomatic vs. Healthy</th>
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<th>HLBs</th>
<th>Smell: Less OJ, chemical, citrus like, rotten Taste: rubber, less flavor, less sweet, medicinal, watery, metallic, bitter, bland/flat, oily, tart/astringent, off-flavor, sour, rotten citrus, no citrus, fermented, old, dusty, green, floral</th>
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Hamlin January 2011

Electronic tongue
Hamlin January 2011

Electronic Nose

Graph showing discrimination index of Conv. HLBa, Maury Boyd HLBs, Healthy, and Conv. HLBs.
Valencia April 2011

Electronic Tongue

Discrimination index = -38

PC2: 56.41% PC1: 89.999%
Valencia April 2011

Electronic Nose
Conclusion

The nutritional programs did not seem to mitigate the off-flavor in the Hamlin samples, nor did they reduce adverse compositional effects, but this was not as clear for Valencia.

In most cases, the panelists could distinguish between healthy controls and a HLB samples that received either conventional or nutritional sprays.

There was no significant difference in taste or smell observed in the ‘Valencia’ harvest for the nutritional treatments, yet there was either a difference between the HLBa and HLBs samples from the conventional treatment compared with the healthy control.

More harvests would be necessary to draw a conclusion. Also the effect of volatiles on juice aroma is under investigation.
Thank you!