Citrus packing: A ‘field-based’ effort to identify baseline vulnerabilities

- Identifying vulnerabilities in citrus packing
  - applicable to tree fruit in general

- Key lessons from environmental surveys
  - not citrus-specific

- Quick overview of citrus “field studies”
At the risk of making myself unwelcome...

Numerous recent recalls and outbreaks all point to the fact that, for your business sustainability, not having a well-managed cleaning and sanitization program AND an environmental testing program in the packing facility is irresponsible, reckless, and frankly insane.

It is important to see the vulnerabilities that others perceive, real or speculated

Hazard Analysis would likely cause evaluator to look at Risk Potential of sporadic contamination associated with power lines and palms as sites for birds and rodents above tree canopy.
Bins on grove soil are likely to occasionally bring Listeria into the packing facility

In non-citrus evaluations during 2014, the potential for Macro Bins resting on soil, especially wet soil, to introduce Listeria to a packing facility was demonstrated. The potential for this to occur with citrus as well seems likely.

IN OTHER FRUIT SYSTEMS... Listeria is found around bins dumps

It would be reasonable to carefully evaluate the situation in your facility.
### Environmental Monitoring in CA Packing Operations Key Findings

- Listeria prevalent in within Zone 2 and 3
  - areas routinely wet
  - Typical chlorine or ClO₂ addition to water doesn’t prevent biofilm buildup
- L. mono persistent following chlorine sprays
  - More aggressive sanitation needed
- L. mono persistent over several months
  - Detectable in dry off-season conditions
  - Rebounds once wet operations re-start
- Some facilities consistently no-detection

### Assume there is a constant assault from Listeria on your facility

Contamination is likely to be low but our specific knowledge is limited. Suslow CRB project for 2015-16 is targeted to address this knowledge gap.
Expect Listeria to be Present Sporadically in a Grove

Tote Cleaning Schedules are Variable
Each Bin Represents a Variable Risk of Introducing Contaminants to Water

The Challenges of Cleaning Fruit Handling and Sorting Equipment are Prominent
Citrus Packers May Have Vulnerable Surfaces; Contact and Non-Contact

Citrus Packers May Have Vulnerable Surfaces; Contact and Non-Contact
### Examples of Outcomes of Produce Handler Environmental Surveys

<table>
<thead>
<tr>
<th>Cooperator Location</th>
<th>Total Swabs</th>
<th>Total Listeria spp. Positives</th>
<th>Total Listeria monocytogenes</th>
<th>Key Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50</td>
<td>18</td>
<td>11</td>
<td>All culture confirmed positives from dry surfaces formerly positive when wet 56 days earlier and dry 26 days earlier</td>
</tr>
<tr>
<td>B</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>All swabs taken from wet areas</td>
</tr>
<tr>
<td>C</td>
<td>28</td>
<td>5</td>
<td>3</td>
<td>All culture confirmed positives from surfaces positive in previous dates</td>
</tr>
</tbody>
</table>

### Listeria Positive Sites: Is your packing operation vulnerable?
Listeria Positive Sites: Is your packing operation vulnerable?
Listeria Positive Sites: Is your packing operation vulnerable?
Listeria Harborage Sites
Is your cleaning and sanitizing program adequate?

Citrus Industry Workshop:
AVOIDING RECALLS – MANAGING FOOD SAFETY RISKS IN CITRUS PACKINGHOUSES
January 28, 2015

Citrus packing: A ‘field-based’ effort to identify baseline vulnerabilities

- Identifying vulnerabilities in citrus packing
- Key lessons from environmental surveys
- Quick overview of citrus “field studies”
If irrigation water directly contacts fruit, what is the expected survival?

As with many crops, attSalmonella survival is greater than attEcO157
On-Tree Fruit Inoculation: 
attrSalmonella Typhimurium $\chi_{3985}$

2014 LREC Field Trial Summary: 
Qualitative Detection...the last survivors

<table>
<thead>
<tr>
<th>% Enrichment Positives</th>
<th>0</th>
<th>5</th>
<th>12</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generic E. coli (Spray)</td>
<td>100%</td>
<td>16.7%</td>
<td>18.3%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Attn Salmonella (Spray)</td>
<td>100%</td>
<td>95.0%</td>
<td>46.0%</td>
<td>15.0%</td>
</tr>
<tr>
<td>Generic E. coli (Feclone)</td>
<td>100%</td>
<td>100%</td>
<td>74%</td>
<td>2%</td>
</tr>
<tr>
<td>Attn Salmonella (Feclone)</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>12.2%</td>
</tr>
</tbody>
</table>
Navel Orange Wash Line Trials Baseline Microbial Disinfection

LREC Citrus Wash and Postharvest Treatment Line

Validation Criteria
Reproducible and reliable postharvest control parameters

Verification Criteria
Measurement and Documentation of a postharvest program matching validation parameters
What happens if I don’t control my recirculating postharvest water ‘microbial load’?

- **Figure A**: Comparison of Total Plate Count and Total Coliform counts between Non-treated Water and Antimicrobial treated water over time. The graph shows a 1000-fold increase in both counts for the Non-treatment conditions.

- **Figure B**: Similar comparison with the addition of a control group, indicating the effectiveness of the antimicrobial treatment.

---

**Pilot Wash Line Trials at UC Lindcove Citrus Research Station**

- Defining validation parameters and process verification standards
## LREC Wash and Treatment Line: Results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$n$</th>
<th>$pH$</th>
<th>Temp (°F)</th>
<th>Free Chlorine</th>
<th>log CFU/orange ± st dev (% enrichment positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attn Salmonella Concentration Post Inoculation</td>
<td>20</td>
<td>N/A</td>
<td>62.6</td>
<td>6.26 ± 0.17</td>
<td>(100)</td>
</tr>
<tr>
<td>10 Hours Post Inoculation</td>
<td>20</td>
<td>N/A</td>
<td>6.93 ± 0.37</td>
<td>(100)</td>
<td></td>
</tr>
<tr>
<td>3 Days Post Inoculation (conc prior to washing)</td>
<td>1</td>
<td>30</td>
<td>2.70 ± 0.71</td>
<td>(100)</td>
<td></td>
</tr>
<tr>
<td>Control (Soak in Water @ ambient temp)</td>
<td>2</td>
<td>30</td>
<td>8.31</td>
<td>1.59 ± 0.59</td>
<td>(100)</td>
</tr>
<tr>
<td>Soak in 200 ppm FAC pH 7.0 @ ambient temp (15s followed by rinse)</td>
<td>4</td>
<td>30</td>
<td>8.3</td>
<td>1.55 ± 0.62</td>
<td>(86.7)</td>
</tr>
<tr>
<td>Soak in 200 ppm FAC pH 7.0 @ 80F (15s followed by rinse)</td>
<td>5</td>
<td>30</td>
<td>8.36</td>
<td>1.68 ± 0.68</td>
<td>(76.7)</td>
</tr>
<tr>
<td>Soak in 200ppm FAC and 1.0% SBC pH 8.3 @ 80F (15s followed by rinse)</td>
<td>7</td>
<td>30</td>
<td>8.34</td>
<td>1.60 ± 0.56</td>
<td>(76.7)</td>
</tr>
<tr>
<td>PW @ 200 ppm FAC pH 7.0 at ambient temp</td>
<td>3</td>
<td>30</td>
<td>8.2</td>
<td>1.37 ± 0.28</td>
<td>(43.3)</td>
</tr>
<tr>
<td>PW @ 200ppm FAC and 1.0% SBC pH 8.3</td>
<td>6</td>
<td>30</td>
<td>8.3</td>
<td>1.31 ± 0.00</td>
<td>(53.3)</td>
</tr>
</tbody>
</table>

N/A: not applicable

LOD: log 1.26 CFU/orange

## Mixed Inoculated and Non-Inoculated Oranges Run at the Same Time

- **attSalmonella $\chi^{3985}$**
  - Below Limit of Detection (12 CFU/fruit)
  - 45.9% enrichment positive – Culture & PCR

- **Non-inoculated**
  - Below Limit of Detection (12 CFU/fruit)
  - 4.5% enrichment positive – Culture & PCR
Spatial Mapping of Stress-adapted attSalmonella enterica Typhimurium χ3985

Results by Fruit
10 out of 16 fruits (62.5%) with 1 positive each

<table>
<thead>
<tr>
<th>Location</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem end</td>
<td>40% (4/10)</td>
</tr>
<tr>
<td>Top</td>
<td>10% (1/10)</td>
</tr>
<tr>
<td>Center</td>
<td>20% (2/10)</td>
</tr>
<tr>
<td>Bottom</td>
<td>20% (2/10)</td>
</tr>
<tr>
<td>Blossom end</td>
<td>10% (1/10)</td>
</tr>
</tbody>
</table>
How many live bacteria are still attached to the rind?

- Recovered from fruit individually
  - Filtration
    - 1 ml
    - 10 ml
  - Centrifugation
    - 1 ml

Between 5 and 25 cells/fruit recovered at end of line

Log-Removal of Inoculated Salmonella by HPW 150 psi

- Pedicel end: 2 log removal (99%)
- Center: 3.5-4 log removal (99.95%)
- Stylar end: 1 log removal (90%)
Are the persistent survivors an experimental artifact due to spatial concentration following inoculation?

Contamination is likely to be low but our specific knowledge is limited
CRB 2015 Objectives

**Postharvest**

- Conduct a baseline survey of indicators and pathogens on incoming and final packed navel oranges during the 2014-15 season with cooperating commercial operations

  - We are approved to conduct a preliminary evaluation of commercial wash-lines towards the goal of science-based and data-based Best Practice guidance for the CA citrus industry

  - We have conducted multiple studies with other diverse commodities using a delayed-detection technique to assess pathogen prevalence well after normal fresh market shelf-keeping expectations

Benefit to Citrus Industry

- We anticipate substantial progress towards a validated postharvest sanitization process for navel oranges

- The CA citrus industry will benefit from developing a rational and sensible low risk profile for oranges
Thank you for your attention