

Resolving Postharvest Harborage Sites of *Listeria* Protects Zone 1 Surfaces

SUMMARY

The 2014-15 California Agricultural Statistics Review places the combined value of oranges, lemons, and tangerines at over \$2 billion dollars and all three fruits are in the Top 15 Specialty Crops by value. Fresh whole citrus has not experienced an incident of recall, illness, or outbreak. CA citrus production practices appear to significantly limit the risk of preharvest contamination. However, concerns voiced across the supply chain towards *Listeria monocytogenes* in packing operations have prompted proactive measures to better understand its prevalence and more carefully assess interventions to sustain this low risk profile. Confidentially enrolled handlers are participating in a detailed survey for indicator *Listeria* and *L. monocytogenes* spanning multiple dates and across two seasons. The outcome will be a model Environmental Monitoring Program (EMP) and guidance in a zone-based Master Sanitation Schedule linked to EMP-outcomes and on-site testing of sanitizer options. From this effort, measurable reductions in *L. monocytogenes* prevalence will result.

OBJECTIVES

The rationale for the project objectives is straightforward. Our experience is that irrespective of an individual packing operation Environmental Monitoring Program using a zone concept, the systematic identification of harborage sites and source-tracking of transient and resident *Listeria monocytogenes* does not occur in the absence of a 'crisis' incident for that company. In this manner, broadly sharing prevalence traits towards protecting against recalls and outbreaks remains opaque. Our project seeks to develop this generalized knowledge by:

1. Developing a baseline spatial mapping profile, among confidentially enrolled handlers, towards the development of a model Environmental Monitoring Program (EMP) for California fresh citrus packinghouses.
2. Assessing the efficacy of selected hard-surface, non-porous, and porous surfaces sanitizers on-site that test positive for genus *Listeria* in enrolled citrus packinghouses.

METHODS

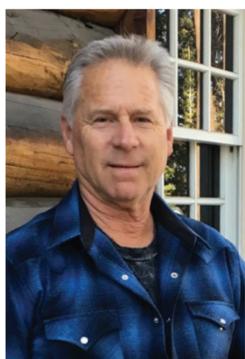
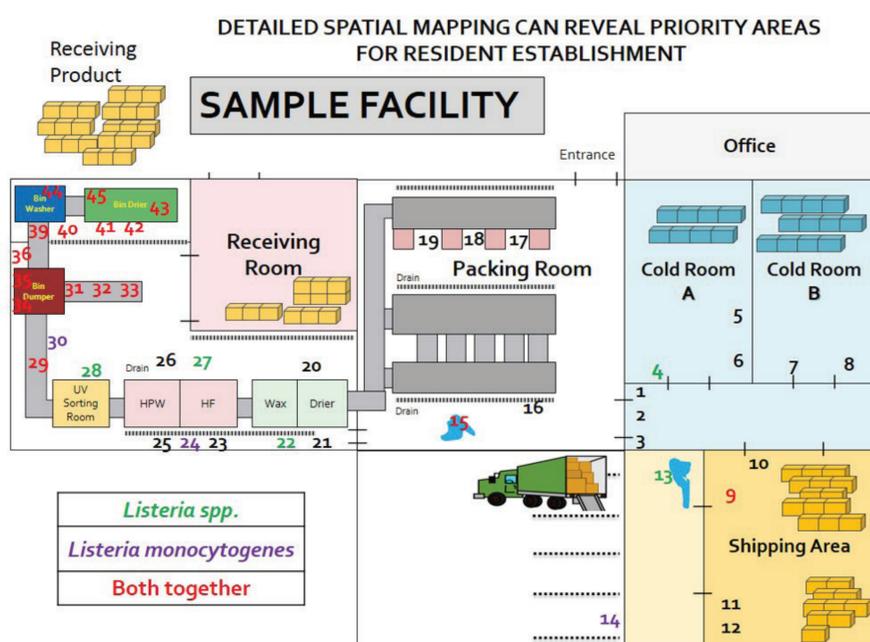
The number of swabs per facility per date will depend on size and complexity of the operation. Each sterile sponge is presoaked with neutralizing buffer in Whirl-Pak® bags (Hydrated Speci-Sponge, Nasco). Following non-selective resuscitation, we have implemented enrichment using an improved *Listeria* media (Actero *Listeria* Enrichment Media). Sponges and probes are enriched with Actero *Listeria* Enrichment Broth and incubated for 24 h at 37 °C. After incubation, enrichments are screened using Roka Atlas® (Roka Bioscience). Positive samples are streaked in triplicate onto CHROMagar *Listeria* (CHROMagar) and incubated for 48 h at 37 °C. *Listeria* strains are screened for diagnostic markers using TaqMan PCR. Conventional multiplex PCR is used for *Listeria monocytogenes* serogroup assignment. A simple assessment of the efficacy of alternative disinfectants being introduced to the produce industry, including RelyOn (potassium peroxydisulfate; sodium chloride; Dupont by PFMBio, Inc.), JC 9450 (Jenfitch, LLC), and Decon 7 (Decon7 Systems, LLC.) will be tested.

RESULTS TO DATE

During this period, 457 samples from non-food-contact surfaces were collected from five citrus packinghouses. Three swab events were conducted in facility 1 (N=100), four samplings in facility 2 (N=160 samples), and one sampling in facility 3 (N=62), facility 4 (N=84) and facility 5 (N=51). Enrichments were processed for molecular and culture screening of *Listeria*. Samples were tested using two platforms, Atlas® (Roka Bioscience) and 3M™ Molecular Detection system (3M). Of 457 samples collected, 40.9% (187) of enrichments showed a positive molecular outcome for *Listeria*, and 39.3% (174) were culture positive. At this point in the culture process, 114 isolates have been confirmed as *Listeria* spp. and 110 as *Listeria monocytogenes*. Approximately 28.2% (49) of culture positive enrichments have yielded both *Listeria* spp. and *Listeria monocytogenes*. Based on molecular and cultural findings, initial spatial mapping profiles for facilities have been developed and tests of sanitizers on repeat positive areas have been initiated.

BENEFITS TO THE INDUSTRY

The immediate beneficiaries are the over 4,500 CA citrus growers and shippers who can reduce their vulnerability to an economically damaging recall or, more seriously, an outbreak. The PI has been conducting highly related research surveys at various tree fruit packing facilities for the past 2 years. These experiences have led to optimized detection and recovery techniques. All isolates of *L. monocytogenes* (*Lm*) are characterized by pulsed-field gel electrophoresis (PFGE) and we have completed the first view of spatial mapping and diversity with 80 isolates by whole genome sequencing (WGS). The two-enzyme PFGE profiles and PCR serogrouping we have completed have provided unique opportunities for spatial and temporal mapping of *Lm* and suggest likely cycling between crop production and packing. Resolving relatedness and diversity characterization by WGS is critical to source-tracking. One immediate outcome from these investigations has been procedural changes at specific zone-sites yielding consecutive positives for *Lm* among enrolled companies.



CONTACT Trevor Suslow
University of California, Davis
Dept. Plant Sciences MS3
tvsuslow@ucdavis.edu
530.754.8313

AUTHORS Adrian Sbodio aosbodio@ucdavis.edu
Janneth Pinzon-Avila jpinzon@ucdavis.edu

ACKNOWLEDGEMENTS

We gratefully acknowledge the technical assistance of David Hill, Mariya Skots, and Lee Ann Richmond in the execution of this project. We gratefully acknowledge the assistance and in-kind support from Roka Biosciences, FoodChek Systems Inc./Actero, 3M Food Safety, the Citrus Research Board, CA Citrus Quality Council, and citrus industry cooperators.

LENGTH OF FUNDING

January 1, 2017 – December 31, 2018