CPS Research 2019: Putting Research into Practice
Bringing research learnings to the produce industry

Welcome

Center for Produce Safety
Produce Marketing Association
United Fresh Produce Association
Western Growers
Dave Corsi
VP of Produce and Floral, Wegmans Food Markets
Chair, Center for Produce Safety Board of Directors
Mission:
Fund the science, find solutions and fuel the change

Purpose:
Providing the science to support produce safety
2019 CPS Research Symposium

June 18-19, 2019, Austin Texas

377 Participants
21 Full research presentations
14 Lightning reports and posters
4 General sessions
Our Presenters
Bonnie Fernandez-Fenaroli
Executive Director
Center for Produce Safety
CPS Research Program

Funding Sources:
• Campaign for Produce Safety – Produce Supply Chain,
• Specialty Crop Block Grant Programs – California, Florida, Washington, Texas

Approval Process
• CPS Technical Committee recommends proposals for funding
• CPS Board provides approval for all projects

2019 Program
• Track 1 – Annual Call
• Track 2 – Challenge Award – Grower’s Risk Assessment Biomarkers Investigative Tool
• Track 3 -
• Track 4
Track 1 – Annual Call for Proposals

Listeria monocytogenes (Lm) in the Supply Chain, Postharvest Preventive Controls, Postharvest Interventions.

Preliminary Proposals - 49
Full Proposals - 20
Total project budget $2.77M
9 projects – US institutions, 2 projects located in Spain
5 new principal investigators
Track 2 – Challenge Award

Real-time pre-harvest crop monitoring tool(s) for animal / plant proximity risk assessment – GRABIT (Grower's Risk Assessment Biomarkers Investigative Tool)
Track 3 – Water Treatment Baseline Program - RFP

Where and when are agricultural water treatments appropriate, and what options are available to growers.

- **Five Regions**
- **Five Prime Awards**
- **Funding Prioritized by Region**
- **Minimum of Five Sites in Region**
Track 4 – Rapid Response

- Research opportunities that will be lost if not implemented quickly
- Industry partner require
- Maximum Project Cost - $50,000 – maybe larger if cash match is available
- CPS funds available – Will provide up to 50% of total project cost as a match to other cash pledges.
- Proposal and Budget must be submitted at the same time.
- Once the proposal and budget is received. A subcommittee of the CPS Technical Committee is formed. A review will be provided within 3-5 days of the proposal receipt.
- The receiving university must be ready to implement a contract quickly.
www.centerforproducesafety.org
Research Topic 1: Ag Water
Establishment of operating standards for produce wash systems through the identification of specific metrics and test methods
Ana Allende (PI) & Mabel Gil (Co-PI) CEBAS-CSIC, Spain

Objectives
• Identify, validate, and verify critical parameters and operational limits
• Evaluate sensor performance for monitoring
• Determine evidence-based standards for common disinfectants (e.g., chlorine, PAA)

Key Findings
• Critical parameters:
  • Chlorine = residual FC and total chlorine, pH, and organic matter
  • PAA = disinfectant level
• Absorbance of water at 254 nm can be used to monitor produce wash water.
• Lower operational limits for FC and PAA vary depending on produce
• PAASense (Palintest, Gateshead, UK) is suitable for measuring PAA
• Chlorosense (Palintest), and a DPD-based method (Spectroquant, Merck, Darmstadt, Germany) are appropriate sensors for FC and total chlorine

Importance/Next Steps
• These findings provide critical information to help operators effectively setup, maintain and monitor their wash water systems.
Remotely-sensed and field-collected hydrological, landscape and weather data can predict the quality of surface water used for produce production

Martin Wiedmann, PI, Cornell University, Channah Rock, Co-PI, Univ of Arizona

Objectives
• Assess variation in the microbial quality of NY and AZ surface waters (SW).
• Quantify generic *E. coli* levels and pathogen presence
• Identify and prioritize factors associated with pathogen presence in SW.
• Develop models to predict SW quality in NY and AZ.

Key Findings
• Water sampling method depends on the organism of concern:
  • pathogenic *E. coli* and *Salmonella* - 24-h Moore swabs
  • *Listeria* - 10-L grab samples
• Utility of *E. coli* appears to be pathogen- & region-specific
• Data on *E. coli* levels - useful for managing EHEC-associated risks in AZ.
• Changes and interactions in environment influence microbial water quality.

Importance/Next Steps
• Optimize ag water sampling protocols
• Design risk management strategies for preharvest surface water use considering environmental influences.
• Develop predictive risk maps using the existing models
FSMA agricultural-water die-off compliance provisions benefit from condition-specific modifiers
Renata Ivanek, Cornell University

Objectives
• Estimate indicators and attenuated pathogen die-off rates on baby-spinach and baby-lettuce under field conditions in 3 different climatic regions.
• Develop predictive models of pathogen die-off under relevant environmental conditions and industry practices and use the model to evaluate the FSMA ag water matrix

Key Findings
• A log-linear die-off rate is not always appropriate
• A segmented log-linear die-off rate is a better representation of observed microbial decline
• The rate of die-off is highly variable and varies by location, trial, bacteria and produce type
• Results do not support uniform die-off rates across locations and over time.
• Assuming uniform die-off rates close to harvest is risky

Importance/Next Steps
• FSMA die-off rate is not always reliable
• Unique environmental conditions and industry practices should be factored into specific die-off rates
**Objectives**

- Determine the occurrence of *C. cayetanensis* in:
  - irrigation waters in Arizona and Texas.
  - raw sewage and treated wastewater effluents in produce-growing areas in Arizona and Texas

**Key Findings**

- *Cyclospora* was found in:
  - influent (raw sewage) samples –46 to 55%;
  - effluent (treated wastewater) samples –26 to 37%
  - irrigation water samples –5 to 13%
- *Cyclospora* occurrence - no seasonality pattern and no correlation between *E. coli* and coliform data
- *Cyclospora* source, viability and infectivity of positive samples were not determined
- Findings indicate there is *C. cayetanensis* infection among both communities
- Persons from these communities – working in produce operations may pose risk

**Importance/Next Steps**

- Identify areas of risk from *C. cayetanensis*
- Rate of *C. cayetanensis* infection among these communities
- Work with industry to raise awareness in precautionary measures to reduce the potential for cross-contamination of fresh produce by infected persons
- Determine the risk of *C. cayetanensis* in effluent (treated wastewater) released into watersheds flowing to fresh produce growing regions in Mexico
Application of chitosan microparticles to eliminate foodborne pathogens in agricultural water that contacts fresh produce

Anita Wright, University of Florida

Objectives

• Investigate practical pre-harvest application of chitosan microparticles (CM) for pathogen reduction in the complex medium of irrigation pond water.
• Optimize use of CM films for post-harvest application to reduce/prevent pathogen contamination on produce.
• Conduct preliminary experiments to investigate CM activity against norovirus.
• Assess cost-effectiveness of chitosan application to ag water.

Key Findings

• Biodegradable, non-toxic alternative to chlorine; mode of action does not appear to promote antimicrobial resistance
• CM consistently produced significant reductions in multiple *Salmonella* serotypes in stationary phase
• CM reduces indicator bacteria to levels that would meet current standards for irrigation water
• CM significantly reduced levels of viruses
• Flocculant properties may contribute to removal of other unwanted components
• Data demonstrate that CM may present a viable alternative for treatment of natural water systems

Importance/Next Steps

• Further explore application of antimicrobial CM films to food products
• Industry collaboration to adapt methodology for practical application
• Expanded validation and preparation method need to be scaled up to be practical for industrial applications and reduce cost
Lightning Round

Projects

• **Development of a model to predict the impact of sediments on microbial irrigation water quality** - Charles Gerba, University of Arizona
  - Pathogens occur in greater concentrations in irrigation canal sediments than in the overlaying water
  - Pathogen resuspension in the water can cause a rapid increase in concentration
  - Project will develop a model to determine when resuspension will occur, which can be used to minimize risk during irrigation event

• **Cyclospora prevalence in irrigation water in fresh produce growing regions** - Gerardo Lopez, University of Arizona
  - Project was funded as a rapid response to last year’s *C. cayetanensis* outbreaks
  - Determine the occurrence and prevalence of *C. cayetanensis* in irrigation waters in fresh produce growing regions
  - Is irrigation water in the US a major source of *C. cayetanensis* contamination to fresh produce?

Importance

• Actively examining two key areas of risk that have been directly tied to recent outbreaks.
• Results should provide actionable information to industry

**CPS Symposium – June 23-24 – La Jolla, CA**
Dr. Trevor Suslow
Vice President, Produce Safety
Produce Marketing Association

Research Topic 2:
Produce Environment
Produce Production Environment

- Approaches to reduce Pacific tree frog intrusion
  - Michelle L. Green; University of South Florida St. Petersburg

- Growth potential of *L. monocytogenes* on non-traditional salad ingredients
  - Amanda Lathrop; California Polytechnic State University - San Luis Obispo

- Are raptors a viable approach to deter wild bird and rodent intrusion?
  - Paula Rivadeneira; University of Arizona, Yuma Agricultural Center

- Environmental monitoring and corrective actions for shade-house systems
  - Trevor Suslow; University of California, Davis Emeritus Faculty

- Establishment of vegetative buffer zones (VBZs) to reduce the risk near domesticated animal operations
  - Siddhartha Thakur; North Carolina State University
Engineering and ecological approaches reduce Pacific tree frog intrusion into leafy green agriculture

Are there environmentally friendly management practices for reservoirs and ponds that would limit reproduction?

Would a better understanding of amphibian behavior within leafy greens production environments lead to possible control measures?
Engineering and ecological approaches reduce Pacific tree frog intrusion into leafy green agriculture

KEY LEARNING
Fence designs with a physical lip or rough surfaces will inhibit frogs from climbing.

Fabric ‘silt fences’ are easily breached

Fencing with a lip are not

Experimental

Grower Version
The effects of storage conditions and the microbiome of non-traditional salad ingredients on the fate of *Listeria monocytogenes*

- Determine if *L. monocytogenes* will grow, survive or die off in fresh-cut broccoli stalk, Brussels sprouts, kale and beet greens at 4, 12, 22 and 35°C

- Inoculated at 2–3 log CFU/g with a 5-strain cocktail of *L. monocytogenes*

**KEY OUTCOME**
- Produce type and temperature influenced *L. monocytogenes* growth
- Differences in rate and peak population increase
- Shredded Brussel Sprout had least growth across temperatures and times
Large variation Among replicates

4°C (39°F)

Log Increase

2.6
2.4
1.8
0.6

L. monocytogenes Log CFU/g

Time Days

0 5 10 15 20 25

Kale
Brussels Sprouts
Broccoli
Beet Greens
Use of raptors to prevent wild bird and rodent intrusion into fresh produce fields

• Non-lethal bird control measures, like sound and visual scare tactics, are minimally successful at best

• New deterrents need to be developed to prevent/reduce bird intrusion and potential for transmitting foodborne pathogens

• Raptors used in other industries and preliminary CPS project in CA
Use of raptors to prevent wild bird and rodent intrusion into fresh produce fields

KEY LEARNING

- Falconry can be an effective tool for deterring nuisance birds in fresh produce fields when used in combination with other tools
  - Drones also used in study

- Growers attracted to concept of falconry but cost is a likely barrier

- Strategic deployment at key vulnerable growth stages of interest
Establishment of vegetative buffer zones (VBZs) to reduce the risk of STEC and *Salmonella* transmission from animal operations to fresh produce on co-managed farms

- Develop a fast-growing and cost-effective vegetative buffer zone (VBZ) to prevent movement of STEC and *Salmonella* from animal production areas (APAs) to fresh produce fields.
Establishment of vegetative buffer zones (VBZs) to reduce the risk near domesticated animal operations
Establishment of vegetative buffer zones (VBZs) to reduce the risk near domesticated animal operations

KEY LEARNINGS

• Lower recovery rates of presumptive *Salmonella* and STEC from samples (soil, air, and fresh produce) closest to the VBZ

• The number of positive environmental samples increases during the summer to fall transition
Dr. Jennifer McEntire  
Vice President, Food Safety & Technology  
United Fresh Produce Association

Research Topic 3:  
Microbiological Tools
Metagenomics to ID viral indicators in the produce chain

• Gloria Sanchez-Moragas, IATA-CSIC, Spain

• Objective
  • By evaluating the microbial communities of irrigation water positive for human pathogens, find indicators

• Key Findings
  • Optimized method
  • No single family that is always present with pathogens, and absent when there are no pathogens

• Importance/next steps
  • Deeper evaluation within families to see if there are suitable indicators
Listeria whole genome sequence data reference sets are needed to allow for improved persistence assessment and source tracking

• Martin Wiedmann, Cornell University

• Objective
  • Understand geographic spread of Listeria, including monocytogenes, in the non-ag environment in the US

• Key findings
  • Over 30% samples were positive for Listeria, and positives were more likely found in the eastern part of the country. 12% were mono, and different species had different distribution patterns.

• Importance/ next steps
  • Whole Genome Sequencing of isolates and comparison to historical produce isolates
Developing cross-assembly phage as a viral indicator for irrigation waters

• Kyle Bibby, University of Notre Dame

• Objective
  • Identify human-specific fecal indicators in irrigation water in 5 states

• Key findings
  • CrAss phage was more readily detected than current standard indicators

• Importance
  • Final report; look for publication
  • Poor correlation with other “indicators” such as generic E. coli
Detection, validation, assessment of risks implied by the VBNC state of enteric bacterial pathogens in fresh produce

• Xiaonan Lu, University of British Columbia

• Objective
  • Develop method for quantifying the viable but non culturable state
  • Determine if cells enter the VBNC state in real life

• Key findings
  • Loop-mediated isothermal amplification coupled with propidium monoazide (LAMP-PMA) method developed
  • Both *E. coli* O157:H7 and *Salmonella* enter VBNC on romaine lettuce

• Importance
  • Final report
  • Increase awareness for interpretation of pre-harvest test results
Rechargeable antimicrobial and antifouling plastics for improved cleaning and sanitation of plastic bins and totes

• Gang Sun, UC Davis

• Objective
  • Develop a coating for RPCs that is antimicrobial and antifouling

• Key findings
  • Coating developed, can be recharged by chlorine
  • Demonstrated lack of cross contamination, and prevention of biofilm formation

• Importance/ next steps
  • Develop manufacturing process for industrial application
  • Seeking industry collaborators to pilot
Dr. Bob Whitaker
Chief Science & Technology Officer
Produce Marketing Association

Research Topic 4:
Packing and Processing
Research Session 4 – Packing and Processing
Preservation of stone fruits by spray application of edible coatings with antimicrobial properties

Kay Cooksey, Clemson University

Objectives:

1. Develop a flow-through system to determine cleaning efficacy on surfaces with flat and topographical features and determine surface role and cleaning procedure in the possible pathogen contamination in stone fruits.
2. Evaluate novel, edible fruit coating formulations with antimicrobial properties that can be developed as brush-independent (spray) applications and can replace traditional wax treatments to maintain fruit integrity and shelf life.
3. Determine efficacy and properties of selected coating formulations in challenge studies in controlled conditions.
Key findings:
1. Developed a flow-through apparatus to create biofilms
2. Found mineral oil coatings (PrimaFresh 220) increased cell attachment to brush filaments.
3. 200 ppm chlorine for 20 minutes achieved 3-log reduction on brush filaments coated with PrimaFresh 220.

Why is this important?
• Recognition that different surfaces needs different treatment
• Flow through biofilm device sets the stage to look at alternative edible fruit coatings and also mechanisms to control biofilms (Lightning Session)
• Potential to replace brushes with spray treatments?
Characterization and mitigation of bacteriological risks associated with packing fresh-market citrus

**Objectives:**

- Provide the CA citrus industry with controls that can be used to reduce or eliminate pathogen cross contamination in wash/drench systems

**Storage**
- 52°F (11°C)
- 24 h to 120 days

**Diagram courtesy of Linda Harris**

- Chlorinated dump tank
  - Sizer
  - Pre-grade

- Potable water
  - Spray
  - Brush rollers

- 3% soda ash tank

- Storage wax

- Imazalil + PAA recirculating
Key Learnings

• Cross contamination is possible under normal simulated use of soda ash
  o Can achieve >5 log reduction of Lm in 3% soda ash at 60C for 1 minute

• No significant difference in impact on Lm with extended use material

• Imazalil with >20 ppm PAA significantly reduces
  o Both *Listeria monocytogenes* and *Salmonella*
  o >5 log reduction – 20 ppm PAA. Exposure time reduced from >3 minutes to >1 minute as temperature increases (60-104F)

Why is this important?

• Demonstrates conditions can be found that accomplish pathogen control and post-harvest protection

• Key is operator's willingness to make changes/monitor variables
Resolving postharvest harborage sites of Listeria protects Zone 1 surfaces

Trevor Suslow, University of California, Davis (now PMA)

Objectives:

1. Develop a detailed baseline spatial mapping profile, among confidentially enrolled handlers, toward the development of a model environmental monitoring program (EMP) and guidance in establishing an environmental-zone Master Sanitation Schedule linked to EMP outcomes for California fresh citrus packinghouses

2. Evaluate the efficacy of emerging, registered hard-surface, nonporous- and porous-surface sanitizers on diverse non-contact surfaces that test positive for genus Listeria in enrolled citrus packinghouses
Key Learnings:

• High prevalence of *Listeria* may be expected on nonfood contact surfaces (NFCS) throughout RAC facilities until significant changes are made
  - Zones 2,3 baseline swabbing
  - 10 facilities (2016-2018)
  - 1,475 swabs
  - 31% molecular positives for *Listeria*
  - 30% culture positives

• Seasonality appears to play a role in prevalence and location at a facility
  - Range of positives varies by date
  - 3-93% molecular, 3-87% cultural positives for *Listeria* spp.
  - 3-28% cultural positives for *Lm*

• Sub-typing is needed to guide traffic and source tracking

• Current cleaning and sanitation regimes, in general, are inadequate

• Frequent or predominant negative test results should be questioned

• Zone 1 (FCS) remain vulnerable
Example of *L. mono* core genome allelic profiles from non-citrus facilities

- Isolates from different facilities belong to same cgMLST clusters
- Some of the cgMLST complexes include strains from several years (persistence) and from different geographically separate facilities
Control of *Listeria monocytogenes* on apple through spray manifold-applied antimicrobial intervention

Meijun Zhu, Washington State University

**Objectives:**

1. Examine the efficacy of selected sanitizers against *Listeria monocytogenes* on the whole apple surface though laboratory testing

2. Verify the selected sanitizer interventions in pilot packing line and multiple commercial packing lines
Key Learnings:

• PAA at a legal concentration was more effective than chlorine-based sanitizers against *Lm* on fresh apples

• PAA at 80 ppm and 30 s contact time resulted in 1.5-2.0 log reduction when applied at an elevated temperature during commercial packing intervention. Efficacy of PAA increased at elevated temperatures (up to 45C)

• PAA had a similar antimicrobial efficacy against *L. monocytogenes* and *E. faecium*. *Surrogate for Lm in sanitizer studies?*

Why is this important?

• Protocol to better control (but not eliminate) Lm on the surface of apples

• *L. monocytogenes* on fresh apples is difficult to eliminate, thus we need to **prevent contamination** throughout the production line
Mathematical modeling tools for practical chlorine control in produce wash process

Daniel Munther, Cleveland State University

Objectives:

• Construct data-informed models which use easy to measure water quality parameters to predict commodity specific organic load and free chlorine decay kinetics during recirculated wash conditions

\[
\frac{dX}{dt} = F(X, Y, t) \\
\frac{dY}{dt} = G(X, Y, t) \\
X(0) = X_0, \ Y(0) = Y_0
\]
Key Learnings

1. % of COD increase is a better predictor of the organic load affecting free chlorine decay than TUR/TDS and even COD
2. Using % of COD increase as input, the models capture fundamental FC dynamics, as predictions hold for multiple scales/produce types

Why is this important?

• Beginning of model development to guide operators on wash water control – rate of FC addition, consistent FC concentration maintenance, when to change water, etc.

• Provide tools to help validate treatments

• More to come – examine impact of continuous FC dosing, pH, products, etc.
What’s it all mean?

• Better to prevent Lm contamination that to try to remove Lm already on the product.

• Listeria sp. Can be found routinely in packinghouse environments. If your EMP is not finding them, you might not be looking hard enough.

• Seasonality can impact Lm prevalence and location within a facility.

• Recurrence of strains and frequency of detection indicates current sanitation practices not as effective as they need to be.

• Re-examining processes, e.g. use of brushes, drenches, to account for emerging knowledge of Lm can lead to mitigations.

• New tools (models) are on the way to permit more consistent control of wash systems
Q&A
Thanks to today’s presenters:

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Hungry for more?

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