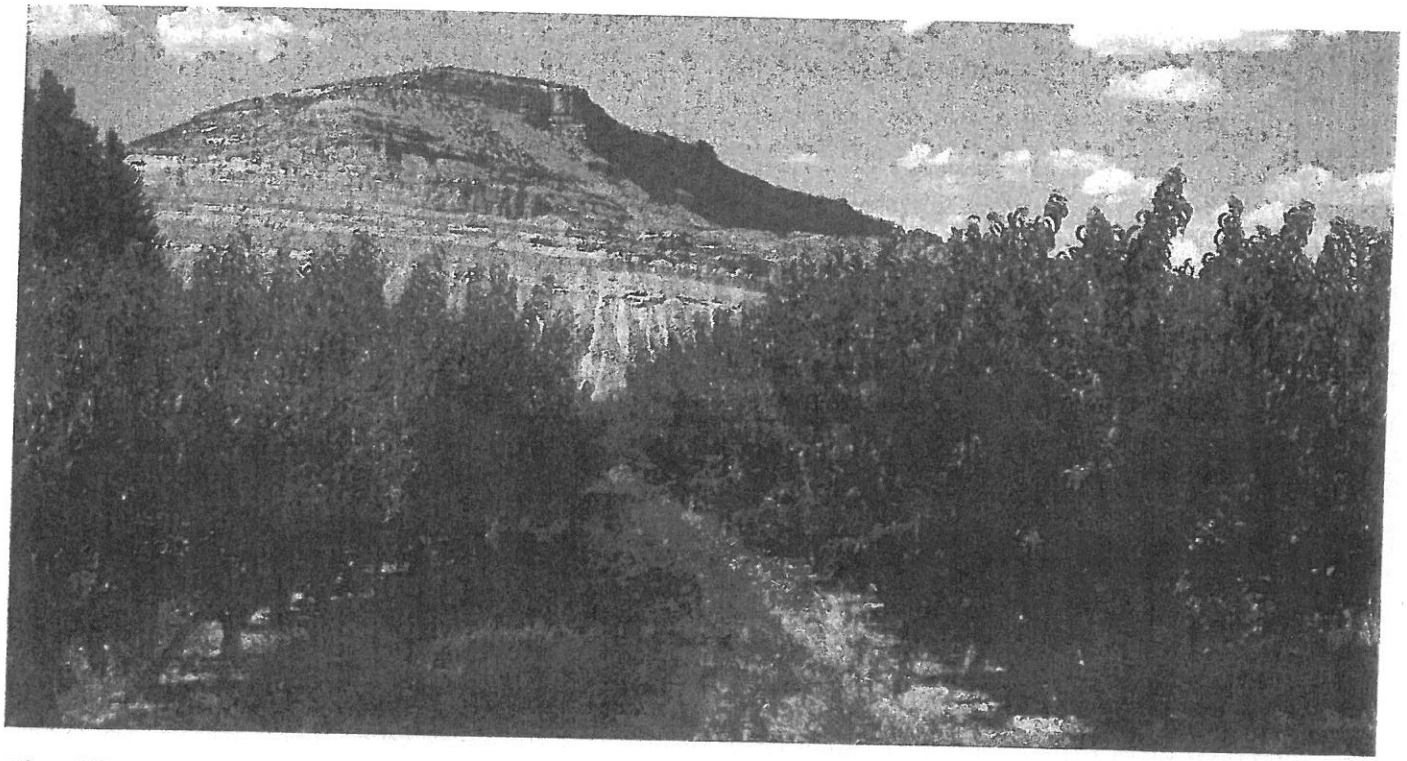
Poster Session



Significance of the Dormant State in the Persistence, Interaction with Growing Plants and Virulence of Shiga Toxin Producing *Escherichia coli*

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Project Date
January 1, 2017 – December 31, 2017

Summary
Shiga toxin producing *Escherichia coli* (STEC) are potentially highly virulent and can cause illness at levels of 10 cells if ingested by a susceptible host. Manure is a significant source of STEC and consequently when applied to land there is an interval of 90 to 120 days before harvest to permit any pathogens to die off. In field trials, it has been demonstrated that STEC die off rapidly within the first weeks of being incorporated into soil but a sub-population persist and can be recovered beyond 120 days. This led to speculation that there is a persistent sub-population of STEC that have enhanced tolerance to stress encountered in the field and possibly post-harvest. In the proposed study the persistent (dormant) state will be studied in STEC. Specifically, the culture conditions that induce the dormant state will be elucidated along with potential genes implicated. Studies will then determine the extent to which dormancy contributes to persistence in soil and resistance to sanitizers. Finally, the virulence of STEC in the dormant state will be determined. The main benefit of the research relates to providing data for risk assessment and also to develop novel methods to make STEC more susceptible to pre- along with post-harvest interventions.

Poster Session

Detection, Validation, and Assessment of Risks Implied by the Viable but Non-culturable (VBNC) State of Enteric Bacterial Pathogens in Fresh Produce

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Project Date
January 1, 2017 – December 31, 2018

Summary
More fresh fruits and vegetables are grown, sold and eaten today than at any other time in history. Unfortunately, outbreaks of food poisoning caused by pathogenic bacteria in fresh produce are also more common than in the past. Products are routinely analyzed to ensure that they are free of such bacteria, but some are very difficult to find because they do not grow on media used by quality control laboratories. These are known as "viable but non-culturable" (VBNC) bacteria. We will develop a new, inexpensive and easy-to-use method based on the well-known PCR reaction to make it possible for quality control laboratories to detect two important pathogens in fresh produce, *Salmonella* and *E. coli*, even when they are in the difficult-to-find VBNC form. The performance of the new method will be checked through field trials and pilot-plant experiments with leaf lettuce. Information from these experiments will be used to reveal how likely fresh produce is to be contaminated with VBNC pathogens during production and after harvest. Therefore, this research will contribute to an important new laboratory method and key information to support on-going efforts by the industry to improve the safety of fresh fruits and vegetables.

Enteric Viruses as New Indicators of Human and Cattle Fecal Contamination of Irrigation Waters *

Contact
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Project Date
January 1, 2015 – December 31, 2016

Summary
The standards used by the produce industry to detect fecal contamination (by indirectly testing for indicator organisms) in irrigation waters are based on tests developed for drinking waters (rather than surface waters) and include risk threshold levels established by the Environmental Protection Agency for recreational (bathing) waters. This contamination threshold is set with little scientific evidence for risk to human health from potentially contaminated irrigation water for food crops. Thus, it may not be appropriate for determining if there is a "risk relevant" level of contamination for cops irrigated with waters tested in this manner. To improve these regulations, we will use novel viral targets that have shown to better correlate with the presence of fecal material and evaluate them for potential use as more sensitive and specific detection methods for evaluating the safety of irrigation waters. This work will evaluate the accuracy of these novel indicator viruses and optimized the methods required for field use. This information may allow the produce industry valuable exposure data on the presence/absence

and quantity of fecal contamination that may be present in irrigation waters and provide much needed improvements to the use of indicator organisms for evaluating irrigation water quality.

Rapid Bacterial Testing for On-Farm Sampling *

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Project Date
January 1, 2015 – December 31, 2016

Summary
Due to the sensitive nature of fresh produce, bacteriological safety tests which requires days for results are not practical tools for food safety. In order for a testing plan to present a pragmatic solution, it must be low-cost, reliable, robust and deliver rapid results. Our labs have been developing diagnostics to be used in non-laboratory settings by utilizing bacteriophages. These viruses can attack specific bacteria, replicate within them and then lyse the host while releasing hundreds to thousands of additional viruses. We are proposing the development of a dipstick for the rapid detection of *Salmonella* spp. in agricultural samples. Following a sample pretreatment, the bacteriophages will be used to infect *Salmonella* spp. in the sample. Within 45 minutes, the increase in bacteriophage can be quantified with a simple lateral flow device resembling a pregnancy test. Preliminary results for our *E. coli* sensor suggest a very low limit of detection (<10 CFU/mL). This project will perform the additional development, optimization and validation steps needed and if successful will empower farmers to perform tests on-farm with rapid results and at low-cost. This ability will then enable more risk-based testing of agricultural waters following heavy rains or high flow.

Developing Cross-Assembly Phage as a Viral indicator for Irrigation Waters ****

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Project Date
January 1, 2017 – December 31, 2018

Summary
Ensuring high-quality irrigation water is necessary to protect the public when consuming minimally processed produce. The highest risk from exposure to contaminated water is due to viruses; however, water quality is currently monitored using bacteria that are poor representatives of viruses. All previous viral indicators are limited by a low abundance (i.e. difficult to detect) in the environment. Recently, a bacteriophage (virus that infects bacteria) named 'cross-assembly phage' (crAssphage) was discovered that is more abundant than all other bacteriophages in the human gut combined. Investigations in the PI's research group have shown crAssphage to be highly abundant in sewage. As crAssphage is a virus, it will be a better representative of viral contamination in the environment. In this investigation, I propose to sample irrigation water samples and measure crAssphage, viruses, and indicators in these samples to demonstrate the correlation of crAssphage and pathogens. I also propose to determine how much sample volume is necessary to accurately measure crAssphage. The development of this viral monitoring tool, catalyzed by funding this project, will enable risk-

managers to have an accurate and abundant indicator of viral contamination. This will ultimately provide greater protection of public health and improve consumer confidence in produce consumption.

Characterization and Mitigation of Bacteriological Risks Associated with Packing Fresh-Market Citrus ***

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Project Date
January 1, 2017 – December 31, 2018

Summary
After harvest, fresh oranges and lemons are sorted, washed and packed for further distribution and sale in packinghouses. Because green and blue molds result in significant losses of citrus fruit during storage and shipping, fungicides are often applied to during packing. Recirculating drench applications are common because they significantly increase fungicide efficacy but they also provide an opportunity for cross contamination or movement of microorganisms throughout the facility, which can be a food safety issue if not appropriately managed. The overall goal of this project is to provide data that the California fresh citrus packinghouse industry can use to support the controls that reduce or eliminate foodborne pathogen cross contamination where citrus fruits are comingled or where recirculating materials come into contact with the fruit. A laboratory component is included to determine, for the most common fungicides, minimum compatible sanitizer concentrations that are effective in eliminating *Salmonella* and *Listeria monocytogenes*. The laboratory data will be verified in a pilot scale citrus packing facility and the results of these studies will be used to prepare documents the industry can use to support the efficacy of their food safety practices.

Evaluation of Sanitizing Treatments for Sizer Carriers in Stone Fruit Packinghouses **

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Project Date
January 1, 2016 – December 31, 2016

Summary
Ensuring the safety of fresh fruit is a top priority of fresh produce packinghouses. The aim of this one-year research project is to evaluate and improve sanitizing treatments for sizer carriers in stone fruit packinghouses. The project will not only describe the potential for sizer carriers to harbor pathogens and allow for their growth under different environmental conditions, but will also define a set of sanitizers and application methods that represent the greatest promise for evaluation at the commercial level. Environmental sampling will be performed in active commercial packinghouses to determine natural microbial loads on fruit contact surfaces of sizer carriers. Subsequently, laboratory inoculation studies will be performed to determine the growth potential of foodborne pathogens on fruit sizer carriers under varied humidity and temperature. Furthermore, the potential of Clean-in-Place (CIP) sanitization will be

evaluated by applying no-rinse sanitizers (steam and aerosol antimicrobial chemicals) to the sizer carriers. Results from this study potentially will be applicable to diverse fresh fruit packinghouses for preventing pathogen cross-contamination in produce packing operations. Findings and recommendations will be reported and/or disseminated through industry meetings and technical publications.

Control of *Listeria monocytogenes* on Apple through Spray Manifold-Applied Antimicrobial Intervention ***

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Project Date
January 1, 2017 – December 31, 2018

Summary
Listeria monocytogenes is listed by the Food and Drug Administration as a 'pathogen of concern' and has been singled out on both ready-to-wash and ready-to-eat produce due to its nature as a true evironmental species. The Pacific region apple industry, led by Washington, suffered a significant loss of income following the *L. monocytogenes* outbreak that was traced back to a California packer. The final FDA Produce Rule and Preventive Controls Rule are challenging apple packers and handlers to develop specific efficacy data for their process controls. The apple industry has an immediate need to begin the process of science-based improvements in *Listeria* control during packing and subsequent storage. The overall goal of the proposed studies is to comparatively assess and validate critical operating parameters for registered, commercially practical, and legally allowed sanitizer(s) against *L. monocytogenes*, and to further seek to verify their efficacy on multiple apple packing lines. The proposed project will develop information for apple producers about the practical efficacy of antimicrobial interventions under commercial packing conditions, resulting in tested and proven methods for spray bar intervention in fresh apples, which will fill critical gaps in the knowledge. The results will be crucial for addressing *L. monocytogenes* safety in fresh apples.

Listeria monocytogenes Growth and Survival on Peaches and Nectarines as Influenced by Stone Fruit Packinghouse Operations, Storage and Transportation Conditions ***

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Project Date
January 1, 2017 – December 31, 2017

Summary
The recent multi-state *Listeria monocytogenes* outbreak associated with stone fruit consumption highlights the potential for stone fruits to serve as a vehicle in *Listeria* transmission. Further, the outbreak also demonstrates the pathogen's ability to persist and survive on stone fruits during handling, storage and transportation. While investigations on the persistence of Listeria have been performed on other

produce, there is a general lack of knowledge on the behavior of pathogens associated with stone fruits. Additionally, each produce type has unique compositional and physical characteristics that require produce-specific management practices. Therefore, to develop stone fruit-specific risk reduction knowledge and preventive controls, this study will investigate the survival and growth of *Listeria* on peaches and nectarines under packinghouse environment, storage and transportation conditions. The study will be performed under conditions simulating stone fruit unloading and staging, waxing and fungicide application, and storage and transportation from the packing facility. It is expected that results from this study will provide quantifiable data on the effect of current practices on *Listeria* survival on stone fruits. Furthermore, identification of food safety risks associated with different steps within the packinghouse continuum will help develop comprehensive preventive controls for foodborne pathogens including *Listeria monocytogenes*.

Cyclospora: Potential Reservoirs and Occurrence in Irrigation Waters ***

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Project Date
January 1, 2017 – December 31, 2018

Summary
Cyclospora has recently been implicated in outbreaks associated with U.S. produce imported from Mexico. Outbreaks have also been linked to drinking water. Information on the sources and occurrence of this organism are very limited. Currently, only humans and possibly primates are believed to be infected by this parasite. Our goal is to determine if produce in the United States is at risk of contamination from irrigation waters contaminated with human sewage (e.g., from faulty/leaky septic systems or compromised sewer pipes) and treated wastewater effluents that could potentially be discharged into surface waters used for the irrigation of food crops. Our specific objectives are to: a) determine the occurrence of *C. cayetanensis* in irrigation waters in Arizona and Texas, which will allow a determination of any risk from *C. cayetanensis* and to identify areas of potential risk; and b) to determine the occurrence of *C. cayetanensis* in raw sewage and treated wastewater effluents in produce producing areas such as Yuma, AZ and El Paso, TX, which will allow for an assessment of the incidence of *C. cayetanensis* infection among these communities. In addition, treated wastewater effluents are sometimes released into watersheds and could potentially impact irrigation waters. This study will allow us to determine if any risks exist from *Cyclospora* in irrigation waters from these two regions.

Establishment of Operating Standards for Produce Wash Systems Through the Identification of Specific Metrics and Test Methods

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Project Date
January 1, 2017 – December 31, 2018

Summary
The main objective of this proposal is helping producers to maintain the quality of the process water in commercial washing systems through control of water quality variables and the selection of adequate test methods for monitoring the process. Water disinfection is one of the most critical processing steps in fruit and vegetable production aimed at preventing cross-contamination. In the packinghouses and processing facilities, it is difficult to treat and maintain properly the quality of the process water because of the variability in the demand of disinfectant, the lack of operational limits and test methods to monitor the process and the different commercial operations. This project will investigate the most common disinfection agents used in packinghouses and processing facilities. Four scenarios have been selected based on different water characteristics including fresh-cut onions (excessive cell exudates, very high organic matter and turbidity), chopped lettuce (high organic matter and low turbidity), baby leaves (low organic matter and low turbidity) and peppers and tomatoes (low organic matter and high turbidity). Operational limits will be established in commercial facilities and lab-scale experiments using inoculated foodborne pathogens. Results obtained should inform producers about the realistic expectations for controlling selected water quality variables in produce washing systems.

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

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Project Date
January 1, 2017 – December 31, 2018

Summary
Fresh citrus is an important global commodity and a major specialty crop in California. The 2014-15 CA Agricultural Statistics Review places the combined value of oranges, lemons, and tangerines at over $ 2 billion dollars and all three are in the Top 15 Specialty Crops by value. Fresh whole citrus has not experienced an incident of recall, illness, or outbreak and CA citrus production practices and regions appear to significantly limit the environmental risk of preharvest contamination. However, recent serious incidents involving the bacterial pathogen *Listeria monocytogenes*, associated with CA apple, have prompted proactive measures to more carefully assess postharvest risks and develop validated interventions for citrus system-wide. Confidentially enrolled handlers will participate in a detailed survey for indicator *Listeria* and *L. monocytogenes*. The outcome will be the development of model Environmental Monitoring Program (EMP) and guidance in establishing an environmental-zone Master Sanitation Schedule linked to EMP-outcomes for California fresh citrus packinghouses. The anticipated outcomes are expected to include a general overview and report-card of the CA citrus packing environment and identification of potential sources of Listeria related to industry growing regions and harvest/postharvest practices. From this knowledge-gap closing effort, measureable improvements in reduced *L. monocytogenes* prevalence will result.

Poster Session

Remotely-sensed and Field-collected Hydrological, Landscape and Weather Data can Predict the Quality of Surface Water used for Produce Production ***

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Project Date
January 1, 2017 – December 31, 2018

Summary
There is a clear need for the development of improved, science-based tools to help reduce pre- harvest introduction of microbial produce safety risks through surface water use. The purpose of this project is (i) to identify and prioritize spatial and temporal risk factors for microbial contamination of surface water, and (ii) to develop geospatial models that predict surface water microbial quality, which will be assessed by quantifying generic *E. coli* and testing for key pathogens (e.g., *Salmonella*). Spatial and temporal variation in water quality will be assessed by repeatedly testing multiple water sources over two years. Publicly available remotely-sensed data (e.g., predominant upstream land-use) will be used to identify factors that are associated with elevated *E. coli* levels, and an increased risk of pathogen detection. Data collection will be performed in two produce growing regions (AZ and NY) to assess the robustness of our models and their translatability to other regions. These data and models will allow growers to identify times and locations where surface water sources are more likely to be microbially contaminated. This will enable growers to better time water use, testing, and treatment to minimize produce safety risks associated with microbially contaminated surface water.

(*) 2014 RFP This project was supported by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA.

(**) 2015 RFP This project was supported by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 15-SCBGP-CA-0046. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA.

(***) 2016 RFP This project was supported by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 16-SCBGP-CA-0035. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA.

(****) 2016 RFP
This project was supported by the Specialty Crop Block Grant Program at the Florida Department of Agriculture and Consumer Services through Grant USDA-AMS-SCBGP-2016. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the FDACS.

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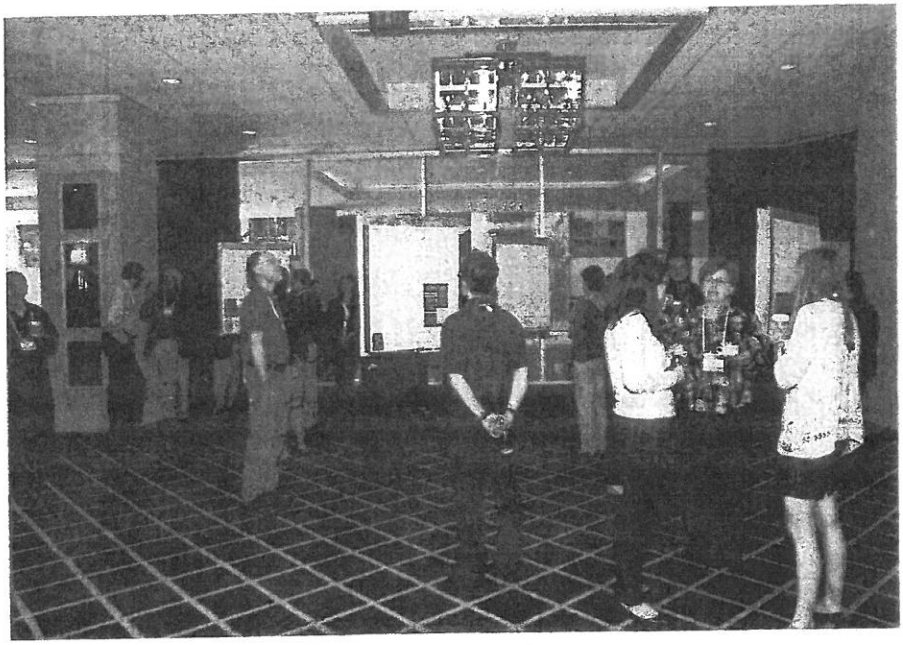

CPS Research Symposium 2016

A Produce Food Safety Timeline

1984 Traceback and lot identification

1994 Transportation SOPs

1995 Machine harvesting

1996 GAP

1998 FDA Guidance - Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables published, Water and Washing

1999 3rd part verification of field food safety programs, Well and Reservoirs testing (E. coli and coliforms)

2000 SS Harvesters, food contact surfaces, food grade containers

2001 Daily on-site sanitation of equipment, Industry Guidance—Core Elements of GAP & Audit Questionnaire published, Ranch buffer zones

2002 Application of GMPs to all field employees, Rodent Control Programs, Air Jumps

2002 Self-contained rest rooms, water, and waster water

2005 Lettuce/Leafy Greens Specific Guidelines

2006 Spinach-related E. coli outbreak

2007 Leafy Greens Commodity Specific Food Safety Metrics and Marketing Agreement

2008 Center for Produce Safety founded

2010 Food Safety Modernization Act passed by Congress

2011 Industry-developed GAP - Harmonized, published, and incorporated into USDA program

2012 FDA publishes Proposed Preventive Control Rules and Produce Rule

2017 Stakeholder Sessions

- Look back
- Discuss knowledge gained
- Engage expert panel to discuss research and look at next steps
- Look at research from four perspectives:
  - ✓Grower
  - ✓Packer
  - ✓Processor
  - ✓Buyer

cps CENTER for PRODUCE SA

At the field level

10 years ago:

- Guidances for specific commodities
- How long do pathogens survive in the field?
- If contamination found, how long before re-planting?
- What types of soil amendments are used and what are the associated hazards?
- What are the real risks represented by irrigation water and delivery systems?
- How do we best test irrigation water sources?
- Are some animals more "risky" than others?

Packing stakeholders

10 years ago:

- GMPs/guidances for some commodities
- Equipment pieced together
- Divergence of practices and operations
- Can cartons be re-used safely?
- Do pathogens transfer from product to container and container to product?
- Are gloves effective protection against pathogen transference?
- Do I need to change my wash water more than once/year?
- What do wash water disinfectants do?
- What is a biofilm?
- Concept of transient versus resident Lm?

Processor Food Safety: Then and Now

- Wash system log reduction vs. controlling cross contamination
- A focus on sanitizer level only vs. multiple process control factors
- Listeria controls: Fresh-cut processing is different from other food manufacturing

A Buyer's Perspective

- What's working?
- What do we need more of?
- What are the opportunities for improvement?