2018 CPS Symposium - Key Learnings

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Introduction:

The Center for Produce Safety (CPS) held its 9th Annual research symposium in Charlotte, NC in June 2018. In November 2018, CPS, United Fresh, Western Growers and Produce Marketing Association partnered to conduct a webinar highlighting the key learnings from the symposium and to answer questions from the audience. The recording of that webinar can be found on pma.com. Additionally, the research summaries of all the research projects presented at the 2018 CPS Symposium can be found on the CPS website.

As we prepare for the 2019 CPS Symposium in Austin, TX in June (link to registration page), I was reminded that I had not yet written down my key takeaways from the 2018 CPS Symposium. Since I have performed that task for the previous eight symposia, I thought I would take this opportunity to do so again, especially since some of these research findings are particularly relevant, given the illness outbreaks and consumer advisories of the last year and the upcoming research presentations scheduled for the 2019 symposium. Below are some of the findings that stood out in 2018.

Executive Summary:

1. Agricultural water remains a key area of industry operational focus and research. Understanding the potential risks associated with different water sources, irrigation water delivery systems and the environmental influences on the microbial quality of open water sources and the impact that can have on the safety of the product if the water contacts the edible portions of the plant are critical.
2. The environment agricultural water passes through and weather events prior to irrigation can impact the microbial quality of the water and should be part of any decision-making process on whether to irrigate with specific open water sources.
3. Wash or cooling water control is a multi-variable process and it is important for operators to validate or prove that their preventive controls are effective in killing pathogens. It is equally important to verify that the disinfection process is properly controlled and operated according to the process parameters you set every day.
4. Validation is such an important concept for the industry. When performing validation experiments to prove the efficacy of a preventive control, operators should be sure that the indicators or surrogates they use are authentic and that they are grown in such a way that puts them in the same physiological state as they would be in if they were in one of our production environments. Often pathogens on a fruit surface or in a drain in a cooling operation are physiologically stressed, i.e. they are not in their ideal environment for survival, so they slow their physiology and turn on defense mechanisms to protect themselves. Sometimes these defense systems also make them more resistant to disinfectants. We want to be sure to have them in this state when we validate treatments to kill pathogens, so we are setting metrics when they are the toughest to kill.
5. It is generally impossible to use live pathogens for validation studies so non-pathogen alternatives are critical. Several projects in 2018 reported results in the search for indicators of fecal contamination and *E. faecium* NRRL B-2354 was described as a useful indicator for Salmonella and demonstrated to be effective in validation of poultry litter heat treatments.

6. *Listeria monocytogenes* (Lm) remains an important area for creating awareness and inciting action in the produce industry. The Lm-focused research programs presented in 2018 at the CPS Symposium cut across several commodities and packinghouse configurations but, in the end, it was clear that operators need to have strong and aggressive sanitation programs and robust environmental monitoring programs (EMPs). Part of a strong sanitation program is understanding the niches within your operation where Lm might take up residence and being prepared to eliminate them. A strong EMP means designing sampling programs that are designed to find Lm if it is there, i.e. sampling places and locations where Lm might escape sanitation chemicals or locations where if Lm was there it might be able to contact the food (e.g. zone 1).
7. An important aspect of sanitation whether at the harvest equipment level on the farm or the display case level at retail is to perform cleaning right after operations end for the day as pathogens are harder to eliminate the longer they are permitted to reside with other organic matter on the surfaces of equipment. It is also important to permit appropriate residence time for sanitizers on cleaned surfaces to permit elimination of pathogens.

Key Learnings:

Agricultural Water – under the microscope. As we went into the 2018 CPS Symposium, agricultural water was already a topic of focus for the produce industry owing to the *E. coli* O157:H7 outbreak associated with romaine coming from the region around Wellton, AZ. A few weeks later, CDC reported that the specific outbreak strain was found in an irrigation canal in Wellton that was in proximity to a large cattle feeding operation five months after the causative cross contamination event(s) were likely to have occurred. Of course, later in 2018, a second *E. coli* O157:H7 outbreak was associated with romaine and that incident was ascribed to the use of irrigation water sourced from a farm pond.

Martin Wiedmann from Cornell University presented his results from a project entitled: "*Remotely-sensed and field-collected hydrological, landscape and weather data can predict the quality of surface water used for produce production.*" The objective of this program is to define and prioritize spatial and temporal microbial contamination risk factors for surface water and develop models to predict surface water microbial quality. While this project is not complete and we expect further results at the 2019 CPS Symposium, some of the major takeaways were:

- Adjacent landscapes and terrain can impact the microbial quality of agricultural water. For example, higher wind speeds correlate with increased incidence of finding STECs in samples and rainfall 96 hours prior to a sampling event results in greater detection of *Listeria monocytogenes*.
- Generic *E. coli* is not a reliable indicator of pathogenic *E. coli* strains
- Increased sample volumes for agricultural water and/or the use of Moore swabs increases the chances of recovering pathogens in an upstate New York watershed.

Michael Cahn from the University of California in Davis provided his final report on a two-year study titled: "*Microbial food safety risks of reusing tail water for leafy green production.*" This project was inspired by drought-related scarcity of agricultural water in some regions of California and the desire by some growers to better understand if tail water might be used for certain production applications. Tail water is a term used to describe water collected from irrigation activities. In effect, irrigation water that is not absorbed into the soil follows the slope of the land and collects in a tail water pond. The question then arises if that water might have become contaminated with pathogens present in the soil as it moved through the field and if re-used might represent a cross contamination hazard. Some key findings were:

- In five of the six tail water reservoirs studies the geometric mean for generic E. coli was less than the acceptable LGMA standard of 126 MPN/100 mL water (<u>note:</u> the LGMA standards in CA and AZ are currently under review and are likely to change).

- The prevalence of STEC, *E. coli* O157:H7 and *Salmonella* (<2% of samples) was lower in tail water ponds than in other portions of the Central Coast watershed.
- Tail water samples are higher in nutrients than well water, but the higher nutrient levels did not improve pathogen survival versus well water.

Things about agricultural water to consider in your produce safety program*:* The last year has seen intense efforts by the leafy greens industry and elsewhere in the produce industry to examine existing agricultural water practices and metrics. In truth, the key findings from Wiedmann were already in our knowledge base prior to 2018. Previous CPS Symposia have highlighted the inadequacy of generic *E. coli* as indicator for human pathogens in open water sources, increased sample volumes are preferred for finding low level contaminations and the environment water passes through and weather conditions can impact the quality of the water. Given recent events and our existing knowledge that pathogen populations decline quickly, but are recoverable for prolonged periods with enrichment, the use of any open water source for irrigation if the water contacts the edible portion of the plant are and should be called into question. It seems that tail water would fall into that category. Current work to define open source water treatments and validate their efficacy certainly take on critical importance as we move forward. For now, it is paramount that growers characterize their ag water sources and delivery systems and how that water is to be used on their commodities and if there is a chance it might contact the edible tissues. Generic *E. coli* may still have value as an indicator of potential contamination for closed water sources like wells. In a sufficiently maintained well, there should be no or very low levels of generic *E. coli*, so any significant presence of generic *E. coli* acts like a canary in a coal mine and can signal that the well has been compromised and bears inspection. Lacking better options for surrogates or indicators, proposals to use total coliforms (TC) as an indicator to validate open water treatment systems may be our best option owing to its hardiness; i.e. if the treatment controls TC, it should control the pathogen.

The data also inform the grower that part of their characterization of their ag water sources involves knowing the environment that water passes through and exists in. Wild animals, animal feeding operations, dairies and others using the system for non-agricultural uses can impact the quality of the water and subsequently the crop it irrigates. In as much as unusual weather can result in run-off into the water source or cause the silt where microorganisms are known to settle at the bottom of the water source to be stirred, a grower needs to take that into account when making determinations on whether to use the water for crop production without further mediations.

What to look for at the 2019 CPS Symposium on agricultural water: The 2019 CPS Symposium will continue to expand our agricultural water knowledge base. Channah Rock, University of Arizona will report on her work; *"[CPS Rapid Response - Yuma Valley](#)"* at a romaine farming site in Arizona examining and water treatment options for open source canals. Renata Ivanek, Cornell University will present here work; *"FSMA agricultural-water die-off compliance provisions benefit from condition-specific modifiers"* examining pathogen die-off rates in water and Martin Wiedmann will expand upon his 2018 presentation with his project, *"Remotely-sensed and field-collected hydrological, landscape and weather data can predict the quality of surface water used for produce production."*

CPS has funded agricultural water treatment projects previously and 2019 will feature two efforts in this area. Anita Wright, University of Florida will share her results thus far on *"Application of Chitosan Microparticles to Eliminate Foodborne Pathogens in Agricultural Water that Contacts Fresh Produce"* and Ana Allende, CEBAS, CSIS, Spain will share her project titled; *"Establishment of operating standards for produce wash systems through the identification of specific metrics and test methods."*

Lastly, our industry faced our first incidence of domestic contamination of romaine with Cyclospora in 2018. It was previously thought that Cyclospora, a parasite, was limited to tropical locations. Gerardo Lopez, University of Arizona will share his work on *"Cyclospora: Potential Reservoirs and Occurrence in Irrigation Waters."*

Wash Water sanitation is about process control. Control of produce wash water systems remains an important industry objective. In recent years, we have come to understand that effective wash water disinfection is dependent on several key variables including pH, chemical oxygen demand (COD), and disinfectant concentration. COD is a measure of the organic compounds in the water; often referred to as the organic load. At the Symposium in 2018, Ana Allende, CEBAS CSIC Spain reported on year one of her two-year project: "*Establishment of operating standards for produce wash systems through the identification of specific metrics and test methods*." This research program is particularly timely given new FSMA Preventive Controls Rule requirements for operators to develop, validate and verify preventive controls for wash water. Wash water control can be a challenge as different systems have unique physical characteristics; e.g. flumes, baths, single pass water, recirculated water, temperatures and different produce items have unique surfaces and shapes. The key findings from this project are:


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- The study evaluated ten different wash lines and pH is often poorly controlled. Even when pH and disinfectant are properly controlled, microorganisms are not totally eliminated.
- COD in wash water drives disinfectant demand and the level of COD is also directly correlated with the accumulation of chlorine dissociation byproducts; e.g. chlorates.
- Very low free chlorine concentrations (1-3 ppm) are effective in controlling inoculated pathogens in process water. However, pathogens grown under laboratory conditions are not necessarily a reliable indicator of naturally occurring pathogen physiological states and may, therefore be more sensitive to wash water disinfectants (see below).
- The type of fresh produce being washed can significantly impact the efficacy of free chlorine in controlling wash water microbial quality.

The 2018 Symposium also featured a presentation from Martin Wiedmann, Cornell University entitled: "*Pathogen physiological state has a greater effect on outcomes of challenge and validation studies than strain diversity.*" The focus of this study is broader than wash water control, but its significant in this section on wash water as it raises an important caution when performing validation studies. One of the key objectives of this work was to provide the produce industry with comprehensive data on how strain or pre-growth conditions affect survival of control interventions and growth on produce commodities. The key findings were:

- The bacterial strains used and the pre-growth conditions; i.e. the conditions used to grow the strains for validation studies greatly influence pathogen, index organism, indicator or surrogate survival against sanitizers or intervention steps and growth on produce.
- Strains obtained from culture collections may not always be what they are advertised to be. So, it behooves researchers or operations choosing strains for validation studies to use whole genome sequencing to verify the strain's identity.
- Given the two previous learnings, it is critically important to question historical validation experiments with a specific focus on how the test strains were prepared and if they were likely in a rapid growth phase that might be more susceptible to the intervention.

Things to consider in your produce safety program*:* The Allende project highlighted the complexities of wash water disinfection and control. The observed variability in pH and disinfectant control are likely more common than not and point to the critical importance of understanding the variables of wash water process control and using data to manage the systems properly. In the new world of FSMA and the Preventive Controls Rule, validating your preventive controls are working is the priority. Any operation where produce is washed or where water is treated with a disinfectant to prevent cross contamination (cooling conveyance, etc.) must have data to confirm their preventive controls are validated for their system and verify that the process was conducted within the parameters defined for the wash system operation. Water can be a great medium for transfer of pathogens. What might be a single leaf contaminated by a pathogen entering a wash system can become hundreds of pounds of contaminated leaves if the water is not properly controlled.

Wiedmann's project is both a reminder that the process of validating preventive controls is one that takes scientific rigor and a warning to make sure we question how the experiments or data we use to validate preventive controls was performed. The results tell us all to be sure the strains we use for any studies we perform are authentic and that they are grown in such a way as to reflect the physiology of the strain in our production environments. It is important to remember that many human pathogens thrive in warm, dark, humid, nutrient-rich environments like the human gut. Our produce environments can be cold, bright with sunshine, dehydrating by winds and nutrient challenged; in other words, stressful, so the pathogens slow their physiology to survive. That survival mode turns on defense mechanisms and can make the bacteria less susceptible to oxidizing disinfectants. So, when we want to test a preventive control designed to kill that pathogen, we want to do that experiment under conditions where the organism is most resistant. Therefore, growing the pathogen up to do the testing means growing it under conditions that stress the bacteria and make it act more like it would under our challenging production environments.

What to look for at the 2019 CPS Symposium on wash water control: Again in 2019 the CPS Symposium will feature new research on wash water management. Ana Allende will be back with her second year results from her project *"Establishment of operating standards for produce wash systems through the identification of specific metrics and test methods."* Daniel Munther from Cleveland State University will describe his work on "*Mathematical modeling tools for practical chlorine control in produce wash process.*" Modeling has the potential to be a valuable tool for predicting disinfectant needs and control. Lastly, Meijun Zhu from Washington State University will present "*Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention.*" We will discuss the first-year results of this work in a later section.

Wash water control part 2 – packinghouses: Packinghouse wash water or fruit treatments based on water (fungicide treatments or waxing) controls are critical areas of produce safety research. These issues have been amplified and extended in recent years owing to sporadic recalls owing to *Salmonella* or *Listeria monocytogenes* (Lm) detection in finished products. The 2018 Symposium featured three research presentations focused on management of fruit packinghouse wash system-based cross contamination risks. Linda Harris from the University of California at Davis presented her project: "*Characterization and mitigation of bacteriological risks associated with packing fresh-market citrus.*" This project examined potential cross contamination risks associated with the use of re-circulated fungicide treatments. The following was discussed:

- Citrus industry wash water and fungicide treatment practices varied from operation to operation. The concentrations of soda ash (lemon packing), pH, temperature and exposure times were variable. The fungicide commonly used, Imazalil, is used to prevent decay but it is not compatible with chlorine products that might be used to disinfect water.
- Neither *Salmonella* nor Lm was killed in heated water (40^{o}C/104^{o}F) within 5 minutes exposure time.
- Both S*almonella* and Lm were killed in 20 ppm PAA at 40^{o}C. In experiments designed to monitor cross contamination from inoculated to un-inoculated lemon fruit, 20 ppm PPA was effective in preventing cross contamination.
- *Salmonella* but not Lm was killed in 20 ppm PAA within 2 minutes at 16^{o}C/60^{o}F. Therefore, when doing validation studies, since Lm is more resistant to PAA than *Salmonella*, if you effectively control for Lm you will also be controlling *Salmonella*.

Moving from citrus to mangoes, Michelle Danyluk, University of Florida examined hot wash treatments commonly used to control fruit flies and mechanisms to control *Salmonella* in her project; "*Factors that influence the introduction, fate and mitigation of foodborne pathogens on mangoes throughout the production chain*." Typically, mangoes for export to the US are treated with hot water (115^{o}F for up to 90 minutes) and then hydrocooled to control fruit flies. This project examined the potential for Salmonella intrusion inside the fruit resulting from these hot water wash treatments and if there are varietal differences (Ataulfo, Tommy Adkins and Kent). The following results were reported:

- Hot water treatment followed by hydrocooling with Tommy Atkins fruits resulted in internalizations of *Salmonella*.
- With Ataulfo, the research uncovered significant internalization of *Salmonella* at the blossom end and midsection compared to other mango varieties.
- If the fruit are permitted to go through a "rest" period (air cooling) after the heat treatment and prior to hydrocooling, internalization is reduced, but if internalization occurs and the fruit are shipped at the standard shipping temperature of 12°C, *Salmonella* can continue to grow.
- The project also focused on spray/brush wash systems with different approaches to disinfection including chlorine, PAA and ozone to control *Salmonella*. The brush wash systems resulted in significant reductions of *Salmonella* within 5 seconds of contact time but there was a survival tail out to 60 seconds.
- Ozone and chlorine dioxide were not as effective in controlling *Salmonella* in this brush system as PAA and sodium hypochlorite at labelled rates.

The idea of finding alternatives to common immersion wash systems was also explored by Meijun Zhu from Washington State University in her project; *"Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention."* This project examined various disinfectants applied via a spray manifold designed for apple washing. Zhu reported the following results:

- Using Granny Smith apples inoculated with Lm and left to dry for 48 hours and then spray washed with water containing chlorine sanitizers, less than 1-log reduction was observed with a 2-minute contact time.
- PAA at 80 ppm and 2-minute contact time yielded a 1.6-log reduction in Lm.
- Lm and *E. faecium* had similar resistances to chlorine and PAA at 2 min contact times.

Things to consider in your produce safety program: The data described for packinghouse wash systems reinforce previous CPS research that shows the positive effect of the use of brushes or spray manifolds to provide a physical force to “scrub” fruits to loosen bound bacteria and then the effective use of wash water disinfectants to kill those bacteria in the water. As we observed in the previous section on processing, wash water management is a complex, multi-variable process and certainly variables like organic materials in the wash water, pH and temperature are important factors to measure to ensure disinfectant efficacy. These research projects also point out the importance of contact time; i.e. the amount of time the product remains in contact with the water that contains the disinfectant. This is an important factor for operators to pay attention to. In general, many packinghouse operations are focused on speed; wash and pack quickly to meet orders and get them on the way to our customers. As these projects demonstrate, we need to also consider the effectiveness of our wash systems and build in the required contact times (either slow the lines or extend the length of the wash line to boost contact or when possible adjust disinfectant concentrations to insure control) to make sure that our systems operate properly.

These projects also remind us to be aware of the possibility of pathogen intrusion, i.e. when pathogens in the water are not completely controlled or killed by the disinfectant and the temperature differential between the fruit and the water is such that water tends to move into the fruit through the blossom end. As discussed in the previous section, it is important to validate the efficacy of our disinfection use in our systems. Every operation will have a different geometry and design for their wash or cooling systems and our validation studies need to be reflective of those differences.

What to look for at the 2019 CPS Symposium on wash water control in packinghouses: At the 2019 CPS Symposium there will be at least three presentations related to packinghouse wash systems. Kay Cooksey, Clemson University will present her program titled; “*Preservation of stone fruits by spray application of edible coatings with antimicrobial properties*.” Linda Harris, University of California at Davis will build on her 2018 presentation with “Characterization and mitigation of bacteriological risks associated with packing fresh-market citrus” as will Meijun Zhu, Washington State University with “*Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention*.”

The search for surrogates and indicator organisms continues. Validation has been a term that has already been used in the previous sections of this paper. As produce safety is evolving to embrace the scientific rigor of validation; i.e. demonstrating that preventive controls effectively manage cross contamination risks, the search for surrogates for human pathogens continues to be a priority. It is really a matter of practicality as it is not advisable to use live pathogens like *E. coli O157:H7* or *Listeria monocytogenes* in production environments to test their control. Therefore, the identification of surrogates; a non-pathogenic organism that shares similar growth and survival characteristics with correlated pathogens or indicator organisms that are not pathogens themselves but are known to be associated with animal or human fecal material, is an important research priority. Therefore in 2018 we heard from Kyle Bibby from the University of Pittsburg on his project "*Developing Cross-Assembly Phage as a Viral Indicator for Irrigation Waters.*" This project is focused on identifying indicators of human viruses in agricultural water. Currently, we do not really know the prevalence of human viruses in ag water nor the extent to which human viruses would be a potential public health hazard if present. The key findings thus far are:

- Cross-assembly phage (crAssphage) may be an indicator for human fecal pollution and human viruses in ag water. A virus may have benefits over bacterial indicators of human fecal material as we would expect them to be present in much higher concentrations and therefore more easily detected and monitored.
- The research team has collected irrigation water samples (approx. 100) from five states and crAssphage has been found in 35% of the samples thus far. The team also test for enterococci and generic *E. coli*. crAssphage does not correlate with indicators of human bacterial pathogens.
- crAssphage does not correlate with coliphage (indicator of *E. coli*).

Kelly Bright, University of Arizona reported on her project, *"Identification of novel indicator organisms to determine the risks of fecal contamination of irrigation waters."* Water samples were collected from two Arizona production areas with an objective of characterizing the microbiome and identifying indicators of pathogen presence. This novel approach to characterizing ag water demonstrated that there are regional and seasonal changes in the microbial populations in water sources.

Julie Meyer, University of Florida, used an existing surrogate, an avirulent *Salmonella* strain, for her project; *"Comparative genomics analysis and physiological assessment of the avirulent Salmonella surrogate relevant to produce safety."* As surrogates are developed for use in validation studies, it is important to characterize them to determine how genetically and physiologically similar they are to their pathogenic cousins. The better we understand that relationship, the more confidence we can have that the preventive controls we implement to control the surrogate will also be effective against the represented pathogen. The highlights of this project were:

- The project looked at what genes in *Salmonella* are essential to permit growth in tomatoes. In basic terms, the research team used comparative genomics to identify genes needed to survive in the tomato plant versus what the pathogen needs to survive in the environment.
- Use of mutant *Salmonella* strains demonstrated that proliferation of *Salmonella* in tomatoes requires genes associated with amino acid and lipopolysaccharide production.
- The studies verified that the surrogate *Salmonella* strain responds similarly to wild-type strains when challenged within growth in tomatoes.
- Longer term, a potential mechanism to breed *Salmonella* resistance in tomato might revolve around approaches to disrupt lipopolysaccharide production as a host's defense to prevent *Salmonella* proliferation.

Lastly, Xiuping Jiang, Clemson University reported on her project; "*Validating a physically heat-*

[treated process for poultry litter in industry settings using the avirulent Salmonella surrogates or indicator microorganisms](#).” In this study, the group examined avirulent *Salmonella* surrogates and indicator organisms to find one that could be used to validate heat treatments to kill pathogenic *Salmonella* for chicken litter, an organic soil amendment commonly used in the produce industry. The outcome of this work was the identification of *E. faecium* NRRL B-2354 as an appropriate surrogate for *Salmonella* and an important tool for validating thermal processing of poultry litter.

Things to consider in your produce safety program: The search for surrogates and indicator organisms has been an arduous journey. There have been successes; among them are *E. faecium* strains used in tree nut roasting programs, generic *E. coli* for deep well agricultural water quality, avirulent *E. coli* O157:H7 and *Salmonella* surrogates for testing persistence and growth in production environments and, as we learned at the 2018 CPS Symposium, *E. faecium* NRRL B-2354 for validating heat treatments of poultry litter. It is particularly exciting to see Jiang’s work on the development of a surrogate that can be used to validate poultry litter composting treatments as pelletized poultry litter is a common soil amendment used across several produce commodities. Composting is really a process where temperature, time and several other factors (e.g. moisture, pH, salt content, etc.) are important to control to ensure pathogen elimination.

More generally, it is important for growers, packers and processors to appreciate the need to validate preventive controls and using surrogates or indicators are important tools for that work. Pulling learnings from the earlier discussed Wiedmann presentation, verifying that the surrogate or indicator you use for validation studies and making sure it is grown under conditions that produce a physiological state that mimics the state found in the production environment is also critical.

The approaches that were employed in Bright’s work using genomic sequencing to identify organisms that might be indicative of pathogens and the Bibby work looking for human viruses that might be indicative of human fecal matter are novel and scientifically interesting. Operationally, we need additional surrogates or indicators to cover the multitude of preventive practices we need to validate, so we need to stay tuned to see what emerges from these programs. In recent times, the focus on finding methods to treat open or surface sources of irrigation water have really been hampered to some extent by the lack of surrogates or indicators that might exist naturally in open water sources can be used to validate those treatments.

Lastly, the concept of breeding tomatoes with resistance to *Salmonella* persistence and growth in the fruit is exciting. Plant breeders have been developing new varieties with resistance to plant pathogens for two hundred years and it seems reasonable that to the extent that human pathogens can persist or even grow in certain produce items; genetic resistance is a reachable goal.

What to look for at the 2019 CPS Symposium on the search for surrogates or indicators and validation tools. AT the 2019 CPS Symposium we will hear further developments from Kyle Bibby, University of Pittsburg on his project “*Developing Cross-Assembly Phage as a viral indicator for* *irrigation waters."* Gloria Sanchez-Moragas from IATA-CSIC Spain will expand on the concept of viral indicators of human fecal material and present her progress on her project *"Metagenomics to identify viral indicators in the produce chain."* If you are attending the 2019 Symposium, listen carefully as I suspect you will hear several of the programs are reliant on using surrogates or indicators. Listen for how they are used, how they are grown and prepared and the treatments they are used for to see how they might relate to your own operational needs.

***Listeria monocytogenes* control: equipment and facility design, sanitation and movement.** In some ways the recent focus on *E. coli* O157:H7 and leafy greens has pushed industry attention away from Lm cross contamination. Fortunately, the 2018 CPS Symposium was rich with research reports on Lm and featured three outstanding programs:

Mary Anne Amalaradjou, University of Connecticut delivered her final report on her project; "*Listeria monocytogenes growth and survival on peaches and nectarines as influenced by stone fruit packinghouse operations, storage and transportation conditions."* This project examined post-harvest contamination and survival of Lm on peaches and nectarines handling at three critical stages: (1) unloading and staging entering the packinghouse, (2) waxing and fungicide treatment and (3) cooling, storage and transport. The following points were made:

- Waxing and fungicide (Fludioxonil, Propiconazole) application experiments were conducted in the laboratory and there were no effects on Lm growth or reduction after 6 hours.
- There was no difference between Lm survival on peaches and nectarines.
- **Lm** survives but does not grow on non-wounded fruit surfaces. Survival is not influenced by variety, common postharvest treatments like waxing, temperature or relative humidity.

  Rolf Joerger, University of Delaware presented his final report on "*Evaluation of the efficacy of antimicrobial agents to prevent the transfer of Listeria monocytogenes from existing biofilms to produce or processing surfaces."* This project focused on conditions during a wash process the limit or kill Lm cells released from biofilms on equipment surfaces. The key findings were:
- Low levels of Lm released from mixed-type biofilms into process water are more resistant to antimicrobials.
- In "clean" water, transfer of Lm to blueberries is controlled at sodium hypochlorite concentrations greater than 10 ppm; i.e. Lm is killed.
- In process water with high solids content, Lm is not eliminated even at concentrations of 250 ppm sodium hypochlorite and can transfer to fruit or vegetable surfaces. grown in soil, attach to mung beans (smooth and rough surface), - get a lot of concentration

Lastly, Trevor Suslow from the University of California at Davis (now at the Produce Marketing Association) discussed his project; "*Resolving postharvest harborage sites of Listeria protects Zone 1 surfaces."* This was a multi-season study of non-food contact surfaces (zones 2 and 3) in a diverse collection of California citrus packing operations in the first year of the program that was extended to zone 1 (food contact) sites in selected operations during year 2. The key learnings are:

- There was variable but predictable presence of Listeria and Lm in all operations on non-food contact surfaces. In over 1,200 swab samples, 8-17% were positive for Lm. There is a high level of diversity for Lm strains in packing operations.
- There were different Listeria strains present in the finished product areas versus the incoming raw product areas. Interestingly, the greening rooms where unwashed product is generally present have low levels of Listeria or Lm. This may be related to the fact that these areas are relatively dry.
- Typical cleaning and sanitation programs were not effective in eliminating established Lm.
- Listeria positives from zone 1 swabs were very low; 3/269 samples positive, none were Lm.

- Listeria spp. and Lm prevalence and distribution is seasonal; i.e. there are more positives in the rainy season and carryover from season to season for certain subtypes was observed.

Things to consider in your produce safety program. While the three research programs that dealt with Lm were diverse in scope, they all ended up with the conclusion that all produce operations across the supply chain need to have aggressive environmental monitoring programs and effective sanitation strategies. We were fortunate in 2018 to have Martin Wiedmann, Cornell University, share his thoughts on Lm risk management in a session titled "*Listeria monocytogenes*: what can be learned from outside the produce industry? Lm is not just a produce issue; it is a food industry challenge. Beef, poultry, seafood and frozen foods have all had their challenges in the past. As I was reviewing my notes on Martin's presentation it occurred to me that they matched up well with the key learnings all growers, packers and processors need to consider for their operations:

- Prerequisite programs like Good Manufacturing Programs (GMPs), preventive controls, sanitary equipment design, standard sanitation operating practices (SSOPs) and environmental monitoring are critical to Lm control for all foods.
- The meat industry has learned that growth niches for Lm are key. If the sanitation program does not effectively reach the niche, Lm can take up residence and subsequently be a source for cross contamination onto food. It is important that equipment is dissembled, cleaned and sanitized or heat treated in a manner to eliminate any contaminating organisms.
- It is always important to create clear separation between raw products and ready to eat (RTE) finished products.
- Keep facilities as dry as possible to help control movement of Lm.
- It is important to know that Lm preventive controls are effective; i.e. validation. Far too often, operators are verifying procedures when they really do not know if those controls work.
- When introducing a new piece of equipment to your operation, make sure the SSOPs for that equipment are validated *before* commercial product is made.
- A risk-based master sanitation schedule is important if you cannot clean everything every day.
- A key learning from the meat industry has been the need for each operation to have a sophisticated environmental monitoring program (EMP). The use of random selection to identify daily or shift-based sampling sites overlaid on the frequency of positive or out of compliance verification testing is crucial. EMPs need to drive preventive action. If you get a positive swab, perform vector swabbing; left, right, up and down and determine where the Lm might have come from. It can be instructive to do swabbing before you sanitize. If you deep clean first and then swab, you might push bacteria back into niches. You can then swab after cleaning/sanitation to measure effectiveness.
- Composite swabbing (combining swabs from multiple locations and combining them into a single test sample) can save costs but it makes it hard to find the source of positive test. It is not recommended for zones 1 or 2.
- The Blue Bell ice cream incident demonstrated the importance of Lm long-term survival; i.e. can make individual consumers sick over a long period of time which will eventually show up on surveillance programs.
- There can be value in having an independent third party do a swab-a-thon to find problem areas. It provides a "break" in the routine practices your operation might have and brings in outside experts to look in places where your employees might not.
- Seek and destroy is a convenient terminology, but it is important to really seek out Lm with aggressive testing and it is equally important to make sure your destroy mechanism really works and if not, perform root cause analysis (RCA) to find out why there is either persistence or re-contamination.

What to look for at the 2019 CPS Symposium on Lm control. The 2019 CPS Symposium will feature further research programs dealing with Lm and its persistence in packing and processing environments. Trevor Suslow, University of California at Davis (emeritus) and now at PMA will discuss his project; *"Resolving postharvest harborage sites of Listeria protects Zone 1 surfaces."* Amanda Lathrop, California Polytechnic State University will present her program; *"The effects of storage conditions and the natural microbiome of nontraditional fresh-cut salad ingredients on the fate of Listeria monocytogenes."* Nitin Nitin, University of California at Davis will present on a novel approach of finding ways to control resident bacteria with his project; *"Rechargeable antimicrobial and antifouling plastics for improved cleaning and sanitation of plastic bins and totes."* There will also be a project by Martin Wiedmann from Cornell University titled *"Listeria whole genome sequencing data reference sets are needed to allow for improved persistence assessment and source tracking"* that will speak to the importance of tracking the movement of Lm in facilities and within regions. Lastly, Linda Harris, University of California at Davis will present her work on *"Characterization and mitigation of bacteriological risks associated with packing fresh-market citrus,"* where she will discuss the search for surrogates and their use in validating sanitation practices.


Photo courtesy of Laura Strawn, Virginia Tech

Contamination can occur across the supply chain. Laura Strawn from Virginia Polytechnic reported on the final year of her research project; *"Control of cross-contamination during field-pack and retail handling of cantaloupe."* This project was focused on the assessing transfer of *Salmonella* and Lm onto cantaloupes in field packing operation from food contact surfaces and examining best practices for sanitation programs to mitigate cross contamination risks. Similarly, a second focus area was to assess cantaloupe handling practices in retail environments and characterize potential cross contamination points. The key findings from this program are:

- At harvest, transfer of *Salmonella* or Lm from contaminated cantaloupes to a food contact surface or from a food contact surface to a cantaloupe was low with both dry and wet inoculum.
- All surfaces are not the same; i.e. rubber gloves are more prone to transfer than cotton or nitrile gloves.
- *Salmonella* and Lm concentrations decline over time but persist longer on dirty, fouled surfaces. The important implication is to perform cleaning and sanitation right after harvest operations have ceased for the day. The longer the bacteria reside in these environments, the harder they be to kill.
- Contact time is very important for Lm kill. It is best to leave sanitizer on the equipment contact surfaces overnight if the equipment is fouled. If the equipment is cleaned, 30-minutes contact time is a good place to start but should be validated.
- The same principals apply to retail food contact surfaces as harvest equipment food contact surfaces. It is important to clean and sanitize retail food contact surfaces regularly to reduce the incidence of Lm. Retail surfaces that were not cleaned and sanitized often had higher prevalence of Lm.

Things to consider for your produce safety program. This project really drives home the industry adage that produce safety is everyone's responsibility. This was a unique project in that it focused on the two ends of our industry; harvest equipment in the field and display cases at retail. Importantly, the same risk factors found in the field if cleaning and sanitation are not performed routinely can also be found in the retail display case. Sanitation is critically important to your operation whether in the field, packinghouse, processing plant or retail store. The cleaning part of sanitation is best done as soon as operations halt for the day to prevent pathogens from becoming embedded in organic matter on a surface or forming biofilms that make them harder to access by the application of sanitizers. The second part of sanitation is the application of sanitizer and contact time between sanitizer and the surface is important. Application of sanitizer and simply rinsing it off and starting operations is not recommended.

What to look for at the 2019 CPS Symposium on supply chain contamination. It is already been cited in this paper, but Nitin Nitin, University of California at Davis will present his project; "*Rechargeable antimicrobial and antifouling plastics for improved cleaning and sanitation of plastic bins and totes*." This program speaks directly to the notion of keeping surfaces clean and it implications for sanitation. As you listen to the 2019 CPS Symposium or review the program on the CPS website, look for other areas of produce safety that span the length of the supply chain and where the importance of executing aggressive cleaning and sanitation programs can mitigate the risks of cross contamination.

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This work is meant to inform and provoke thought with an eye towards inspiring readers to examine their own produce safety programs and using the research to make improvements. It is not meant as a directive on what must be done to produce safe food. Produce safety needs to be determined on an operation by operation basis; there are no one size fits all solutions. If you have additional questions, please feel free to contact Dr. Bob Whitaker, PMA Chief Science and Technology Officer or Dr. Trevor Suslow, PMA Vice President of Food Safety.